



## Detection of Varicella Zoster Virus (VZV) in the Benign and Malignant Breast Tumors by Polymerase chain Reaction

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### ABSTRACT

This study aimed to investigate the frequency of Varicella Zoster Virus (VZV) in 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 were collected from patients from June 2011 to February 2012. DNA was extracted from all samples and the infection with VZV was examined by PCR technique. The results obtained in this study showed that 3 out of 24 carcinoma samples (12.5%) were infected by VZV, while the number of fibroadenoma samples infected by this virus was 3 (12.5%). Based on the results obtained by this study, VZV was observed in both benign and malignant tumors. However, no association was observed between VZV infection and the formation of malignant or benign tumors based on the Chi-square test. Although it has been proven that VZV plays role in occurrence of latent infections after chemotherapy, no relationship was confirmed between this virus and breast benign and malignant tumors. Future studies are needed to elucidate the possible role of the virus in the disease. It is concluded that VZV is detectable in both malignant and benign tumors of the breast and the virus may play a role in the pathophysiology of breast tumors. However, further studies are needed to confirm the role of VZV in tumor formation.

### 1. 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, milk producing tissue and in unglanular tissue. Breast cancer is one of the most prevalent cancers in women (Parkin et al., 2005). Every year, a large

number of women are afflicted by breast cancer, of which the disease is fatal for a number of sufferers (Khorshid, 2011). According to the data presented by the US National Institute of Cancer, one out of every 8 women are inflicted with breast cancer (Wong et al., 2002). Although genetic factors, such as mutation in the BRCA1 and BRCA 2 genes, play an important role in the occurrence of the cancer (Miki et al., 1994; Wooster et al., 1995), other

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susceptible factors cannot be neglected. For example, the risk of suffering from breast cancer increases with age (Abbasi et al., 2009; Alamelumangai et al., 2012). Almost, three quarters of the cases of affliction occur in women who are more than 50 years old. It has been shown that family history of breast cancer, puberty before the age of 13 years, menopause after the age of 51 years, women who have never become pregnant and those who have become pregnant for the first time after the age of 30 years, suffering from obesity, particularly after menopause, poor diet (Rand et al., 2009), excess alcohol consumption, and viral infection are among the most important risk factors (Wooster et al., 1995).

The studies carried out in two recent decades have provided the role of viruses in the occurrence of cancers. For example, it has been shown that EBV plays role in Burkitt's lymphoma (Epstein et al., 1964; Irshaid et al., 2010), and nasopharyngeal carcinoma (Nikakhlag et al., 2010), KSHV in Kaposi's sarcoma (Boulanger et al., 2001), HTLV in lymphoma (Poiesz et al., 2001; Philips et al., 2001) and papilloma virus in skin cancer (Zaravinos et al., 2009).

Varicella zoster virus has a double stranded DNA and icosahedral symmetry. This nucleocapsid has been surrounded by a covering being of the lipid (Arvin, 1996). VZV looks similar to herpes simplex virus, has no animal reservoir, and proliferates in human embryo culture with cytopathic effects and produces specific intra-nucleus inclusion bodies (John and Gnann, 2002). Amplification of a small fragment of VZV genome by PCR is the most common technique to detect this virus in clinical samples (Druce et al., 2002).

This study aimed to investigate the frequency of VZV among females with benign and malignant breast tumors. Because of the lack of documented information regarding the frequency of the Varicella Zoster Virus in the benign and malignant tumors, the current study was designed and executed.

## 2. Materials and Methods

Paraffin embedded tissue samples of the breast carcinoma (n=24) and fibroadenoma (n=24) were collected from the pathology ward of Toos and Firozgar hospitals of Tehran, Iran in 2011-2012. The carcinogenicity of the samples was diagnosed

with the aid of the pathologist and based on the Richardson classification system.

### 2.1. Protocol of deparaffinization of the samples

At first, ten 5 $\mu$  cutting were provided from the paraffin blocks by the sterile microtome blade (N35) and transferred to the sterile containers. In order to deparaffinize, the samples were left in a xylene solution (Merck-Germany) for 30 minutes. To dehydrate the samples, they were placed for 10 seconds in ethanol solutions with the concentrations of 100%, 80%, 60%, and 40% (Merck-Germany), respectively. The tissue obtained was then transferred to sterile micro tubes and stored under -20°C until the process of DNA extraction.

### 2.2. DNA extraction

Extraction of DNA from the tissue was implemented according to the instructions of the manufacturing company (Qiagene, Lot No: 11872534, Cat No: 51306). The purity of the extracted DNA was analyzed based on absorbance of the extracted DNA at 260 and 280 nm wavelengths by biophotometer (Eppendorf-Germany).

### 2.3. 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'-TCC AAC ATC AAC ATC TTG GT-3'; and b2-microglobulin-R:5'- TCC CCC AAA TTC TAA GCA GA-3' (Zaravinos et al., 2009). Each reaction was performed in a total volume of 25  $\mu$ l, which contained 13  $\mu$ l of molecular biology-grade water (Sigma Aldrich Company LTD., USA), 2.5  $\mu$ l of 10 $\times$ PCR buffer (Promega, USA), 1  $\mu$ l of 10 pmol of forward and reverse PCR primers, 1  $\mu$ l of 10 mM dNTPs (Promega, USA), 0.5  $\mu$ l of smart taq DNA polymerase (Promega, USA), 1  $\mu$ l of 50 mM MgCl<sub>2</sub> (Promega, USA) and 5  $\mu$ l of DNA template. The negative control tube contained the same PCR reagents as above but had 5  $\mu$ l 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. An aliquot of all PCR products was run on a 1.5% (w/v) agarose gels with a 100 bp DNA ladder (Fermentas-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).

#### 2.4. PCR method

Specific Primers produced by TAG Copenhagen (Denmark) were used to amplify the EBV gene. The sequences of forward and reverse primers were 5'- ATGTCGGTACAACATCAACT -3' and 5'-CGATTTTCCAAGAGAGACGC -3', respectively (Zaravinos et al., 2009).

Each reaction was performed in a total volume of 25 µl, which contained 13 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 MgCl<sub>2</sub> (Promega, USA) and 5 µl of DNA template. The negative control tube contained the same PCR reagents as mentioned above but had 5 µl of water substituted for the DNA template. 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. An aliquot of all PCR products was run on a 1.5% (w/v) agarose gels with a 100 bp DNA ladder (Fermentas-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).

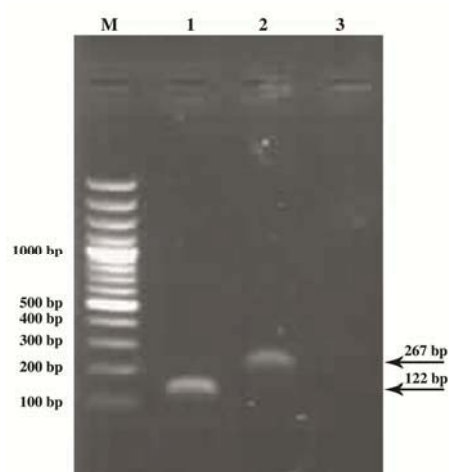
#### 2.5. Statistical analysis

Chi square test was used to determine whether there was any significant difference between the frequency of VZV in the carcinoma and fibroadenoma samples and its relationship with the breast cancer (SPSS software 17).

### 3. Results

The number of patients who suffered from the carcinoma based on age groups was: below 35 years old, 5 patients (21%); 35 to 55 years old, 12 patients (50%); and over 55 years old, 7 patients (29%). The average tumor size in 6 patients (25%) was smaller than 2 cm and in 18 individuals (75%) the average tumor size was larger than 2 cm regarding the lymphatic glands under than arms, involvement of these glands was not diagnosed in 6 individuals (25%) But, the involvement of lymphatic glands was observed in 18 individuals (75%). Among the studied carcinoma samples, 22 samples of ductal carcinoma (92%), one sample of the lobular carcinoma (4%) and one sample of the mucinous carcinoma (4%) were diagnosed. Four patients (16.6%) suffered from stage I malignant tumor, 9 patients (37.5%) from stage II, and 11 patients (45.9%) were diagnosed with a stage III. Regarding the demographic information of patients afflicted with fibroadenoma, only the age of the patients was available. 17 individuals (71%) suffering from fibroadenoma were in the age group under 35 years and 7 individuals (29%) were in the age group of 35-55 years.

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 267 bp, respectively (Figure 1).



**Figure 1.** VZV amplification products analyses in a 1.5% agarose gel stained with ethidium bromide. lane M: molecular marker 100bp, lane 1 human beta-globin gene, lane 2 viral DNA and lane 3 negative control.

In summary, three samples (12.5%) were positive from the point of view of the presence of VZV among the 24 patients. Among 3 patients, two patients were in the age group 35-55 years and one patient was in the age group over 55 years old. Each of the 3 patients infected with the virus also suffered from the ductal carcinoma and no involvement of the lymph node. Two individuals infected with virus suffered from stage III while stage I was diagnosed in one patient. Regarding the tumor size, 1 sample was larger than 2 cm in diameter, while the two samples were below 2 cm. Out of 24 samples of fibroadenoma, three samples (12.5%) had the genome for this virus with this patient placed in the age group under 35 years. The statistical analysis did not show a significant relationship between the frequencies of this virus in the samples studied.

#### 4. Discussion

The role of some viruses belonging to Herpesviridae family has already been confirmed in human malignancies. For example it has been shown that HSV2 plays a role in cervix cancer (Yang et al., 2004; Tsai et al., 2005), EBV in Burkitt's lymphoma (Irshaid et al., 2010) and nasopharyngeal carcinoma (Nikakhlag et al., 2010), CMV in thyroid tumor (Tsai et al., 2005) and KSHV in Kaposi's sarcoma (Boulanger et al., 2001). However, only few studies have reported that other members of this viral family such as VZV in tumorigenesis.

VZV, as a member of Alpha-herpes virinae sub-family, causes two different diseases. Similar to other members of this sub-family, this virus is able to enter into latent stage in nervous tissues following the primary infection. It causes chicken pox upon primary infection, which is a very contagious disease and is common among children (Grant et al., 1993). Reactivation of latent virus in ganglion cells causes shingles, which is more common among adults. In addition to these diseases, VZV can cause respiratory, neurological, and skin defects in people with immunodeficiency and even those with healthy immune system (John and Gnann, 2002).

Although the study performed by Gelb and Dohner in 1984 showed that VZV is able to transform mammalian cells *in vitro*, however, its DNA has not been detected in breast or any other

type of malignant tumors. The results obtained by the current study show that 25% of both examined benign and malignant tumors were infected with VZV. Therefore, this is the first report regarding detection of VZV in breast tumors.

The presence of this virus in breast tumor cells can be attributed to the lymphocytic infiltration around the tumors. Studies on herpes viruses have shown that their presence in breast tumors can be due to infiltration. For instance, the study performed by Murray and his colleague in 2003, Perigoue and his colleague in 2005 on frequency of EBV in breast tumors, the presence of EBV in tumors was attributed to the infiltration of lymphocytes into the tumors. Moreover, Vonsover et al. in 1987 detected VZV's DNA in peripheral blood lymphocytes.

#### 5. Conclusion

It is thus concluded that VZV is detectable in both malignant and benign tumors of the breast and the virus may play a role in the pathophysiology of breast tumors. However, further studies are needed to confirm the role of VZV in tumor formation.

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