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Molecular Detection of Bovine Leukemia Virus (BLV) in Patients with Breast Cancer in Khartoum State, Sudan

Ahmed MHM[1*], Sami EAA[2], Elhasen Abdalla MMA[1], Israa MO[1], Hussein El ARM[1], Isam ME[2] and Khalid AE[1]

[1]*Department of Virology, Central Laboratory, The Ministry of Higher Education and Scientific Research, Khartoum, Sudan*

[2]*Department of Microbiology and Parasitology, Faculty of Medicine, University of Khartoum, Khartoum, Sudan*

[*]**Corresponding author:** Ahmed MHM, Department of Virology, Central Laboratory, The Ministry of Higher Education and Scientific Research, Khartoum, Sudan, Tel: +249909600049, E-mail: ahmedmudathir@gmail.com

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Abstract

Background: Breast cancer is the most common type of cancer to be diagnosed in women, and represents the fifth leading cause of death from cancer among them. Viruses with oncogenic potential such as human papilloma virus (HPV), mouse mammary tumor virus (MMTV), Epstein-Barr virus (EBV), and bovine leukemia virus (BLV) are suspected to have a role in the pathogenesis of this cancer.

Objective: Molecular detection of Bovine Leukemia Virus (BLV) in patients with breast cancer in Khartoum State, Sudan.

Methods: Paraffin embedded blocks of tumor specimen from 52 Sudanese patients with breast cancer were collected from Omdurman teaching Hospital, Sudan, during the period from March to June 2018. PCR was used to investigate the presence BLV in these specimens.

Results: the results showed that only 2 samples tested positive for BLV.

Conclusion: The incidence of BLV in breast cancer patients in Khartoum State was documented through the molecular detection of retro-transcribed DNA. Generally, these findings are useful for future studies since there is no information available about BLV infection in humans in Sudan.

Keywords: BLV; Breast Cancer; Khartoum State; Sudan

Introduction

Breast cancer is the most common type of cancer to be diagnosed in women; roughly 182,000 women are diagnosed with breast cancer every year in the United States, accounting for around 26% of all incident cancers in women [1]. It is the fifth most common cause of death from cancer in women [2].

Many risk factors have been studied for possible association with breast cancer, such as age, female gender, genetic factors, hormonal factors, and environmental factors. However, the molecular mechanisms related to the pathogenesis of breast cancer are still poorly understood [3].

External factors also play major roles in the initiation, development, and progression of cancer. The International Agency for Research on Cancer (IARC) reports that biological carcinogens cause 18-20% of cancers [4]. Among these, the main subjects are viruses, especially those that are known to have oncogenic potential such as human papilloma virus (HPV), mouse mammary tumor virus (MMTV), Epstein-Barr virus (EBV) and bovine leukemia virus (BLV) [5].

Bovine Leukemia Virus (BLV) is known for infecting bovine cattle. Its prevalence in these animals varies between countries, and with reports of 60% throughout the world [6]. It is associated with a persistent lymphocytosis characterized by the nonmalignant polyclonal expansion of B-cells albeit 5% of these animals develop a leukemia or a lymphoma [7,8]. In addition, some authors proposed the possibility of transmission to other species, including humans [9] as BLV has been found in human blood [10]. Many studies were subsequently done to look for association between BLV and breast cancer and indeed a significant association has been found between BLV and breast cancer in multiple case control studies [11- 15].

Statistical Analysis

The presence of BLV-like sequences in tumors was tested for possible association with clinico-pathological data (age, histological type, stage of the cancer). All analysis was carried out using Microsoft Excel 2013.

Materials and Method

Patient Criteria and Specimen Collection

Paraffin embedded blocks of tumor specimens from 52 Sudanese patients with invasive ductal carcinoma breast cancer were collected from Omdurman Teaching Hospital Sudan; during the period from March to June2018. The collected specimens were stored at room temperature till tested.

DNA extraction

Specimen deparaffinization and DNA extraction were carried out as recommended in the protocol provided with the DNA extraction kit (Acrogene, USA).

PCR

The reaction was performed in 25 µl volume using Maxim PCR PreMix tubes (Intron, Korea). The volume included: 5 µl master mix, 1 µl of forward primer, 1 µl of reverse primer, 13 µl of distilled water and 5 µl of retro-transcribed DNA

Primer sequences, below, were from the tax region of the BLV genome. Their genomic location is shown in base pair (bp) numbering according to GenBank accession #EF600696.

Forward (bp 7310±7329): 5'-ATGTCACCATCGATGCCTGG-3'

Reverse (bp 7423±7404): 5'-CATCGGCGGTCCAGTTGATA-3'

Cycling parameters were: 1 cycle of 93 °C for 10 min, 57 °C for 1.5 min, then 30 cycles of 92 °C for 30 seconds, 57 °C for 1.5 min, and 69 °C for 2 min, followed by final extension at 69 °C for 10 min [12].

The PCR products were electrophoresed on 2% agarose gel containing ethidium bromide and visualized under UV light.

Results

2 samples tested positive for BLV. No association with clinical or pathological data (age, histological type, stage of the cancer) was found.

Gender of tested patients	Females
Average age	55 years
Disease subtype	Invasive ductal carcinoma

Table-1: Characteristics of patients

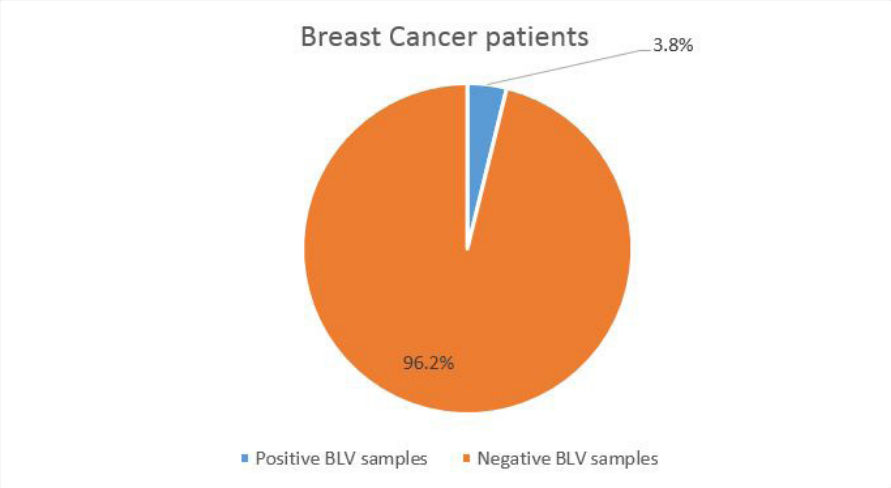

Figure 1: Pie chart demonstrating percentage of positive and negative samples aracteristics of patients

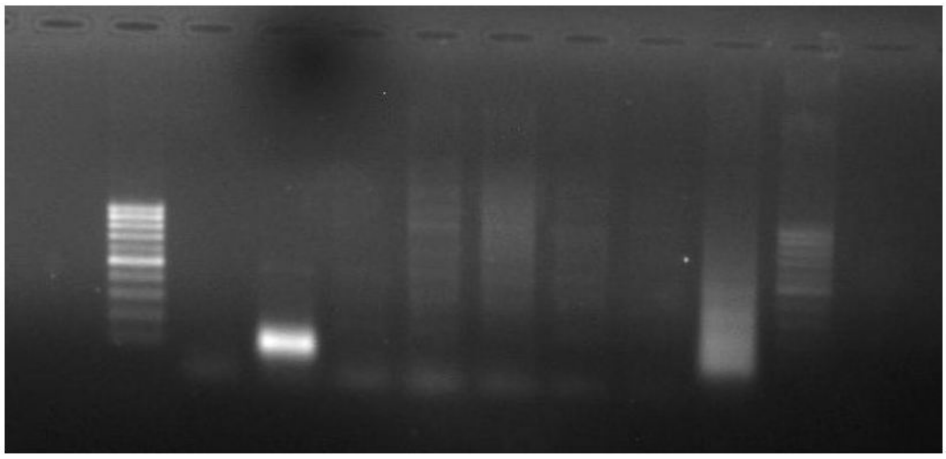

Figure 2: Gel electrophoresis of positive BLV sample

Discussion

Little is known about the viral causes of breast cancer and their epidemiology in Africa. And even much less is known about the epidemiology of these viruses in Sudan. This study was done to screen for the presence of BLV in tissue samples of Sudanese women with breast cancer. The study has not considered the possible means of transmission of BLV, but only aimed to show the prevalence of BLV in Breast cancer tissue from these patients. Only 2 samples tested positive for BLV (4%), a very low percent compared to that found in other similar studies done in different continents. For example, in a case control study done in USA, the frequency of BLV DNA in mammary epithelium from women with breast cancer (59%) was significantly higher than in normal controls (29%) while in women with premalignant breast changes the frequency of BLV DNA was intermediate (38%) between that of women with breast cancer and normal controls [11].

Another study in Australia reported detection of retro-transcribed BLV DNA in breast tissue of 40/50 (80%) of women with breast cancer versus 19/46(41%) of women with no history of breast cancer. For 48 of these subjects, paired breast tissue samples removed 3-10 years apart in two unrelated procedures; were available. For 23/31 (74%) of them; in which the first specimen was diagnosed as nonmalignant (benign or premalignant) and the second as malignant; BLV was already present in benign breast tissue 3-10 years before the malignancy was diagnosed. This is consistent with the supposition of a causative temporal relationship between BLV infection and subsequent development of cancer [12].

In another study that investigated the presence of BLV genome in healthy (n = 72) and cancerous (n = 72) paraffin-embedded samples of breast tissues from women in south Brazil, BLV DNA was found more frequently (30.5%) in breast cancer tissue than in healthy breast (13.9%). There was no association between BLV DNA and other tumor prognostic biological markers such as hormonal receptors, HER2 oncoprotein, proliferation index, metastasis in sentinels' lymph nodes, and tumor grade and size. These findings suggest that BLV should be considered a potential predisposing factor to breast cancer in women [13].

Furthermore, a similar study was done in Colombia where106 tissue samples were collected of which 53 were cancer positive samples and 53 were negative samples for this pathology. DNA from these samples was then amplified, sequenced and phylogenetic analysis was done in order to verify BLV gene segment, presence and origin [15]. The results showed that 43 (40.5%) of the total samples were positive for BLV. In the case group the virus was found in 35.8% of the samples while in the control group BLV presented in 45.2% of the samples. Phylogenetic analysis confirmed BLV presence and had shown a high homology between amplified gene sequences obtained from human breast tissues and those coming from bovine cattle with leukosis reported by GenBank. This study concluded that the presence of BLV genes in humans and its location in breast tissue can be confirmed [15].

From all of the above mentioned studies it is clear that there is significant association between BLV and breast cancer. Our study indicated the presence of BLV in breast tissue, but further wider work will be needed to test for significant association with breast cancer in Sudanese women. Finally, this study is the first study in Sudan to screen for BLV in humans; it can serve as a baseline for future studies to look for BLV prevalence in Sudanese populations, and to search for the possible means of transmission

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