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The Use of Carbohydrate Antigen (CA) 15-3 as a Tumor Marker in Detecting Breast Cancer

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Abstract: This study was carried out to determine the sensitivity and specificity of serum CA 15-3 as a marker in detecting and monitoring treatment in, breast cancer patients. One hundred and ten patients comprising 35 known breast cancer patients, 75 suspected cases and 20 controls entered the study. Blood samples were taken before and after treatment from the 35 known cases as well as the 75 suspected cases from which biopsy specimens were also taken. Serum CA 15-3 was measured by BioCheck CA 15-3 Enzyme Immunoassay. There was a significant difference between the concentration of serum CA 15-3 of the 35 known breast cancer patients before and after treatment ($p<0.05$). Out of the 75 suspected cases, 46 had breast cancer and 29 had benign breast disease (histologically proven). There was a strong positive correlation between the level of serum CA 15-3 and the histopathology results of the biopsies ($r = 0.518$). The mean serum CA 15-3 concentration of the 46 patients (80.6+70.2 U mL^{-1}) was significantly higher ($p<0.05$) than that of the 29 patients with benign breast disease (12.0+9.0). The sensitivity and specificity of the serum CA 15-3 in detecting breast cancer was 76.1 and 100%, respectively at a cut-off of 35 U mL^{-1}. Serum CA 15-3 was found to have a value in the early detection and monitoring of treatment of breast cancer in Ghana.

Key words: Biopsy specimen, benign breast disease, breast malignancy, non-invasive detection, pre-clinical state

INTRODUCTION

Breast cancer is a life-threatening malignancy which is most common among women and the second leading cause of cancer death in women today (Elzagheid, *et al*., 2006). The earlier this cancer is detected, the earlier the opportunity for treatment and hence the lower the risk of death. Currently, women suspected of having breast cancer are most likely to have a biopsy taken for histological investigation, since only a histological examination of biopsy can confirm or rule out cancer. But a large number of people who undergo biopsy do not have cancer, yet they go through the anxiety and trauma of the procedure and its complications afterwards.

Numerous serum tumor markers have been described for breast cancer, including members of the MUC1 family of mucin glycoproteins (e.g., CA 15-3, BR 27.29 and MCA, CA 549), Carcino Embryonic Antigen (CEA), oncoproteins (e.g. HER-2/c-erbB-2) and cytokines (e.g., tissue polypeptide antigen and tissue polypeptide-specific antigen) (Tondini *et al*., 1989).

It is generally agreed that tumor markers in breast cancer patients are not a tool for primary diagnosis because of their low sensitivity and specificity (Tondini *et al*., 1989).

The carbohydrate antigen CA 15-3 however, has been proven to be the most sensitive and specific tumor marker for breast cancer. But there have been differing views on its sensitivity and specificity to recommend it for screening, diagnosis, staging, or surveillance after primary treatment for breast cancer [American Society of Clinical Oncology (ASCO) Tumor Markers Expert Panel, 2001].

Nevertheless, it is used to detect distant recurrence after primary treatment in breast cancer patients, where it often increases before clinical symptoms become evident (Mohammad Nejad *et al*., 2006). The aim of this study was to determine the specificity and sensitivity of serum CA 15-3 for the detection of breast cancer in Kumasi, Ghana by comparing the levels of serum CA 15-3 in breast cancer patients before and after treatment and also in suspected breast cancer patients with the histopathological findings.

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The objective was to determine the levels of serum CA 15-3 to enable early and non-invasive detection of breast cancer in the preclinical state and to monitor treatment of breast cancer patients.

A total number of 110 female patients who visited the Peace and Love Clinic at Oduom, in Kumasi from November, 2007 to February, 2008 were involved in the study. These consisted of 35 known previously confirmed (by histological examination) breast cancer patients and 75 suspected (unconfirmed) breast disease patients. Twenty (20) apparently healthy females were used as controls. Patients examination, specimen collection (both biopsy and blood specimens) were performed with patients consent and in accordance with the Helsinki Declaration. Each patient was given a questionnaire to complete.

About 3 mL of venous blood sample was taken from each of the 110 patients as well as the 20 healthy controls. All samples were processed to obtain sera. For the 35 confirmed breast cancer patients, samples were collected before and three months after treatment (surgery, chemotherapy and radiotherapy). Serum samples were kept at 20°C and were brought to room temperature before assay.

Quantitative determination of serum CA 15-3 concentrations were made using BioCheck CA 15-3 Enzyme Immunoassay. Values greater than 35 U mL^{-1} were considered abnormal.

Tissue biopsies were surgically removed by a qualified surgeon from the 75 unconfirmed breast disease patients and sent to the histopathology laboratory for processing. The processed tissues were stained with Haematoxylin and Eosin (HE). Microscopic examination of each stained sample was made by a pathologist. The histopathological findings were documented for each patient.

Statistical analyses were performed using SPSS 15.0 statistical software and GraphPad Prism 5 for Windows. The Paired-Sample t-test and Independent-Sample t-test were used to compare the serum CA 15-3 concentrations, the age, histopathological results and the menopausal status. Results were considered significantly different at $p<0.05$. The Receiver Operating Curve (ROC) analysis was used to evaluate the reciprocal relationship between sensitivity and specificity of the serum CA 15-3 using the GraphPad Prism 5. A cut-off value of 35 U mL^{-1} was used. Results were obtained at 95% confidence interval.

The mean age of the 110 subjects used in the study was 46.2±12.0 years with minimum and maximum ages being 21 and 80 years, respectively. Out of the 110 subjects, 59(53.6%) were of post-menopausal status and 51(46.4%) were of pre-menopausal status. Also, 35(31.8%) of them were known breast cancer patients and the other 75(68.2%) were suspected cases.

Out of the 35 known breast cancer patients 11(31.4%) of them had normal serum CA 15-3 levels and the other 24(68.6%) had higher than normal serum CA 15-3 levels. After treatment, 32(91.4%) out of the 35 known cases had normal serum CA 15-3 levels and the remaining 3(8.6%) still had higher serum CA 15-3 levels.

Out of the 75 suspected cases, 38(50.7%) had normal serum CA 15-3 levels and the remaining 37(49.3%) had values above normal. The histopathological results of the biopsies demonstrated that 29(38.7%) out of the 75 suspected cases had benign breast disease and 46(61.3%) had breast (malignant) cancer.

Of the 29 benign cases, 27(93.1%) had normal serum CA 15-3 levels, with the remaining 2(6.9%) subjects having values above normal. With the 46 malignant cases, 11(23.9%) had normal serum CA 15-3 levels and 35(76.1%) of them had values higher than normal.

The mean values of serum CA-153 for the various categories of patients are shown in Table 1. There was a significant difference between the serum CA 15-3 concentrations of patients before and after treatment ($p<0.05$) (Table 2). There was also a strong positive correlation between the level of serum CA 15-3 and the histopathology results of the biopsies ($r = 0.518$).

Table 1: Mean levels of serum CA 15-3 for the various breast disease categories (U mL^{-1})

Categories	N	Mean	SEM	SD	Min.	Max.
Age (years)	110	46.2	1.1	12.0	21.0	80.0
Suspected cases (U mL^{-1})	75	54.4	7.4	64.3	5.4	342.8
Suspected patients with benign histopathology	29	12.0	1.7	9.0	1.6	41.0
Suspected patients with malignant histopathology	46	80.6	10.4	70.2	10.6	342.8
Suspected post-menopausal cases	42	64.8	11.4	74.1	6.7	342.8
Suspected pre-menopausal cases	33	41.0	8.2	46.8	5.4	173.2
Known breast cancer patients before treatment	35	54.6	11.4	67.3	7.1	384.2
Known breast cancer patients after treatment	35	22.3	4.2	24.9	5.3	125.6
Control samples	20	13.0	1.0	4.6	7.1	22.5

N = Total No., SD = Standard Deviation, SEM = Standard Error Mean, Min. = Minimum, Max. = Maximum

Table 2: Paired sample correlation of serum CA 15-3 for the various categories of patients

		N	Correlation coefficient ®	p-value
Pair 1	Age and CA 15-3 of suspected patients	75	0.209	0.072
Pair 2	Known breast cancer patients before and after treatment	35	0.896	0.000
Pair 3	CA 15-3 of suspected patients and histopathology	75	0.518	0.000
Pair 4	CA 15-3 and menopausal stage	75	0.185	0.112

N = Total No., Sig. = Significant value

Table 3: Paired sample test CA 15-3 of patients with malignant and CA 15-3 of patients with benign histopathology

				95% confidence interval of the difference				
	Mean	SD	SEM	Lower	Upper	t-test	df	Sig.
Pair 1	64.6	73.9	13.7	36.5	92.7	4.7	28	0.000

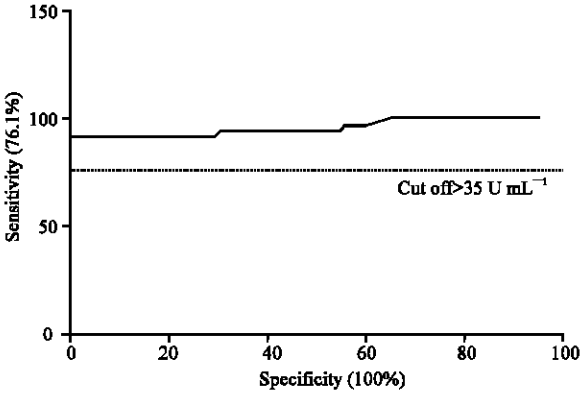

Fig. 1: ROC curve of serum CA 15-3 of controls and breast cancer patients

There was no correlation between the serum CA 15-3 concentrations and the menopausal status of the patients (r = 0.185). Similarly, there was no correlation between the age of patients and the serum CA 15-3 concentration of patients (r = 0.209).

There was a significant difference between the serum CA 15-3 of patients with malignant histopathology and the serum CA 15-3 of patients with benign histopathology (p = 0.000), by Paired-Sample t-test (Table 3).

The ROC analysis of the serum CA 15-3 concentrations of the control subjects and those of the patients with breast cancer (malignant histopathology) showed the serum CA 15-3 to have a sensitivity and specificity of 76.1 and 100%, respectively at a cut-off of >35 U mL^{-1} (Fig. 1).

One area under intense research is a search for an ideal tumour marker for the detection of breast cancer when the tumour is still small. At the moment, only a histological examination of a biopsy can confirm or rule out cancer. This study has shown a strong positive correlation between the serum CA 15-3 concentrations of patients and their histopathology results (r = 0.518). There was also a significant difference between the serum CA 15-3 of patients with breast cancer and the serum CA 15-3 concentrations of the patients with benign breast disease (p<0.05). A study conducted by Lee *et al.* (2004), however, found no significant difference between them.

There was a significant difference between the serum CA 15-3 concentrations before and after treatment (p<0.05). In monitoring a patient longitudinally, small increases or decreases in tumor marker concentrations can be indicative of early recurrence of the disease or response to therapy, respectively (Cheli *et al.*, 1998). It can therefore be said from this study that, serum CA 15-3 can be a useful marker in the monitoring of treatment for breast cancer.

However, there was no significant correlation between the age and the concentrations of serum CA 15-3 of the patients (r = 0.209). This observation is consistent with the study done by Keyhani *et al.* (2005). Nevertheless, a study by Lumachi *et al.* (2000), found a correlation between the age and the CA 15-3 levels in the patients.

Also there was no significant difference between the serum CA 15-3 concentrations of the patients at the post-menopausal stage and that at the pre-menopausal stage (p>0.05). This observation is consistent with the study done by Vizcarra *et al.* (1996). However, the study conducted by Duffy (1999), found a significant difference between the concentrations of serum CA 15-3 in post-menopausal patients and that of pre-menopausal patients.

The sensitivity and specificity of serum CA 15-3 in detecting breast cancer using a cut-off value of 35 Um L^{-1} was 76.1 and 100%, respectively. However, from the research of Keyhani (2005) sensitivity and specificity of serum CA 15-3 were 14 and 92.3%, respectively. This disparity may have occurred due to the different assay procedure and a cut-off value of 30 U mL^{-1}. Since sensitivity and specificity are both high in this study, serum CA 15-3 can be considered to be of help in early detection of breast cancer.

In conclusion, serum CA 15-3 can be used in preliminary screening for breast cancer and can also be used for the monitoring of treatment and the detection of distant recurrences after primary treatment.

In screening for breast cancer, patients with abnormal concentrations would be considered as having the cancer and those with concentrations within normal range would have a histological examination of their biopsy tissues for confirmation. Also, serial sampling for determination of concentrations of serum CA 15-3 would be done, when monitoring for efficacy of treatment, as serum CA 15-3 has a long half-life.

However, further prospective studies, using greater numbers of patients are required before serum CA 15-3 can be recommended for routine clinical use.

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