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Full Length Research Paper

Decrease in expression of some proteins among breast cancer patients in Sudan comparison to healthy tissues

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Breast cancer is one of the most common cancers in women, and rated the second most common cancer and a significant cause of death in females in the Sudan. This study aims to identify tumor protein that elicits humoral immune responses in breast cancer patient in comparison to tissues from healthy individuals as well as from normal tissues of the cancer patient. Serum samples and breast cancer tissue specimens were collected from breast cancer patients (n = 9) and from healthy individuals (n = 5) at Khartoum Teaching Hospital. Breast cancer tissues were homogenized in PBS, centrifuged and the supernatants were lysed in 2X SDS-PAGE sample buffer. The preparation then boiled and the resulting supernatants were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot analysis. Total proteins were separated on SDS-PAGE and transferred to the nitrocellulose paper, then analyzed by immunoblotting for total proteins and serum antibodies using serum from patients with breast cancer and from healthy individuals to enhance humoral immune responses. The SDS-PAGE analysis showed an increase in size of protein bands in the normal control tissues than from breast cancer patients. The Western blot analysis of breast cancer tissues with the serum from breast cancer patients specifically detected major bands than in the normal tissues from the healthy tissues of cancer patients or from healthy individuals. Beside the major bands, additional bands have been detected in breast cancer tissues with the serum from breast cancer patients. The reactive auto-antibodies in patient's tissues bound to the circulating tumor antigen in patient's serum and immune complexes would result by Western blotting indication of strong immune response to these proteins. The present study demonstrated that there was a clear decline in the expression of some proteins among breast cancer patients which has been confirmed by strong immune reactions in the Western blotting analysis.

Key words: breast cancer , protein expression, SDS-PAGE, Commasie brilliant blue, Western blot**.**

INTRODUCTION

Breast cancer is a worldwide health problem notably in women. An estimated 1.7 million new cases have been diagnosed in the year 2012 (12%), thus rated as the second most common cancer (Ferlay et al., 2014). Risk

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factors of breast cancer include older age, a high-fat diet, alcohol intake, obesity, environmental factors such as tobacco use, radiation, shift work, being unmarried and city residence. Prevalence rates are usually advanced in North American and Northern European countries, intermediate in Southern and Eastern European and South American countries, and lowest in Asia and Africa (Kelsey and Horn-Ross, 1993).

According to Globocan estimates, the top most common cancers in both sexes are breast, non-Hodgkin lymphoma, leukemia, esophagus and colorectum (Elamin et al., 2015). Increase rates of cancer in Sudan is believed to be as a result of migraton of people from countryside to cities with resultant changing lifestyle and behavioral changes that include tobacco chewing and smoking, unhealthy dieting, lack of physical activities and according to the age of population (Elamin et al., 2015). Of 1255 women from central Sudan diagnosed with breast cancer between 1999 and 2006, 74% were <50 years old or premenopausal; invasive ductal carcinoma was the most common pathology (82%) and women presenting with stage III or higher tumors that had already metastasized, while ductal carcinoma in situ was the least prevalent (0.5%) finding (Elgaili et al., 2010). A study of 20 patients suffering from breast cancer in Sudan examined germ line and somatic mutation in their BRCA2 exon 11 and the p53 tumor suppressor gene and results indicate that both regions may play a limited role in the pathogenesis of breast cancer in those patients (Masri et al., 2002).

It has been established that cancer is immunogenic. Moreover, multiple tumor antigens have been identified in cancer patients. Therefore, humoral immune response existent in cancer patients could permit the discovery of persons exposed to the malignant transformation of somatic cells (Lu et al., 2008). In breast cancer, auto-immunity has been shown against several proteins such as P53 (Murray et al., 2000), heat shock protein 90 (Stephanou et al., 1998), c-erbB-2/HER2/Neu (Menard et al., 2000), CA15-3 and cumin-related antigens (Mommers et al., 1999) and MUC1 (Gourevitch et al., 1995).

There is at present much need to identify markers for the detection of breast cancer. This research aimed to identify tumor protein that elicits humoral immune responses in breast cancer patient in comparison to tissues from healthy individuals as well as from normal tissues of the cancer patient. There is substantial evidence for a humoral immune response to cancer in humans which is demsonstrated by the identification of antibodies against a number of intracellular and surface antigens in patients with various tumor types (Davidoff et al., 1992; Diaz-Zaragoza et al., 2014).

MATERIALS AND METHODS

Ethical consideration

Ethical approval of the study was obtained from the Ethical committee of the Institute of Endemic Diseases University of Khartoum.

Samples collection

All patient samples were obtained from Khartoum Teaching Hospital between March and July, 2010. The age of the patients ranged between 18 and 65 and they came from different states (Khartoum, Jazeera, Kordofan, Darfur and North). Samples ware contains tumor tissues, non-tumor tissues and serum from breast cancer patients, and control tissues and serum from healthy individuals. The tissue samples were then snap frozen in liquid nitrogen and stored at –80°C.The serum samples were collected, aliquoted and stored at –20°C, and then was subjected to the SDS-PAGE (Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis) and western blood analysis.

SDS-page

Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) was done by the study of optimized protocol for protein extraction from the breast tissue that is compatible with two-dimensional gel electrophoresis (Olena et al., 2011). The study follows this technique as a reference. Breast cancer tissues were homogenized in PBS, centrifuged, and the supernatants lysed in 2X SDS-PAGE sample buffer. The preparation then boiled and the resulting supernatants were subjected to SDS-PAGE. The supernatants were transferred into the Mini-V 8-10 vertical gel electrophoresis system and the tank was filled with electrophoresis buffer. The separation of the protein was carried out at a constant voltage of 80 V for 2 h. The resolving gel was then transferred to the staining solution and was put on a shaker for 24 h at room temperature. The staining solution was then replaced by the destaining solution, which was changed until the protein bands became clear. Total proteins were separated on SDS-PAGE and transferred to the nitrocellulose papers.

Western blot analysis

Western blot analysis was done by the study of Caspase 3 in breast cancer used primary breast carcinoma, breast fibroadenoma, and normal breast tissue samples (Norma et al., 2003). The study follows the above technique in this study. Two nitrocellulose papers were incubated in a blocking solution (2% gelatin in PBS) for 1 h at room temperature. Then the blocking solution was discarded and the membrane was washed by washing PBS-T. Then one paper was incubated in the primary antibody solution (human serum from breast cancer patients) and the other one was incubated in the primary antibodies solution (human serum from healthy human). The two papers were washed 3 times with washing solution, and the two papers with primary antibody were incubated in secondary antibody solution (conjugate) of anti-human-globulin and then both nitrocellulose papers were washed with PBS-T and the substrate solution was then added. The reaction was stopped by washing the papers in distilled water then dried and photographed.

RESULTS

SDS-page

The study of SDS-PAGE preparations of proteins showed difference in the expression of protein in the tumor and normal tissues (Figures 1 and 2).

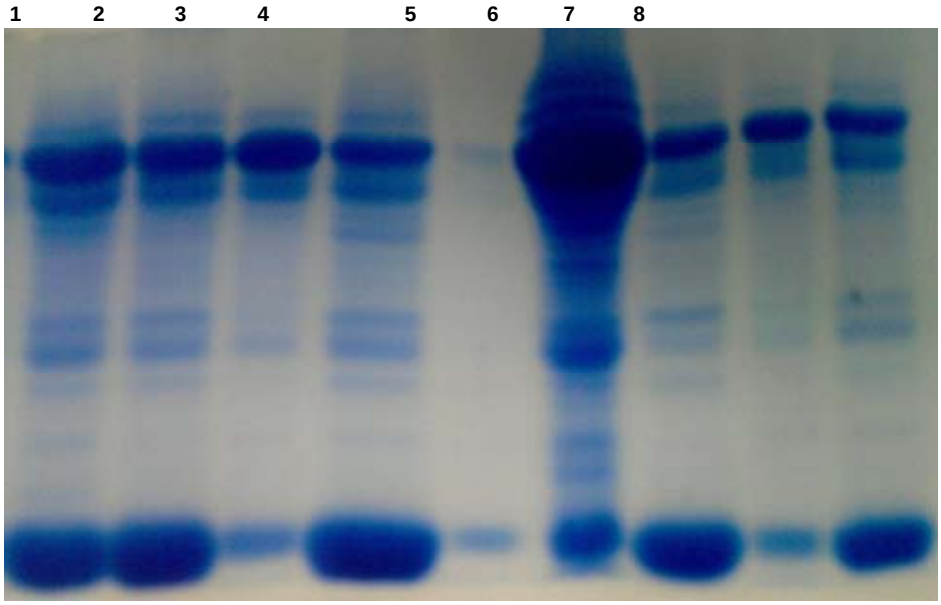

Figure 1. The SDS-PAGE protein profile of breast cancer patients stained with commasie brilliant blue. Lanes 1, 2, 3, 4 normal tissues (from breast cancer patients about 2 cm from the local of disease diagnosed by histopathology as normal tissues) (5, 6, 7, 8, disease tissues from breast cancer patients from the local of disease diagnosed by histopathology as disease tissues) Lanes 1,2,3,4 showed high expression of proteins compeer with the lanes 5,6,7,8 disease tissues.

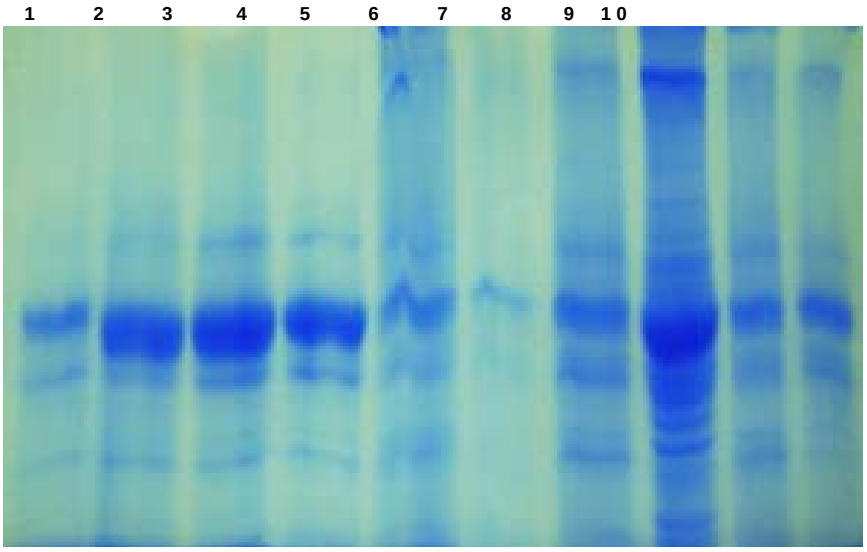

Figure 2. The SDS-PAGE protein profile of breast cancer patients and health individuals stained with Commasie brilliant blue. Lanes 1, 2, 3, 4, 5 breast cancer tissues 6, 7, 8, 9, 10 healthy individuals' tissues. The result in this test also showed high expression of proteins in healthy individuals than the breast cancer patients.

Western blotting

The Western blot analysis of breast cancer tissues with the serum from breast cancer patients specifically detected major bands in both normal and tumor tissues. There are low immunoreactions between disease patient's tissue and healthy individual's serum (Figures 3, 4 and 5).

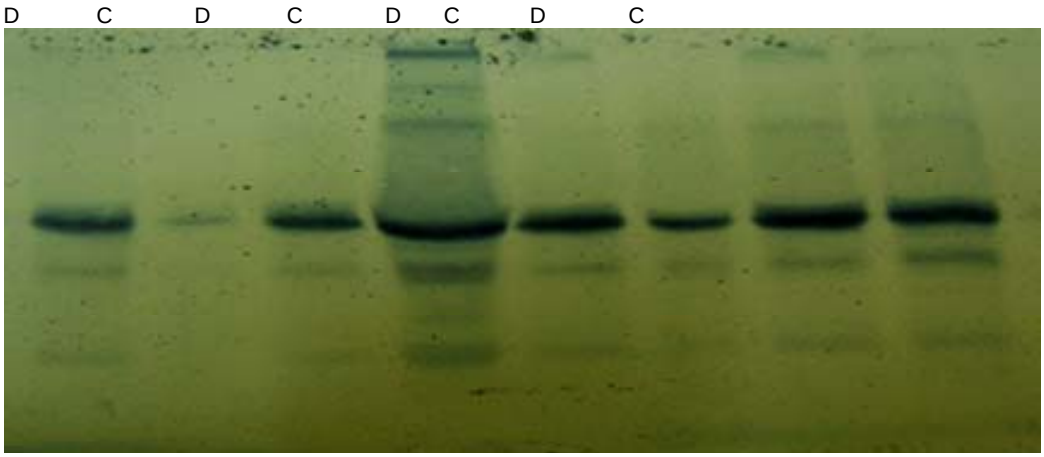

Figure 3. Western blot analysis of breast cancer tissues (with serum from breast cancer patient). D - Diseased tissues from breast cancer patients. C - Normal tissues from breast cancer patients. In this test we showed detection of bands in both disease and normal tissues from breast cancer patient's indication of strong immune response to these proteins between disease patient's serum and disease patient's tissue.

Figure 4. Western blot analysis of diseases tissues from breast cancer patients with serum from healthy individuals.

DISCUSSION

Most breast cancer tissues contain proteins components that are uncommon in normotypic breast tissues derived from control and healthy individuals. It has reported that breast cancer tissues are heterogeneous in regard to quantitative and/or qualitative variation in protein pattern as seen in 8.5% PAGE preparations (Black et al., 1976). SDS-PAGE proteins extracted showed up-regulation of protein levels in the control tissues (patients and healthy individuals), and decrease the level of some proteins (Barathidasan et al., 2013). Human tissue kallikrein 14 (KLK14) a novel extracellular serine protease is expressed to several diseases, primarily cancer and it is believed to be implicated in several facets of tumor progression, including growth, invasion and angiogenesis (Borgono et al., 2007). Contrary to these findings, none of the extracted markers showed increase levels in cancer patients examined in the present studies.

The reduction in the expression of some proteins was observed in breast cancer patients during the extraction of the proteins; strong immune reactions were shown in Western blotting indicative of immune responses to these proteins. The study findings have to be linked to a recent

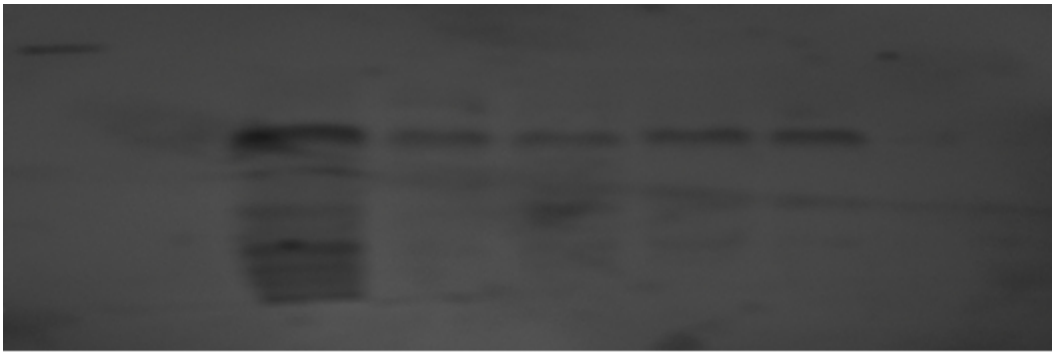

Figure 5. Western blot analysis of healthy individuals' tissues with serum from breast cancer patients. Also, the test showed low immunoreactions between disease patient's serum and healthy individual's tissue.

study which has assessed serine/glycine relationships at the protein level in regards to clinical outcomes (Noh et al., 2014). These concluded that the most abundantly expressed serine/glycine metabolism-related protein in basal-like TNBC tissues was tumoral PHGDH, and expression levels of stromal SHMT1 and tumoral PHGDH were inversely correlated with clinical prognostic factors (Noh et al., 2014). The goals of the present study need to understand tumor immunity and developing strategies for cancer immunotherapy.

Conflict of Interest

The authors have not declared any conflict of interest.

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