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Comparison of CEA and CA15-3 Markers with Serotonin, Ceruloplasmin and Copper in Breast Cancer Recurrence after Chemotherapy

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ABSTRACT

Background: The serotonin, copper, and ceruloplasmin markers are altered in various cancers, including breast cancer. It has been reported that these markers have the potential to be used in the study of cancer recurrence. The purpose of this study was to compare the levels of serotonin, copper and ceruloplasmin besides the routine breast cancer markers such as CEA and CA15-3 in the blood sample of patients with invasive ductal breast cancer, before and after chemotherapy.

Methods: This study was performed on 30 patients with breast cancer. Blood samples were taken from the patients before and after chemotherapy. Necessary data including age, tumor grade and status of Her-2, ER, PR receptors were obtained from patient records. Serotonin, CEA and CA15-3 levels were measured by ELISA method. Ceruloplasmin and copper were measured by nephelometry and colorimetric methods, respectively.

Results: Results showed a decrease in serotonin, ceruloplasmin, copper, CEA and CA15-3 after treatment but only the levels of serotonin and ceruloplasmin showed a steady decrease. No significant relationship was observed between tumor grade and ER-PR, Her-2 receptors.

Conclusion: This study showed that chemotherapy resulted in steady decline in serotonin and ceruloplasmin levels but this decrease was not steady in levels of CA15-3 and CEA. Therefore, if our results are confirmed by further research, they can be considered as a viable alternative to routine markers in cancer recurrence after chemotherapy.

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INTRODUCTION

Breast cancer is by far the most frequently diagnosed cancer and cause of death among women. According to statistics from 2021 in the United States,

it is estimated that breast cancer with 284,200 new cases is ranked first in new cases of cancer.¹ Currently, the most common pathological factors used to assess a patient's condition include tumor size, lymph node status, tumor grade, estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor-2 (HER-2) status.^{2,3}

Serum tumor markers have attracted increasing attention for their use in the screening and monitoring of different types of cancer.⁴ Among several tumor markers, CEA and CA15-3 are the two most widely

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used markers for diagnosis and follow up in the treatment and recurrence of breast cancer. Numerous studies have been performed to quantitatively evaluate the serum levels of these two tumor markers, which have identified a wide range of cut-off values for predicting the prognosis of poor survival in breast cancer.⁵⁻⁸ Beyond these routine markers, introducing new markers may help early diagnosis and monitoring the treatment of patients with breast cancer.

Serotonin (Ser) (5-hydroxytryptamine; 5HT) is a monoamine neurotransmitter which mediates a wide range of physiological actions in the human body. Serotonin is implicated in psychiatric and neurological disorders and also plays a fundamental role in tumor growth, differentiation and gene expression. It acts as a growth factor for several types of tumor and non-tumor cells.⁹ Increased tryptophan metabolism via the Ser pathways could, therefore, be linked to malignant progression in breast cancer.^{10, 11} Fröbe *et al.* studied plasma free serotonin (HT5) as a new tumor marker in breast cancer patients and reported that serotonin levels were increased among those who had recurrence after treatment.¹² In addition to its known functions, serotonin is known as a mitogenic agent for a wide range of natural cells such as vascular smooth muscle cells, muscle cells, lung fibroblasts, renal mesenchymal cells, liver cells, etc.⁹ A recent study confirmed the plasma serotonin as a predictor for recurrence and poor prognosis in colorectal cancer patients.¹³

Ceruloplasmin (CP) is an acute phase protein which is normally synthesized in the liver.¹⁴ Serum Copper (Cu) and CP have been reported to be useful markers of disease activity in patients with Hodgkin's disease, Non-Hodgkin's lymphoma, acute leukemia, gastrointestinal tract cancer, lung cancer and breast cancer.^{15, 16} CP is suggested to have a role in cancer since it is involved in angiogenesis and neovascularization. Previous studies reported that CP mRNA exists in human colon and breast cancer cell lines.¹⁷ The aim of the current study was to investigate the potential of Ser, CP and Cu beyond the CA15-3 and CEA in breast cancer recurrence after chemotherapy.

Methods

Patients

Among patients with invasive ductal carcinoma who underwent surgery at Omid Hospital of Urmia, Iran (May 2019 - May 2020), and those who referred to this hospital for chemotherapy, 30 individuals were randomly selected. The patients with severe systemic or cardiovascular disease, other malignant tumors, hematologic diseases, liver and kidney dysfunction, infection, pregnancy, smoking, and taking NSAIDs were excluded from the study. All participants were aware of their participation and had willingly signed a consent form. The study protocol was approved by the

Medical Ethics Committee of Urmia University of Medical Sciences.

Sampling

Blood samples (6 ml) were collected from each patient at 3 different times (before chemotherapy, before the 4th cycle of chemotherapy, and before the 6th cycle of chemotherapy) and centrifuged after clotting. Serum was harvested and stored at -70°C until used.

Chemotherapy program

Chemotherapy was performed in a course of 6 cycles for each cycle of 21 days. Adriamycin and Cyclophosphamide were used for all patients in the first three cycles of chemotherapy and Taxotere and Taxol were used in the second three cycles of chemotherapy. In addition, Herceptin was prescribed to HER2 positive patients.

Assessment of serum serotonin

The serum serotonin was determined by serotonin competitive ELISA kit (abcam; cat No. ab133053) following the manufacturer's instructions. Absorbance values were observed at 405 nm. The results were expressed as nanograms per milliliter.

Assessment of serum ceruloplasmin

The immunonephelometry method was used to measure the serum ceruloplasmin by NEPHSTAR Ceruloplasmin (CER) Kit (Cat No. DK018) according to the manufacturer's protocol.

Assessment of serum copper

The serum copper was measured by colorimetric assay kit (Sigma-Aldrich Co, Cat No. MAK127), according to the manufacturer's instructions. The method utilizes a chromogen that forms a colored complex specifically with copper ions. The intensity of the color was measured colorimetrically at 359 nm. The range of linear detection was 7 µg/dL (1.0 µM) to 300 µg/dL (47 µM).

Clinical pathology outcomes

Patients' clinical information including the expression patterns of Estrogen Receptor (ER), Progesterone Receptor (PR), HER-2, stages of tumor, and age were obtained from the patients' files and the results of their pathology tests.

Assessment of serum CA15-3 marker

The measurement of serum CA15-3 was been done by DIAMETRA kit (REF: DKO055). Absorbance (E) was read at 450 nm against Blank.

Assessment of serum CEA marker

The CEA levels in patients' serum were determined quantitatively using CEA EIA Kit (Padtan Elm Co, Iran) according to the standard protocol of manufacture.

**Table 1.** Changes of study markers during chemotherapy

	Pre- chemotherapy	Post- chemotherapy (3 cycle)	Post- chemotherapy (5 cycle)	F
CA 15-3	14.813±0.990	12.514 ±0.789 ^a	13.008 ±0.794	14.03
CEA	1.182 ±0.111	0.948 ±0.094 ^a	0.921 ±0.113	5.24
Cu	116.161 ±3.292	108.460 ±2.952 ^a	106.486 ±2.903 ^{a, b}	17.06
CP	34.628 ±1.09	31.266 ±0.939 ^a	29.666 ±0.858 ^{a, b}	62.36
Ser	131.477 ±7.153	113.059 ±5.889 ^a	106.164 ±5.906 ^{a, b}	41.73

a: compared with pre-chemotherapy, b: compared with 3 cycles of chemotherapy; p<0.01. Cu: Copper, CP: Ceruloplasmin, Ser: Serotonin

Statistical Analyses

All analyses were performed by Statistical Package for the Social Sciences (SPSS, V.23). The normality distribution of data was determined by Kolmogorov-Smirnov test. In normal distribution, paired T- test and repeated measures ANOVA test were used to examine the changes of serum levels of these factors. The $P<0.05$ was considered statistically significant.

Results

Demographic and clinical characteristics of the patients

All patients in this study were female with a mean age of 52.03 years (ranging from 32 to 86 years). According to the clinical information, most of the patients were at stage II (63.3%) and only 4 patients were at stage III (13.3%). Furthermore, it was observed that most patients were ER and PR positive (73.3% and 70 %, respectively) but HER-2 negative (56.7 %).

Changes in study markers during chemotherapy

As can be seen from Table 1, the serum levels of Ser, CP, and Cu levels were steadily decreasing significantly after each cycle of chemotherapy. Levels of serum CA15-3 and CEA significantly decreased only after 3 cycles of chemotherapy and serum CA15-3 levels increased insignificantly after 5 cycles of chemotherapy.

According to the results, the alternations of these markers seemed to be associated with the time points of sampling. The reduction efficacy of chemotherapy for CP, Ser, Cu, CA15-3, and CEA were 68.3%, 59%, 37%, 32.6%, and 15.3%, respectively.

According to our data, HER-2 showed a non-significant inverse relationship with the average level of all factors except Cu (R: 0.051, P value 0.791). Also, Ser, CA15-3 and CEA showed a non-significant inverse correlation with tumor grade (R: -0.087, P :0.649; R: -0.039, P :0.837; and R: -0.160, P :0.397, respectively).

The results showed that there was no significant difference in the levels of Cu, CA15-3, CEA, CP and Ser between patients with HER-2, ER, and PR receptor positive and negative before and after chemotherapy (data not shown). The results also showed that there was no significant difference in the

levels of Cu, CA15-3, CEA, CP and Ser in patients with different stages of breast cancer (stages I, II, and III) (data not shown).

DISCUSSION

The purpose of the present study was to evaluate the status of routine markers (CEA and CA 15-3) in breast cancer patients during chemotherapy and compare them with CU, CP and Ser as possible markers in monitoring chemotherapy.

There are many different treatments for cancer, including nanoparticles¹⁸, herbal medicines¹⁹, chemical drugs, etc. Each treatment shows different effects in specific patients and the same results are not achieved in all cases. Therefore, it is important to follow up and evaluate cancer recurrence with reliable markers. CEA and CA15-3 as tumor markers are widely used in the diagnosis and monitoring of breast cancer. Yijie Fu reported that CEA and CA15-3 increased only in malignant tumors and their values did not change in benign tumors.²⁰ Several studies demonstrated that these markers do not have enough sensitivity and specificity.

According to the results reported by Agrawal *et al.*²¹, ductal breast cancer is associated with increased serum CEA and normal levels of CA15-3. However, Duffy *et al.*, in a study measuring CA15-3 to evaluate the treatment process in patients with breast cancer, considered the use of CA15-3 marker appropriate only in advanced breast cancer.²² In a review study, Mirabelli *et al.* showed CA15-3 was not suitable to use as a routine marker due to lack of sensitivity and specificity in the early stages of the disease.²³ They also showed that CA15-3 marker was a more specific marker than CEA, and noted the CEA marker was unreliable due to its low sensitivity and lack of specificity in distinguishing benign patients from healthy individuals. CA15-3 also had low specificity and sensitivity in STAGE 1 and 2 of cancer.²³

In the present study, by comparing the changes of CEA to CA15-3 before and after chemotherapy, a greater decrease in CEA values was observed. CA15-3 levels appear to be affected by surgery and CEA levels appear to be affected by chemotherapy. The findings of the current study are consistent with those



of Shooshtary *et al.* who reported the treatment process (surgery and chemotherapy) reduced the serum level of CA15-3 more than the serum level of CEA.²⁴

In the current study, serotonin levels decreased following chemotherapy. There are several possible explanations for this result. The most likely reason is the induction of monoamine oxidase enzyme expression. This finding corroborates the results reported by Gordon *et al.* who suggested that chemotherapy induces the expression of monoamine oxidase enzyme.²⁵ In the present study, serotonin was reduced as a biomarker for monitoring the treatment process, but this reduction cannot be attributed to the effectiveness of chemotherapy drugs.

Frobe *et al.* found that in patients who responded to the initial treatment but had a recurrence of the disease, the plasma serotonin levels were significantly higher than in the control group, but that the level of CA15-3 remained in the normal range.¹²

A review study by Siddiqui found that serotonin is a growth factor for a variety of tumor and non-tumor cells.⁹ This study, referring to the role of serotonin in carcinogenesis and tumor growth, can explain why serotonin increased in patients in our study. Xia Y *et al.* introduced preoperative plasma serotonin elevation as a functional prognostic biomarker for recurrence of colorectal cancer.¹³

In the present study, serum ceruloplasmin and copper levels were significantly reduced during chemotherapy. This finding is in agreement with findings reported by Ohanlon *et al.* who showed the acute phase response can be activated in a variety of

malignancies.²⁶ Shenkin also noted an increase in copper controlled by interleukin-1, interleukin-6, and TNF, which could be a reason for high levels of copper before starting chemotherapy.²⁷

However, the findings of the current study do not support some previous research such as Schapira who showed that ceruloplasmin levels in metastatic breast cancer patients increased again after an initial decrease after chemotherapy.²⁸ Due to these contradictory results, further studies in this regard seem necessary. However, our results are broadly consistent with earlier results regarding copper and ceruloplasmin such as the study conducted by Vaidya on breast cancer patients.²⁹

CONCLUSION

In conclusion, the present study was designed to determine the effect of chemotherapy on Serotonin, Ceruloplasmin, copper, CEA and CA15-3 as biomarkers of breast cancer. One of the most significant findings to emerge from this study is that Serotonin showed a steady decrease compared to other markers during chemotherapy, but further studies are needed to evaluate serotonin as a marker in monitoring breast cancer recurrence after chemotherapy.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this paper.

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