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Research Article

Molecular Detection of Mouse Mammary Tumor Like Virus (MMTV-like) in Breast Carcinoma Patients in Sudan

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ABSTRACT

Background: Since the discovery of MMTV virus and its association with mammary tumors in mice, there has been growing evidence that a virus with 95% DNA identity to Mouse Mammary Tumor Virus (MMTV) may have a role in human breast cancer.

Aim: To determine the prevalence of the MMTV-like virus in Breast Cancer tissue specimens in Khartoum State, Sudan by using semi-nested PCR.

Objective: To document the existence of MMTV in BC in Sudan.

Methods: A retrospective cross-sectional study is used for molecular detection of mouse mammary tumor virus-like sequence. Fifty paraffin-embedded tissue samples of breast cancer tissue were collected from Omdurman Teaching Hospital Histopathology Department. The information in connection with these samples included age, sex, gender, breast cancer type and grade. The specimens were de-deparaffinized using xylene and then rehydrated in graded ethanol concentrations and deionized water. The DNA was extracted using a Qiagen kit (USA) according to the manufacturer's instructions. Semi nested PCR was used to amplify 190-bp MMTV like sequence. Data analysis was performed using Microsoft Excel.

Results: The presence of MMTV-like sequence was investigated in 50 breast cancer tissue samples collected from Sudanese women previously diagnosed with breast cancer by using semi-nested PCR analysis. MMTV-like sequences were detected in 18 (36%) of the 50 breast cancer specimens.

Conclusion: The study provides the first evidence on the high prevalence of MMTV-like sequences in Breast Cancer tissue obtained from Sudanese female patients.

Keywords: Breast cancer; Mouse mammary tumor virus-like sequences; Sudan

INTRODUCTION

Breast cancer (BC) is the second most common cancer in women worldwide, with nearly 1.7 million new cases diagnosed in 2012. This represents about 12% of all new cancer cases and 25% of all cancers in women. It is the fifth most common cause of death from cancer in women [1]. According to the American cancer society and the national cancer institute over 250,000 new cases of invasive breast cancer will be diagnosed each year in women and over 2,400 in men in the USA [2].

Several risk factors have been put forward for the development of BC, such as age, length of reproductive life, nulliparity, obesity, and the inherited genetic predisposition; however, the etiological and molecular mechanisms related to the breast cancer pathogenesis remain poorly understood [3].

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External factors also play major roles during 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 candidates are viruses 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].

Antigens immunologically related to Mouse Mammary Tumor Virus (MuMTV) and the major envelope glycoprotein, gp52 of MuMTV, were early on identified in tissue sections of human mammary carcinomas using serological technique [6-11]. Subsequently, other studies suggested that a virus similar to the Mouse Mammary Tumor Virus (MMTV) was associated with breast cancer in humans with prevalence ranging from 0 to 74% in BC cases in several countries around the world, including 0% in Japan, Austria and the United Kingdom, 0.8% in Vietnam, 4.2% in Mexico, 17% in China, 31% in Argentina, 38% in Italy, 40% in United States, 42% in Australia, and 74% in Tunisia [6,12]. It is interesting to note that in Asia where the incidence of breast cancer is much lower than in North America, MMTV infected samples were many times lower too [7,13].

Further, the presence of a Human Mammary Tumor Virus (HMTV) has been reported whose sequences are 90% to 98% homologous to MMTV. This retrovirus (MMTV-like Virus) is found in greater frequency in mammary tumors, and it is capable of integrating and becoming expressed in mammary tissue. However, MMTV or HMTV are not always found in normal breast tissue. For example, HMTV sequences have been found in 40% of breast cancerous tissues in both American and Australian women, whereas viral sequences were detected only in 1% of the non-affected mammary tissues from the same patients [6,12].

MATERIALS AND METHOD

Patients and tissue samples

Paraffin-embedded blocks of tumor specimens from 50 Sudanese female patients diagnosed with breast cancer were obtained from Omdurman teaching hospital, department of pathology, during the period from, April to June 2018. Ethical approval to perform this study was granted by the Sudan international university microbiology department. Samples were selected randomly from the pool of the department stored samples.

Specimen deparaffinization

Two 20 µ sections were cut from each tissue specimen block by the same person to avoid cross-contamination, the microtome block was cleaned and the blades were replaced between specimens. All Specimens were deparaffinized by adding xylene for one hour and then serially washed by ethanol 100%, 80%, 60%, and 40% respectively, then dipped in deionized water for 10 seconds for re-hydration.

DNA extraction

DNA was extracted using Qiagen kit (USA) according to the manufacturer's instructions. Extracted DNA was then stored at -20°C until used.

Semi nested polymerase chain reaction (PCR)

The PCR performed by processing the extracted DNA with primers that are specific for the gene-like sequences of MMTV. Approximately 200 ng of template DNA were used in the first stage of PCR with outer primers 1X (5`TGCGCCTTCCCTGACCAAGGG-3`), 2 NR (5`GTAACACAGGCAGATGTAGG-3`) to amplify a 356 bp segment. Primer pairs used for the second round were: 5F (5`-GTATGAAGCAGGATGGGTAGA-3`) and 2 NR, amplifying a 190 bp inner sequence. 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 forwarding external primer, 1 µl of reverse external primer, 15 µl of distilled water and 3 µl of DNA. Thermocycling was performed by denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 60°C for 30 s, elongation at 72°C for 45 s and a final elongation step at 72°C for 10 min. PCR assays were performed in triplicate to assure reproducibility of the reactions After amplification, the PCR products were electrophoresed on 2% agarose gel containing ethidium bromide and visualized under UV light.

Statistical analysis

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

RESULTS

Twenty-one samples (42%) were from women of the age group 20-40 years old, 20 samples (40%) from the age group 41-60 years old, and 9 (18%) in the age group 61 years old and above (Table 1). Based on histopathology, 49 (98%) of the samples were diagnosed as invasive ductal carcinoma while 1 (2%) was diagnosed as phyloides. Seven out of 49 tumors (14%, 3%) were classified as stage 1, 25 (51%) stage 2, 17 (34.7%) as stage 3 (Table 2) and one specimen was not classified. By using semi-nested PCR analysis, MMTV-like sequences were detected in 18 (36%) out of the 50 breast cancer specimens tested. The highest prevalence of positivity to MMTV-like sequences was detected in the age group 41-60 years old (50%) and in stage one (42,8%) and stage 2 (41,2%) groups of cancer samples with no detectable significant differences according to age, type, or stage of cancer. The distribution of MMTV like sequences positive cancer samples among various groups (according to age, type of cancer and stage of cancer) (Tables 1 and 2).

Table 1: Distribution of MMTV-like cases and positive results according to age group.

Age (years)	Number of cases	Number of MMTV like positive cases (%)	p-value*
20-40	21	5 (24)	0.214
41-60	20	10 (50)	
61 and above	9	3 (33.3)	
Total	50	18 (36)	

Table 2: Distribution of MMTV-like positive results according to cancer type and histological stage.

Variables	Number of cases	Number of MMTV-like positive cases	p-value
Cancer type			
Invasive ductal carcinomas	49 (98%)	17 (34.6%)	
Phylloides	1 (1%)	1 (100%)	0.178
Histological grade			
Grade 1	7 (14.3%)	3 (42.8%)	
Grade 2	25 (51%)	7 (28%)	
Grade 3	17 (34.7%)	7 (41.2%)	0.602

DISCUSSION

This study investigated the association of MMTV-like sequence with breast cancer in Sudanese women. The study has not considered the possible means of transmission of MMTV-like sequences, but only aimed to show the prevalence of MMTV like the sequence in Breast cancer tissue from these patients. However, it is suggested that MMTV like sequence is an exogenous virus transmitted from mice to humans [8,14] as it was reported that the incidence of breast cancer correlated with the geographic distribution of various species of wild mice. The common house mouse *Mus domesticus* as compared with *Mus musculus* is thought to shed a higher viral burden of MMTV [15]. The former is the predominant species in western Europe and North America, where the highest prevalence of breast cancer is found, whereas the latter species reside in countries with a low prevalence of breast cancer [15]. *Mu musculus* (sisi meaning small as it is called locally) and rats (*Rattus rattus*) are widespread in Sudanese households. (Asma Mohamed, faculty of science, university of khartoum personal communication). In addition, MTTV transmission among humans can be effected through saliva and milk.

In the present study, MMTV-like sequences were detected in 36% of Sudanese BC patients. This prevalence of MMTV-like in BC is close to the results reported in areas with a high incidence of BC, especially in the United States and Italy where MMTV-like sequence was identified in 36% and 38% of BC respectively [9,16].

Our Results showed higher prevalence than those reported in some other countries e.g. in Tunisia (14%), China (16.8%) and Saudi Arabia (5.97%) [17]. This finding may be due to the fact that these countries have lower incidences of BC than our country [10,11,18,19]. However, our finding was much lower than those reported in Morocco where MMTV-like sequences were identified in 57.14% of BC and in Australian BC specimens where the sequences were detected in 56% of BC specimens [12,13,20,21].

In the current study, the analysis of our data parameters showed no significant correlation (p-values more than 0.05) between the presence of MMTV-like sequences and age groups, histological type and histological grade. These findings are in agreement with several previous reports [14,15,22].

CONCLUSION

Our findings record for the first time the presence of MMTV-like sequence in BC of Sudanese women. Although no significant associations between the virus infection and BC were detectable, the high prevalence (36%) of MMTV sequence in our samples suggests that MMTV infection might be a contributing factor in the development of breast cancer in Sudan. Hence, further detailed country wide studies with larger sample sizes are needed to investigate the extent of this virus infection in other regions of the country to be more able to properly evaluate its association with breast cancer in Sudan.

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