7- Amos AS, Mckarty DJ and Zimmet D. (1997). The rising global burden and its complications: estimates and complicates the year of 2010. *Diabet Med.* **5:**81-85.

8- Wild S, *et al.* (2004). Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care* **27**(4):1047-53.

9- Pieper-Bigelow C, Strocchi A, Levitt MD. (1990). Where does serum amylase come from and where does it go? *Gastroenterol Clin North Am.* **19**:793-99.

10- Reddy YN, And Shankaraiah P. (2011), Amylase Expression in Indian Type2 Diabetic Patients. *J. Med. Sci.* **11**(7):280 – 284.

11- Wendy Arneson, Jean Brickell. (2007). *Clinical Chemistry: A Laboratory Perspective,* Australia.

12- Stuart J, and Kenny MW. (1980). Blood rheology. *J. Clin Pathol.* **33**(5):417–429.

13- Taniyama, H, Hirayma K, Kagawa Y, Kurosawa T, Tajima M, Yoshino T, and Furuoka H. (1999). Histopathological and immunohistochemical analysis of endocrine and exocrine pancreas in twelve cattle with insulin – Dependent Diabetes Mellitus (IDDM). *J. Vet. Med. Sci.* **61**:803-810.

Evaluation of Circulating CA15-3 Tumor Marker Among Sudanese Women With Breast Cancer

Munsoor M. Munsoor*, Noha Algily, Mohammed Abdulrahim

*College of Medical Laboratory Science, Sudan University of Science and Technology-Khartoum, Sudan.

ABSTRACT

In this study blood samples were collected from 100 patients with breast cancer from Radio and Isotopes Center in Khartoum (RICK) as case group and 50 apparently healthy individuals as control group, during the period from March to December 2011. The study aimed to assess the role of CA15-3 as a marker of breast cancer. The level of CA15-3 level was assessed in patients previously diagnosed as breast cancer before dose of chemotherapy and then at intervals of 3 months (3, 6 and 9 months) after dose of chemotherapy. The level of CA15-3 was detected by two monoclonal antibodies (115D8 and DF3) and measured by ELISA. The level of CA15-3 showed significant difference when compared with control ($p<0.05$). The level of CA15-3 after 3 months of chemotherapy dose showed insignificant difference compared to level of the marker before chemotherapy ($P>0.05$). The level of CA15-3 after six and nine months of chemotherapy dose showed significant difference compared to the level before chemotherapy ($P<0.05$). The results also showed a significant relations between level of CA15-3 and age of patients, family history of the disease and hormonal therapy ($P<0.05$). This study concluded that the level of CA15-3 was elevated in association to metastasis of breast cancer in the patients examined.

المستخلص

فى هذه الدراسة تم جمع 100 عينة دم من نساء مصابات بسرطان الثدي من المترددين على مستشفى الأشعة والنظائر فى الخرطوم بالإضافة الى 50 عينة أخرى من أشخاص أصحاء تم إستخدامهم كمجموعة ضابطة فى الفترة من مارس الى ديسمبر 2011 . هدف الدراسة كان تقييم دور CA15-3 كعلامة تشخيصية فى سرطان الثدي. إستخدمت ELISA لتعيين مستوى CA15-3 فى المرضى قبل وبعد تلقيهم العلاج الكيماوي بثلاث أشهر وستة أشهر وتسعة أشهر وذلك فى وجود نسختين من الأجسام المضادة لــ CA15-3. أظهرت النتائج قبل العلاج الكيماوي أن مستوى CA15-3 زاد زيادة معنوية فى المرضى مقارنة بالمجموعة الضابطة. بعد العلاج بثلاثة أشهر لم يتأثر المستوى ولكن مستوى CA15-3 زاد بعد ستة وتسعة أشهر من العلاج الكيماوي. كما أوضحت النتائج ان هنالك علاقة بين مستوى CA15-3 وكل من عمر المريض، تأريخ المرض فى الأسرة وتلقي العلاج الهرموني وخلصت الدراسة الى أن زيادة مستوى CA15-3 توافقت مع إنتشار سرطان الثدي فى المرضى المشاركين فى هذه الدراسة.

KEYWORDS: CA15-3 tumor marker, breast cancer, chemotherapy.

INTRODUCTION

Breast cancer is the common onset malignancy in women. Up to 12% of women are diagnosed as having breast cancer. About 3.5% of women die because of this disease [1]. The presence and extent of lymphnode involvement is currently the best established prognostic indicator of this disease. Other prognostic indicators include estrogen and progesterone receptor status, tumor grade and growth rate, DNA ploidy, invasiveness and aggression [2]. Tumor markers are substances identified in the circulation of patients with malignant disease, which may be used in diagnosis (early detection and differential diagnosis), prognosis and follow up [3]. The CA15-3 protein is a mucin belonging to a large family of glycoproteins encoded by the muc1 gene that are heterogeneously expressed on the apical surface of normal epithelial cell types, including those of the breast. It is a breast cancer- associated antigen that is defined by its reaction with monoclonal antibodies 115D8 and DF3 [4]. The potential uses of CA15-3 in clinical practice fall into two categories improved or more accurate diagnosis and increased convenience or cost effectiveness [5]. The CA15-3 is considered to be the most sensitive test in detecting metastatic breast cancer [6]. This protein may be a useful marker for the diagnosis of secondary breast cancer [7]. The most obvious role is a direct substitution for a bony scan [8]. The present work was designed to evaluate CA15-3 level in Sudanese breast cancer patients.

MATERIALS and METHODS

Blood samples were collected from 100 patients attending Radio and Isotopes Center- Khartoum (RICK). The level of CA15-3 in patients was compared to level of CA15-3 of 50 normal healthy persons (control) of matching age and body weight. Informed consent was obtained from all the participants. Controls consisted of staff members of the hospital with no previous history of breast cancer and other cancer related diseases. The clinical data obtained include history of barest cancer in the patient's family, clinical examination and hospital follow up records.

Sample collection and processing

After informed consent and use of alcohol antiseptic for the skin (70% ethanol), 3mL of venous blood was drained and poured directly into a centrifuge tube for serum preparation. Serum was separated and kept frozen at -20°C until use.

CA15-3 ELISA Assay

The CA15-3 ELISA assay followed the sandwich principle, where captured antibodies were directly adsorbed into a substrate. The detected antibodies were labeled with an enzyme, which upon addition of the substrate produces colored product that quantifiable by absorbance analysis. The ELISA kit includes microwell plates pre-coated with murine monoclonal anti- CA15-3 antibody, sample diluents, enzyme conjugated diluents, tetramethyl benzidine (TMB) solution and stop solution. A range of CA15-3 reference standards (15-24 µg/ml) were used directly (undiluted). This assay was performed following manufacturer's instructions. Briefly, 200µl of CA15-3 standards was dispensed into the appropriate microwells, gently mixed for l0 seconds, and incubated at 37ºC for 1hour.

The wells were rinsed 5 times with double distilled H_2O, and 200µl of enzyme conjugate reagent was dispensed into each well followed by mixing for 10 seconds and incubation at 37ºC for 1 hour. The wells were re-washed and 10µ/l of TMB reagent was dispensed into each well and gently mixed for 10 seconds. The wells were incubated at room temperature for 20 minutes, then in the dark for 20 minutes and finally, 100µl of stop solution was added to each well to terminate the reaction. The plates were gently mixed for 30 seconds then read at 450 nm with a Bio-Rad microtiter plate reader [9].

Statistical analysis

Level of CA15-3 was expressed in international units per liter (U/L). The means obtained by Student T test for the case and control levels. Correlations of CA15-3 with patient age, family history of the disease and hormonal therapies were assessed by independent T Test, also one way ANOVA test was used.

RESULTS

Levels of CA 15-3 in patients and control

The level of CA15-3 in postoperative patients with breast cancer has increased significantly when compared to that of control (P<0.005) (table 1).

Level of CA15-3 after chemotherapy application

The level of CA15-3 after 3, 6 and 9 months of chemotherapy application in post-operative patients was significantly increased compared to the level before onset of chemotherapy treatment (table 2).

Association of CA15-3 with family history, age and hormonal therapy

The results of this study showed association between level of CA15-3 and individual factors such as family history, age of patient and hormonal therapy.

Serum level of CA15-3 was elevated in patients who have a family history positive to breast cancer and age higher than 50 years. But the level of CA15-3 became gradually lower in patients who commenced hormonal therapy (table 3).

Table 1: Level of CA15-3 in subjects

Subjects	Number	Means of CA15-3
patients	100	93.462*
controls	50	8.004*

*P value< 0.001.

Table 2: Level of CA15-3 in patients after 3, 6 and 9 months of chemotherapy

Chemotherapy	Intervals	Means of CA15-3
Before treatment	At zero time	56.417
After treatment	3 months	59.777*
	6 months	66.899**
	9 months	93.462**

*P value > 0.05.

** P value < 0.05.

Journal of Science and Technology Vol. 14
ISSN -1605 – 427X
Natural and Medical Sciences (NMS No. 1)
mena@sustech.edu

June /2013

Table 3: Association of CA15-3 level with family history, age and hormonal therapy

Variables	Age/ years		Family history		Therapy	
	< 50	>50	No	Yes	No	Yes
Means of CA15-3	1.53	59.77*	1.37	45.17*	59.77*	1.63
Number	47	53	63	37	37	63

*P<0.05

DISCUSSION

The role of tumor markers in the management of breast cancer patients is limited to patients with advanced disease, since these are seldom abnormal in early disease or with local recurrence. Thus tumor marker analysis is appropriate in previously diagnosed patients at high risk for recurrence to detect early disease dissemination and in patients with metastasis disease to evaluate therapeutic response [10]. In general, changes in tumor markers accurately and consistently reflected changes in disease status at the pertinent issue, whether the use of tumor markers in clinical practice will lead to more effective treatment remain controversial [11]. CA15-3 was used widely as a marker of distant metastasis in breast cancer. In this study, the level of CA15-3 was detected in postoperative patients with breast cancer to indicate the recurrence of disease [12]. The results in this study showed that the level of CA15-3 in the normal healthy individual (control) was within the usual reference ranges which were reported to be from zero to 20 UIL [12]. But the serum level of CA15-3 was significantly increased in breast cancer patients compared to that of control. In this respect our results agreed with the conclusion of earlier work on CA15-3[12,13]. Our results on post-operation patients pointed to a possible recurrence of breast cancer in spite of chemotherapy application. This observation was well recognized by the successive increase of the level of CA15-3 in the 3, 6 or 9 months following operations of breast cancer excision. The increment of the antigen level which coincide chemotherapy application indicated that the CA15-3 was secreted from sites other than those treated by chemotherapy. This phenomenon results from metastasis action of the breast cancer. In this respect our results agreed with previous works of Mylonas and his co-workers [14] and confirm the positive predictive values for CA15-3 in breast cancer patients and its use as a sensitive marker for metastasis of this type of cancer. In this study the patients who have family history of breast cancer showed high plasma level of this marker (table 3). These results however, indicated an inheritance root for this disease and in an agreement with the results of John Hopper, who reported that, women with close relatives who have been diagnosed with breast cancer have a higher risk of developing the disease [13,14]. In this study the patients whose ages over 50 years were more frequent (74%) while those below 50 years less frequent (26%). This observation is in an agreement with previous work reported that more than 44.000 people were

diagnosed with breast cancer each year in the UK and 80% of all cases occur in over 50 years of age [15,16]. In our study there was an association between breast cancer and hormonal therapies. This is indicated by the observation that level of CA15-3 was successively decreased in sera of patients who received hormonal treatment. This result is consistent with the result reported recently in Jordanian patients with breast cancer whose CA15-3 level was decreased immediately following estrogen and progesterone therapies [16].

ACKNOWLEDGEMENT

REFERENCES

1- Naume B, Wiedswang G, Borgen E. (2004). The prognostic value of isolated tumor cells in bone marrow in breast cancer patients. *Clin ancer Res.* **10**:3091-3097.

2- Norberg T, Klaar S, Karf G. (2001). Increased *p53* mutation frequency during tumor progression-results from a breast cancer cohort. *Cancer Res.* **61**:8317-8321.

3- Sotiriou C, Neo SY, McShane LM. (2003). Breast cancer classification and prognosis based on gene expression profiles from a population-based study. *Proc Natl Acad Sci USA* **100**:10393-10398.

4- Howell A, Cuzick J, Baum M. (2005). Results of the ATAC (arimidex, tamoxifen, alone or in combination) trial after completion of 5 years' adjuvant treatment for breast cancer. *Lancet*. **365**:60-62.

5- The 9th International Conference on the primary therapy of early breast cancer in St. Gallen, Switzerland. (January 2005).

6- Chang AE, Skibber JM. (2006). Evidence-based management of breast cancer. *In*: *Oncology. An Evidence-based Approach*. Springer, USA.

7- Bofin AM, Ytterhus B, Martin C. (2004). Detection and quantitation of *HER-2* gene amplification and protein expression in breast carcinoma. *Am. J. Clin. Pathol.* **122**: 110-119.

8- Ross JS, Fletcher JA, Linette GP. (2003). The *Her-2/neu* gene and protein in breast cancer 2003: biomarker and target of therapy. *Oncologist* **8**(4):307-325.

9- Pinder SE, Ellis IO, Galea M. (1994). Pathological prognostic factors in breast cancer. III. Vascular invasion: relationship with recurrence and survival in a large study with long-term follow-up. *Histopathology* **24**:41-47.

10- Kato T, Kameoa S, Kimura T. (2003). The combination of angiogenesis and blood vessel invasion as a prognostic indicator in primary breast cancer. *Br. J. Cancer* **88**:1900-1908.

11 Schoppmann SF, Bayer G, Aumayr K. (2004). Prognostic value of Lymphangio - genesis and lymphovascular invasion in invasive breast cancer. *Ann. Surg.* **240**:306-312.

12 Kroger N, Milde-Langosch K, Riethdorf S, Schmoor C, Schumacher M, Zander AR, Loning T. (2006). Prognostic and predictive effects of immunohistochemical factors in high-risk primary breast cancer patients. *Clin Cancer Res.* **12**(1):159-68.

13- Fehm T, Becker S, Pergola-Becker G, Kramer B, Gruber I, Sotlar K, Kurek R, Wallwiener D, Solomayer E. (2006). Influence of tumor biological factors on tumor cell dissemination in primary breast cancer. *Anticancer Res.* **24**(6):4211-4216.

14- Mylonas I, Makovitzky J, Jeschke U, Briese V, Friese K, Gerber B. (2005).