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STUDY OF TUMOR MARKERS IN BREAST AND OVARIAN CANCER PATIENTS

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ABSTRACT

Screening for breast and ovarian cancers are required due to the late stage at diagnosis of cancer and poor survival. Serum CA125 and CA15-3 were important cancer detecting agents in patients with ovarian and breast cancers, respectively. CA15.3 and CA125, by itself, has been shown to be a usefully prognostic marker collectively with routine histopathological markers like tumor size, grade and stage in the management of breast cancer. The study (Breast cancer) was carried out on 25 subjects comparing 12 normal healthy volunteers and 13 patients of breast cancer with the range of 30-65years. The mean age of all breast cancers was 47.75 ± 3.19 years with minimum and maximum ages being 30 and 65 years respectively. The CA 15.3 values in serum ranged from 49.25-730.8 U/ml in the breast cancer group and 5.89-20.10 U/ml in control group. The study (Ovarian cancer) was carried out on 32 subjects comparing 13 normal healthy volunteers and 19 Patients of ovarian cancer with the range of 30-60years. The mean age of all ovarian cancers was 45 ± 2.0604 years with minimum and maximum ages being 30 and 60 years respectively. The CA 125 values in serum ranged from 40.6 – 59.028 U/ml in the ovarian cancer group and 5.89-20.15 U/ml in control group. The study demonstrated a correlation between the stage of breast cancer and ovarian cancer with CA 15.3 and CA125 positivity rate respectively. The higher the cancer stage, more likely that CA15.3 and CA125 will be elevated. The incidence of CA15.3 and CA125 in early stage disease indicates that it can be used in screening and diagnosis. It was also discovered that

CA15.3 and CA125 were more sensitive in detecting the disease earlier before the clinical evidence of the disease. The treatment (Radiation and chemotherapy) results were more reducing level of CA15.3 in breast cancer and CA125 in ovarian cancer with the response to the treatment. A raised CA15.3 and CA125 may prompt the physician to exclude the presence of metastatic disease. Hence an initial estimation of CA15.3 and CA125 should be considered for the routine investigation in the management of patients with breast cancer and ovarian cancer respectively. Statistical analysis was performed by using SPSS 15.0 statistical software and Graph Pad Prism 5 for windows. The Unpaired t-test was used to compare the serum CA 15.3 and CA 125 concentration, the age, histopathological result and menopausal status.

KEYWORDS: Breast and ovarian cancer, Tumer Marker, Malignancy, Screening and Diagnosis

INTRODUCTION

Tumor markers are biochemical substances found in the presence of cancer and produced either by the tumor itself or in response to (para)neoplastic conditions, such as inflammation. Tumor markers can be found in a variety of bodily fluids and tissues and include hormones and several subgroups of (glyco)proteins, such as oncofetal antigens (which are normally expressed during fetal life), enzymes and receptors. They are used for diagnosis, assessment of therapeutic efficacy, and detecting recurrence during follow-up. The most limiting factor in the clinical use of tumor markers is the lack of sensitivity and specificity because the majority of markers are tumor associated rather than tumor-specific; elevated levels can occur in different types of malignancies as well as in benign and physiological conditions.[1] Moreover, early diagnosis and treatment of recurrences that are solely detected by the use of tumor marker alone has not shown survival benefit.[2] Tumor markers are substances that are produced by cancer or by other cells of the body in response to cancer or certain benign (noncancerous) conditions. Most tumor markers are made by normal cells as well as by cancer cells; however, they are produced at much higher levels in cancerous conditions. These substances can be found in the blood, urine, stool, tumor tissue, or other tissues or bodily fluids of some patients with cancer. Most tumor markers are proteins. However, more recently, patterns of gene expression and changes to DNA have also begun to be used as tumor markers.[3-5] Tumor Markers are biochemical substances elaborated by tumor cells either due to the cause or effect of malignant process. These markers can be normal

endogenous products that are produced at a greater rate in cancer cells or the products of newly switched on genes that remained quiescent in the normal cells. A tumor marker produced by the tumor and, when present in significant amounts, indicates the presence of a cancer. They may be present as intracellular substances in tissues or may be released into the circulation and appear in serum.[1-4] Continuing search for suitable tumor markers in serum, tissue and body fluids during neoplastic process is of clinical value in the management of patients with various malignancies.

Ovarian cancer is the fifth leading cause of cancer deaths among U.S. women and has the highest mortality of any of the gynecologic cancers.1 It accounted for an estimated 26,600 new cases and 14,500 deaths in 1995.1 The lifetime risk of dying from ovarian cancer is 1.1%.1a The overall 5-year survival rate is at least 75% if the cancer is confined to the ovaries and decreases to 17% in women diagnosed with distant metastases.2,3 Symptoms usually do not become apparent until the tumor compresses or invades adjacent structures, ascites develops, or metastases become clinically evident.4 As a result, two thirds of women with ovarian cancer have advanced (Stage III or IV) disease at the time of diagnosis.

Breast cancer is the most common cancer in women worldwide, with nearly 1.7 million new cases diagnosed in 2012 (second most common cancer overall). This represents about 12% of all new cancer cases and 25% of all cancers in women. Screening for breast and ovarian cancers are required due to the late stage at diagnosis of cancer and poor survival. Serum CA125 and CA15-3 were important cancer detecting agents in patients with ovarian and breast cancers, respectively. CA15.3 and CA125, by itself, has been shown to be a usefully prognostic marker collectively with routine histopathological markers like tumor size, grade and stage in the management of breast cancer. So, attempt has been made to carryout study of correlation between the stage of breast cancer and ovarian cancer patient with CA 15.3 and CA125 respectively.[6-9]

MATERIAL AND METHODS

The study was carried out at ‘Late Dr.Venkatrao Dawle Medical Foundation, Ambajogai, Dist-Beed, Maharashtra’. The study was performed in accordance with the ethical standard laid down in an appropriate version. Stage II, III and IV patients with breast and ovarian cancer were taken for the present study. The present assessment covering period of nine months which includes 13 subjects for breast cancer and 19 subject for ovarian cancer. The

entire entire cancer patient ranging from 30-70 Yr. old & 12 and 13 subjects were taken as healthy control for breast and ovarian cancer respectively. Age matched normal healthy individual not suffering from any inflammatory diseases with no major illness in the recent past formed the control group. Patients having systemic disease such as hypertension, Diabetes mellitus or any infections were excluded from the study. For patients with neoplasm, the original pathological report was reviewed when available and additional information, including duration of illness, state of disease, areas of metastases and therapy noted (case history of patients were obtained which furnished detailed regarding their habits and symptoms).

Blood samples (3ml) were collected from normal subject (normal subject for breast and ovarian cancer study) only once and from patients of breast cancer and ovarian cancer at three times, before beginning of the treatment and two more samples were taken from patients with diagnosed breast cancer and ovarian cancer who undergoing the treatment. The second samples were drawn on 30th day and the third samples were drawn on 45th day of the treatment.

Serum level of CA 15.3 and CA 125 were determined by direct chemiluminometric technology, which uses constant amount of two antibodies using BAYER KIT. Marker concentrations greater than cut –off were considered as ‘elevated’, the rest were considered as ‘normal’ (Cutoff levels of 35 U/ml for CA 15.3 and 36 U/ml for CA 125).

Specimen collection and handling

Serum was the recommended sample type for this study. The following recommendations for handling and storing blood samples are furnished by the national committee for clinical laboratory standards.

- All blood samples were collected observing universal precautions for venipuncture. Allowed sample to clot adequately before centrifugation.
- Kept tubes stoppered and upright at all times.
- Before placing samples on the system ensure that samples are free of fibrin or other particulate matter remove particulates by centrifugation at 1000 x g for 15 - 20 minutes.
- Samples should be free of bubbles.

Instrument Used

IMMULITE 1000 System

Diagnostic Kit Used

CA-125 and CA-15.3

BAYER HEALTH CARE DIAGNOSTIC DIVISION,DELHI

SUPPLIER:-Health Diagnostics Centre,

Muncipal Complex,

Opposite Ambedkar Garden,

Latur (M.S.) India.

Assay procedure

The IMMULITE 1000 system automatically performs the following steps.

1. Dispensed 50µL of sample into a cuvette.
2. Dispensed 50µL of Lite reagent and 250µL of solid phase and incubates for 7.5 minutes at 370C.
3. Separated, aspirated and washed the cuvettes with reagent water.
4. Dispensed 300µL each of acid reagent and base reagent to initiate the chemiluminescent reaction.

Reagents used in breast cancer

Reagent	Volume	Ingredients
Lite Reagent	5.0 mL/ reage nt pack	Polyclonal rabbit anti-CA15.3 antibody (~400 ng/mL) labeled with acridinium ester in phosphate buffered saline with protein stabilizers, sodium azide (0.12%), and preservatives
Solid Phase	25.0 mL/ reagent pack	Monoclonal mouse anti-CA15.3 antibody (~120 ❍g/mL) covalently coupled to paramagnetic particles in phosphate buffered saline with protein stabilizers, sodium azide (0.11%), and preservatives
CA 15.3 Diluent	5.0 mL/ reagent pack	Bicine buffer, gelatin, and BSA with preservatives and sodium azide (0.1%)

Assay principle

Assay was standardized against an internal reference standard of partially purified CA 15.3 and CA 125. It was two site sandwich immunoassay using direct chemiluminometric assay technology, which uses two purified monoclonal mouse antibodies specific for CA-125. The first antibody, in the Lite reagent, is directed toward the M 11 antigenic domain and is labeled with acridinium ester. The second antibody in the solid phase is directed toward the OV125

antigenic domain and is covalently coupled to paramagnetic particles. Assay was standardized against an internal reference standard of partially purified CA 15.3 and CA 125. A test performed to quantitate an analyte by using an antibody antigen binding reaction. Antibodies binds to antigen were very specific in binding. Antibody forms covalent linkage with acridinium ester.

Sample volume

This assay requires 50 ❍L of sample for a single determination. This volume does not include the unusable volume in the ample container or the additional volume required when performing duplicates or other tests on the same sample.

Assay procedure:

1) Prepare the sample contain for each sample and place barcode labels on the sample containers are required.
2) Load each sample container into rack, ensuring that the barcode label are clearly visible through the slot in each rack.
3) Place the racks in the entry queue.
4) Start the entry queue, if required.

Reagents used in ovarian cancer

Reagent	Volume	Ingredients
Lite Reagent	5.0 mL/ reagent pack	Monoclonal mouse antibody to CA125(~2.4 ❍g/mL) labeled with acridinium ester in phosphate buffer with protein stabilizers, sodium azide (<0.1%), and preservatives
Solid Phase	25.0 mL/ reagent pack	Monoclonal mouse antibody to CA125 (~0.044mg/mL) covalently coupled to paramagnetic particles in phosphate buffere with protein stabilizers, sodium azide (<0.1%), and preservatives.
CA 15.3 Diluent	5.0 mL/ reagent pack	Human serum albumin with sodium azide (0.1%) and preservatives

Statistical Analysis

Statistical analysis was performed by using SPSS 15.0 statistical software and Graph Pad Prism 5 for windows. The Unpaired t-test was used to compare the serum CA 15.3 and CA 125 concentration, the age , histopathological result and menopausal status. Result 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 and CA125 using the Graph Pad Prism 5. Student't' test (unpaired means) was employed to find out the statistical significance. The level of Serum CA15.3 and CA 125 of normal healthy subjects were compared with that of patients of Breast cancer and Ovarian cancer before treatment and level of CA15.3 and CA 125 of Breast Cancer and Ovarian cancer before treatment and after treatment were also compared.

Treatment protocol

All patients of ovarian cancer were treated by cycles of chemotherapy comprising carboplatin and paclitaxel, while patients with breast cancer were treated with Fluorouracil along with the radiation therapy as per stage of cancer and age of patient in the prescribed manner.

RESULTS AND DISCUSSION

On Breast Cancer Patients:

The study was carried out on 25 subject comparing 12 normal healthy volunteers and 13 patients of breast cancer with the range of 30-65years. The mean age of all breast cancers was 47.75 ± 3.19 years with minimum and maximum ages being 30 and 65 years respectively. The CA 15.3 values in serum ranged from 49.25 - 730.8 U/ml in the breast cancer group and 5.89-20.10 U/ml in control group.

Table: 1. Showing value of serum CA-15.3 in normal subject and Breast cancer group.

Biomarker	Group	Number	Mean	Std. Deviation	Std. Error	P value
CA 15.3	control	12	10.86	4.0129	1.1584	P <0.0004
	Patients	13	301.10	244.48	67.8048	

In this study CA 15.3 level was estimated of 14 patients. The majority of patients was in stage 3 (50%), no patients with stage 1 disease had found (0%), patients in stage 2 disease (29 %), patients in stage 4 disease (21%). All patients with breast cancer were elevated CA15.3 as per the stage of their cancer.

Table: 2. The distribution of patients according to clinical stages

Stage	No. of Patients	Percent
I	0	0
II	4	29
III	6	50
IV	3	21
TOTAL	13	100

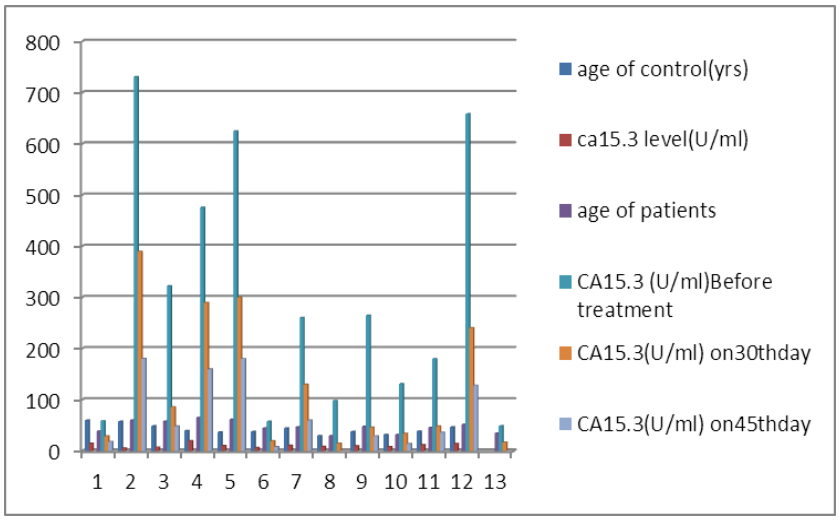
Table: 3. Shows mean pattern of serum CA15.3 levels with study parameters.

Study parameter	Subjects	CA15.3(U/ml) Mean + SE	Significance
Stages	Control (12) Stage I & II (4) Stage III & IV (9)	10.87 105.86 405.52	0.0001

It was apparent that the higher the disease stage, the higher is the frequency of abnormal CA15-3 values, when compared to those in stage I.. The relationship between elevated CA15.3 and clinical stage was found to be statistically significant ($p<0.001$) (Table 3.).

This confirms that CA15.3 assay is valuable in evaluating the progression of disease. This observation is consistent with the study done by Adachi I et al. (1992). This study is comparable with the results from various analysis regarding CA15.3 and tumor stages (Kerian et al., 1989; Gion et al., 1991; O'Hanlon et al.1995).[10, 11-12, 15-16] Serum CA15.3 has been shown to be elevated in 95% of cases where metastasis existed (James et al.2001). However, as compare to the other study, in our study 100% patients showed an elevation in level of serum CA15.3.These value support the fact that CA15.3 is more valuable in follow-up of patients with metastatic breast cancer. In some other studies, the prognostic impact of CA15.3 was independent of tumor size and axillary nodal status (Shering et al., 1998; Kumpulainen et al., 2002; Duffy et al., 2004).[17-21, 22, 23]

The average level of CA15.3 before treatments was 301.10(±67.80) and during the treatments (Radiation and chemotherapy) on 30th day was 126.73(±36.42) and on 45th day 78.66(±19.20). The mean serum CA15-3 level dropped after chemotherapy. There was a significant difference between the serum CA15.3 concentration of patients before & after the treatment ($p<0.005$). In some other studies, monitoring serum CA15.3 levels during first line chemotherapy or hormone therapy in advanced breast cancer patients could provide prognostic information independently from tumor response. A significant relationship was found between disease response and CA15.3 level, Bartsch et al (2006); Tampellini et al. (2006). In monitoring patients, small increase and decrease in tumor marker concentration can be indicative of early recurrence of the disease or response to therapy respectively, Cheli et al (1998). It can therefore be said that, serum CA15.3 can be a useful marker in monitoring of the treatment for the breast cancer.



There was a also strong positive correlation between the level of serum CA15.3 and the histopathological results of the biopsies obtained by co-workers. There was a significant difference between level of serum CA15.3 of the patient with malignant and level of serum CA15.3 of the control subjects. The ROC analysis of the serum CA15.3 concentration of the control subjects and those of the patients with breast cancer (malignant histopathology) showed the serum CA15.3 to have sensitivity and specificity 79 and 100% respectively, at a cut off of 35U/ml. However, from the research of Keyhani M.et al. (2005) sensitivity and specificity of serum CA 15.3 were 14 and 92.3% respectively and the research of Margaret T et al.(2008)were 76.1 and 100% respectively. This disparity may have occurred due to the different assay procedure and a cut off value of 35U/ml. Since sensitivity and specificity are both high in this study.

The present study demonstrates that CA 15-3 chemiluminometric assay was a highly sensitive marker for Stage II, III and IV cases of breast cancer. Therefore, we believe that CA 15-3 can be used as a screening test for breast cancer. On the other hand, CA 15-3 measurements may have a diagnostic value for metastatic breast cancer since the level of antigen is remarkably high in these cases.

Age and level of Serum CA 15.3 in Control subject		Age Of the Patient	Level of Serum CA15.3 Before treatment (U/ml)	Level of Serum CA15.3 After Treatment (U/ml)	
Age	15.3 Level			30 Days	45 Days
60	14.8	39	58.7	29.18	18.5
51	5.89	60	730.8	389.7	180.52
49	7.5	58	322.2	86.3	48.7
40	20.1	65	475.9	289.9	160.2
37	10.5	62	625.18	300.2	180.2
38	6.72	45	58.2	19.9	8.2

45	11.15	47	260.7	130.12	60.24
30	8.84	30	98.5	15.2	
38	9.9	48	264.8	46.5	29.5
32	8.62	32	131.2	34.4	14.4
39	12.17	46	180.2	48.4	36.54
47	14.2	52	658.72	240.6	128.3
--	--	35	49.25	17.15	--

Ovarian Cancer patients

The study was carried out on 32 subjects comparing 13 normal healthy volunteers and 19 Patients of ovarian cancer with the range of 30-60years. The mean age of all ovarian cancers was 45 ± 2.0604 years with minimum and maximum ages being 30 and 60 years respectively. The CA 125 values in serum ranged from 40.6 – 59.028 U/ml in the overian cancer group and 5.89-20.15 U/ml in control group.

Table1. Showing value of serum CA-125 in normal subject and ovarian cancer group.

Biomarker	Group	Number	Mean	Std. Deviation	Std. Error	P value
CA 125	Control	13	11.0953	4.6697	1.2951	0.0005
	Ovarian Cancer	19	303.98	270.8084	62.1276	

For the 19 Patient CA 125 level were estimated. The majority were stage 3 (58%) , no patients with stage 1 disease had found (0%), patients in stage 2 disease (31 %), patients in stage 4 disease(11%). All patients with ovarian cancer were elevated CA125 as per the stage of their cancer as their levels are compared histopathological reports of biopsies those were performed by coinvastigators. The distribution of CA125 levels according to clinical stages was shown in table no.

Table 2. Stage-wise distribution of Ovarian Cancer

Stage	No. of patients	Percent
I	0	00
II	6	31
III	11	58
IV	2	11
Total	19	100

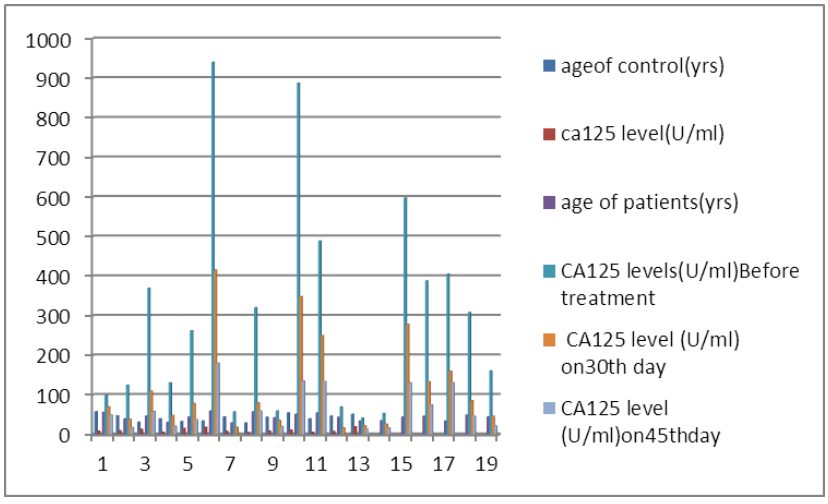
However, as compared to the other study, Jacobs I et al. (1999), Zurawski VR et al.(1988), in our study 100% showed an elevation in level of serum CA125.These value support the fact that CA125 is more valuable in follow-up of patients with metastatic ovarian cancer.

In some other studies, Einhorn N et al.(1986), Einhorn N et al.(1990), Einhorn N et al.(1992)[24-26] monitoring serum CA125 level was elevated above reference levels in only 50% of clinically detectable early stage disease and is not infrequently elevated in patients with benign ovarian tumors.[24-26] Adding one or several markers to CA 125 for use as a composite marker (CM) would improve diagnostic performance if sensitivity were improved with no loss in specificity.

The average level of CA125 before treatments was 303.99(±62.13) and during the treatments (Radiation and chemotherapy) on 30th day 119.40(±27.37) and 45th day 68.10 (±12.27). The mean serum CA15-3 level dropped after treatment was started. There was a significant difference between the serum CA125 concentration of patients before & after the treatment.

According to Bast et al. (1985)[27] decreasing concentration of serum CA125 after therapy was associated with good response to therapy. Increasing or static serum CA125 concentration was associated with either tumor progression or static disease. In another study, Keneman et al. (1994)[28] have suggested similar changes in serum CA125 concentration which matched well with tumor mass progression and regression. A study conducted by Vander Burg et al.(1993) have shown a significant decrease, generally having of the serum CA 125 post therapeutically and was shown to correlate well with clinical response in 87% of 237 patients from their study, however, our study correlate clinical response in 100% of 19 patients.

The utility of serum CA125 monitoring in ovarian carcinoma patients was extremely helpful in defining the response to therapy and detecting an early recurrence of the disease.



There was a also strong positive correlation between the level of serum CA125 and the histopathological results of the biopsies. There was a significant difference between level of serum CA125 of the patient with malignant and level of serum CA125 of the control subjects. The ROC analysis of the serum CA125 concentration of the control subjects and those of the patients with ovarian cancer (malignant histopathology) showed the serum CA125 to have sensitivity and specificity 68 and 100% respectively, at a cut off of 35U/ml.

However, from the research of Mishra S et al. (2006)[29] sensitivity and specificity of serum CA 125 were 58 and 96% respectively and the research of Falcao A et al.(2005)were 76.1 and 100% respectively. This disparity may have occurred due to the different assay procedure and a cut off value of 35U/ml.Since sensitivity and specificity are both high in this study.

The present study demonstrates that CA 125 chemiluminometric assay was a highly sensitive marker for Stage II and III cases of ovarian cancer. Therefore we believe that CA 125 should be used as a screening test for the ovarian cancer. On the other hand, CA 125 measurements may have a diagnostic value for metastatic ovarian cancer since the level of antigen is remarkably high in these cases.

The serum CA125 levels were significantly higher in patients with ovarian cancer than in the control group ($P < 0.001$). The diagnostic sensitivity and specificity, predictive values and accuracies were calculated for each marker.

Age and level of Serum CA 125 in Control subject		Age Of the Patient	Level Of Serum CA125 Before treatment(U/ml)	Level Of Serum CA 125 After	
Age (Yrs)	CA 125 Level			30 days	45 days
58	9	57	99.4	70.2	50.04
48	10	40	125.19	38.51	18.09
32	13.9	47	370.3	110.6	58.5
40	6.6	31	131.2	48.51	22.31
33	15.9	45	262.9	78.4	38.2
35	18.5	60	940.6	415.9	180.4
45	8.3	30	58	18.5	
30	5.79	58	320.8	80.5	59.7
44	9	43	60.38	35.8	20.75
55	12	52	888	348.7	136.05
40	6.6	55	489	250.21	134.2
47	8.5	44	70.51	17.15	
52	20.15	35	42.51	22.2	14.2
--	--	36	54.2	26.5	17.8

--	--	45	598.02	278.9	130.9
--	--	47	388.72	134.1	75.48
--	--	35	405.9	160.9	131.4
--	--	50	308.5	86.5	47.2
--	--	45	161.6	46.6	22.52

SUMMARY AND CONCLUSION

Screening for breast and ovarian cancers are required due to the late stage at diagnosis and poor survival. Serum CA125 and CA15-3 were important cancer detecting agents in patients with ovarian and breast cancers, respectively. Elevation of CA125 and CA15-3 level correlates with malignant and non-malignant conditions. Moreover, a series of individual characteristics affect the serum level of these markers. The study demonstrated a correlation between the stage of breast cancer and ovarian cancer with CA 15.3 and CA125 positivity rate respectively. The higher the stage, more likely that CA15.3 and CA125 will be elevated. The incidence of CA15.3 and CA125 in early stage disease indicates that it can be used in screening and diagnosis.

CA15.3 and CA125, by itself, has been shown to be a usefully prognostic marker collectively with routine histopathological markers like tumor size, grade and stage in the management of breast cancer. It was also discovered in this study that CA15.3 and CA125 were more sensitive in detecting the disease earlier before the clinical evidence of the disease. We also conclude that treatment (Radiation and chemotherapy) results were more reducing level of CA15.3 in breast cancer and CA125 in ovarian cancer with the response to the treatment. A raised CA15.3 and CA125 may prompt the physician to exclude the presence of metastatic disease. Hence an initial estimation of CA15.3 and CA125 should be considered for the routine investigation in the management of patients with breast cancer and ovarian cancer respectively.

This prospective study shows that in pretreatment marker-positive patients 1) the changes in serum tumor marker levels after the start of therapy correlate with the response to therapy; and 2) a greater than 20% reduction in the tumor marker levels was a favorable predictive factor for TTP (longer time-to-progression) during systemic therapy. When the pretreatment serum level of these markers is over the respective cut-off value, sequential measurement of them may be useful for evaluating the efficacy of treatment as well as monitoring the outcome of patients with advanced breast cancer and ovarian cancer.

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CONFLICT OF INTERSET

Authors declare that there is no conflict of interests.

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