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Evaluation of the A-Amylase Enzyme, Carcinoma Antigen (C.A 15.3), and Some Biochemical Variables in Sera of Woman with Breast Cancer Patients

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Received: 15 August 2023 **Accepted: 9 November 2023**

Published: January 2025

DOI: https://dx.doi.org/10.24237/ASJ.03.01.812C

Abstract

The objective of this study was to assess the levels of various markers and enzymes in the blood serum of breast cancer patients at different stages of the disease, as compared to healthy women. The markers evaluated in this study were amylase, C.A 15.3, ALT, AST, ALP, urea, creatinine, and zinc. The study revealed that C.A. 15.3 is a reliable marker for monitoring metastatic breast cancer. The levels of this marker were significantly higher in women with breast cancer at all stages of the disease, particularly in the advanced stages. The study also revealed that the levels of the alpha-amylase enzyme increased with the progression of the disease, which makes it a vital diagnostic sign of breast cancer. the results demonstrated an increase in the levels of AST, ALT, ALP, urea, and creatinine and a decrease in zinc levels with the development of the disease.

Keywords: Amylase Enzyme, Carcinoma Antigen CA-15.3, Breast cancer.

Introduction

Breast cancer is a prevalent malignancy that affects women globally, resulting in a significant number of fatalities. In 2015, breast cancer was responsible for 570,000 deaths, and it continues to be a significant health challenge. Every year, approximately 1.5 million women worldwide are diagnosed with breast cancer, making up 25% of all cancer patients. The United States is expected to see an estimated 252,710 new cases of breast tumors in women in 2017. Breast

cancer is a metastatic type of cancer that can spread to other organs, such as the bone, liver, lung, and brain, making it a dangerous disease. Early detection of the illness is critical in achieving a favourable prognosis and a high survival rate [1]. Carbohydrates are the main ingredients in the human diet, and the main component is sugars. It plays an essential role in power supply. The carbohydrates must first be broken down in to monosaccharides by specific enzymes in the digestive system, since only sugars can do that it is absorbed into the intestinal lumen. α Glucosidase and α-amylase are the main enzymes that Participant in the digestion of carbohydrates[2]. α-amylase is an enzyme that plays a key role in the breakdown of complex dietary carbohydrates into smaller molecules such as oligosaccharides and polysaccharides. These molecules are then converted into monosaccharides by α-glucosidase. Amylases are classified into three subtypes, namely α, β, and γ, with the first receiving significant research interest. Compared with β-amylase, α-amylase shows a higher rate of catalytic activity [3]. The most prevalent enzyme in the saliva of humans and many other animals is alpha amylase (EC 3.2.1.1). Salivary amylase is secreted predominantly in the parotid gland in humans. Its activity varies greatly both between and within a person. The polysaccharide's -(1,4)- glycosidic linkages are known to be broken by the enzyme. Moreover, it is crucial for the fundamental biomechanics of humans, aiding the metabolism of carbohydrates [4]. Medical technologies have made significant strides in the early detection of breast cancer. Digital mammography, contrast-enhanced magnetic resonance imaging of the mammary glands, and soft imaging have proven effective methods. Cancer antigen 15-3 (CA 15-3) is the most widely used tumour marker in breast cancer detection. Furthermore, saliva testing is gaining momentum as a supplementary tool for screening and diagnosing significant systemic disorders such as breast cancer. Saliva can serve as a source of biomarkers instead of relying on blood or other biological fluids. Its primary advantage lies in its non-invasive and simple collection process. Studies on saliva transcriptome and proteome analysis have demonstrated its enormous potential for breast cancer screening.[5] Breast tumour is a complex ailment that presents a significant degree of inter- and intra-tumoral heterogeneity. Consequently, a personalized approach is imperative to ensure optimal patient response to treatment modalities.[6] It is the type of non-invasive breast cancer that affects the breast duct alone and is the most prevalent. Ductal comedo carcinoma is

an illustration of ductal carcinoma that develops in situ. [7] The prevalence of breast cancer in women is greatly affected by pregnancy. Research studies have proven that there is a strong relationship between pregnancy and the possibility of breast cancer, especially the first pregnancy. Studies have shown that women who give birth to their first child before 33 weeks of pregnancy are twice as likely to develop breast cancer. [8] A mucinous glycoprotein called CA15-3 is one of the byproducts of the Mucin1 (MUC-1) gene. Nearly all epithelial cells have MUC-1, and colon, breast, ovarian, lung, and pancreatic malignancies are frequently linked to MUC-1 overexpression[9]. Cancer survivors are prone to facing numerous physical and mental health challenges in the long run. Stress is a well-known factor that significantly affects both aspects of health. In the case of breast cancer survivors, they may encounter various stressors throughout their cancer journey. These stressors can be subtle, such as anxiety about the reactions of loved ones, or chronic, continuing after treatment due to fear of recurrence or death. One way to measure stress-related biomarkers is by focusing on the enzyme alpha-amylase, which is a biomarker of stress in the sympathetic nervous system [10]. A practical approach to examining the impact of Stress with breast tumours on physiological mechanisms is to investigate secretion patterns of relevant biomarkers α-amylase (sAA)[11].

Material

This study was conducted at Al-Amal Cancer Hospital within Baghdad Medical City, Iraq, from November 2022 to March 2023, and included 120 Iraqi female patients with breast cancer. Their ages range from (30-70) years. The diagnosis of each case was determined by clinical examination by an oncologist in the hospital and verified by radiological and laboratory examination. The names of the patients, their ages, and the number of doses for patients with breast cancer were taken. The number of doses ranged between (1-12) doses, depending on the patient's medical history. Thirty academics and medical professionals were included in this study as controls. Their ages range from (30-70) years. To be a control, a person had to be free of breast cancer and other autoimmune diseases with the following conditions excluded from this study by history: cardiovascular disease, chronic liver disease, diabetes, thyroid disorders, pregnancy and other autoimmune diseases. Because these diseases may affect the results.

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Methods

(10) mL of blood was drawn from each control group and subject before and after treatment. The blood was transferred into regular tubes, allowing clotting for 30 minutes. The blood was centrifuged for 10 minutes. Use serum to measure C.A15.3, ALP, AST, ALT, urea, creatinine, and zinc. Enzyme activity was studied in samples collected from patients using a Roche/Hitachi Cobas, Analyzer smart-120 device .The data of the study was analyzed statistically using the (spss 24)

Results & Discussion

This study is consistent with several studies that found that blood amylase levels in pre-treatment breast cancer patients for the four stages (I, II, III ,IV) that were higher than control levels (81.80±6.57,109.20±10.05,135.60±12.99,175.60±80 respectively) were significantly increased ($P \leq 0.01$) as shown in Figure (1)

The activity of α-amylase was significantly increased and reached its maximum value in metastatic breast cancer, which is consistent with the literature data for all enzymes, there was a statistically significant increase in activity characteristic of breast cancer[5]. People with breast cancer have a higher level of α-amylase in their blood. Cloutier et al showed the internalization and transit of pancreatic amylase by enterocytes resulting in an increased level of amylase in the blood. [12]

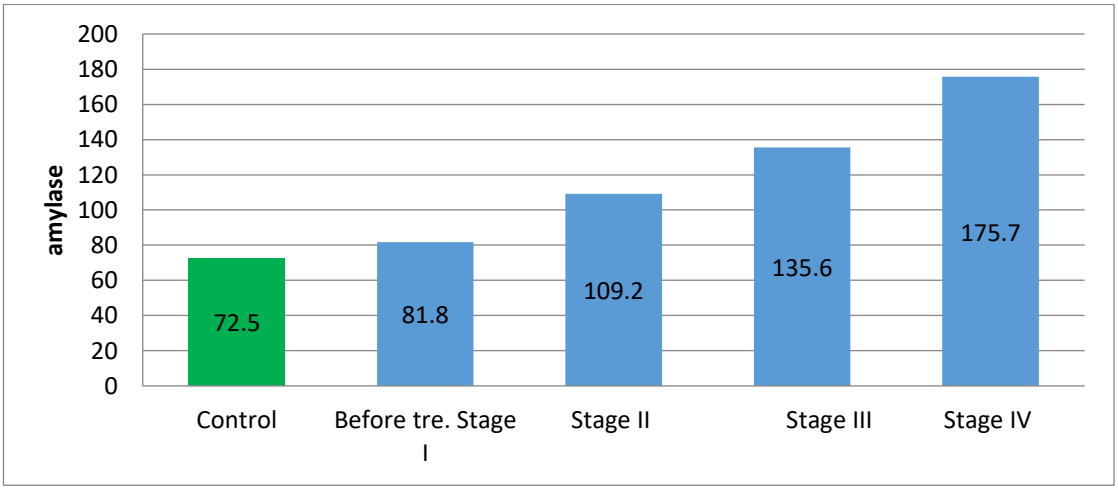

Figure 1: The mean and SD of amylase in control and patients

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The activity of amylase enzymes was studied on (20) before treatment and 30 as healthy controls, and it is shown in the Table (1)

Table 1: The level of amylase in patients before treatment and control.

Patients groups	Amylase(Mg/dl)	P-Value
Control	42±10.89	
Before treatment. Stage I	81.80±6.57	≤0.01
Before treatment Stage II	109.20±10.05	≤0.01
Before treatment Stage III	135.60±12.99	≤0.01
Before treatment Stage IV	175.60±80	≤0.01

Results C.A15.3 indicate that there is a high significant ($P \leq 0.01$) in the serum of post-treatment breast cancer patients (30.63 ± 8.89) compared to that in healthy controls (11.80 ± 4.70).

It was found that the levels of C.A 15.3 in serum of post-treatment as well as before-treatment Breast Cancer, patients, at stage, (II,, III,,IV) were higher than control levels (28.20 ± 2.86, 58.40 ± 7.40, 86.80 ± 15.65, respectively) were significantly increased ($P \leq 0.01$) as shown in Figure (2)

CA 15-3 is a reliable tumorigenic the marker in breast cancer patients with recurrence and distant metastases has the highest levels in patients with metastatic breast cancer (stage IV)[13].this study is consistent with Biao Geng et al who reported that CA15-3 is associated with tumor expansion and metastasis. CA15-3 was elevated in patients with multiple metastatic sites compared with patients with a single metastasis. As well as with Ali HQ et al when CA15-3 was evaluated with early diagnosis and treatment of breast cancer patients. They place great importance on the important association between CA15-3 and primary tumor volume [14].

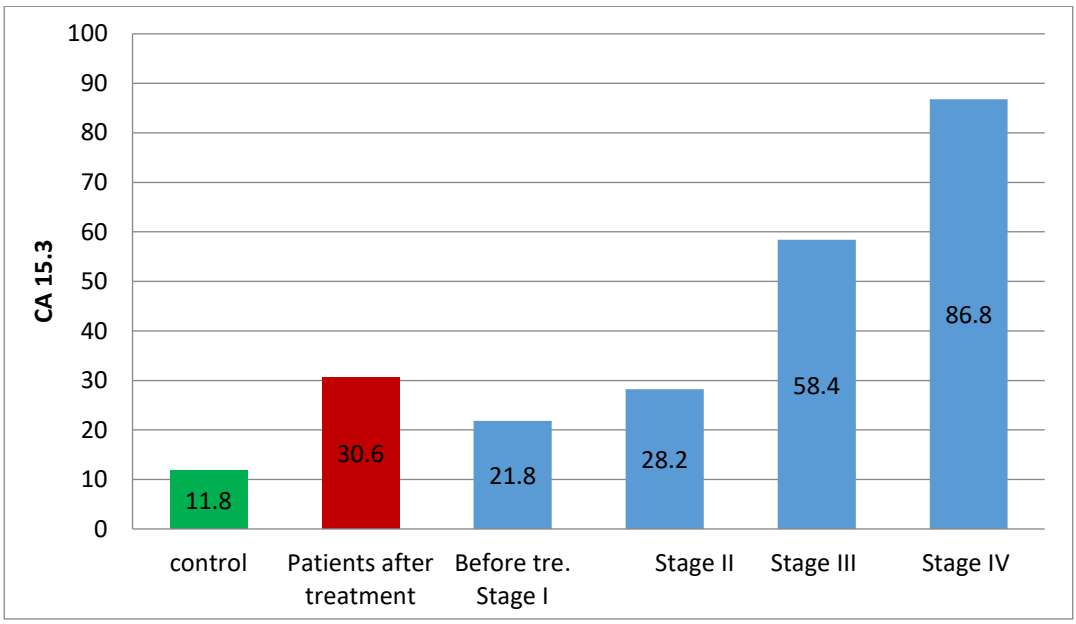

Figure 2: The, mean and SD of C.A15.3 in control and patients

The activity of cancer antigen (C.A 15.3) was studied on 120 patients with breast cancer (20) before treatment,(100) after treatment and 30 as healthy controls, and it is shown in the Table (2)

Table 2: The level of C.A15.3 in patients after treatment and patients before treatment and control.

Patients groups	C.A15.3(U/ml)	P-Value
Control	11.80±4.70	
Patients after treatment	30.63±8.89	≤0.01
Before treatment. Stage I	21.80±3.76	>0.05
Before treatment Stage II	28.20±2.86	≤0.01
Before treatment Stage III	58.40±7.40	≤0.01
Before treatment Stage IV	86.80±15.65	≤0.01

The activity of enzymes (AST, ALT, ALP) was studied on 120 patients with breast cancer (20) before treatment, (100) after treatment and 30 as healthy controls, and it is shown in the Table (3)

Table 3: The activity of serum Enzymes in patients after treatment and patients before treatment and control.

Patients groups	AST,(U/L)	P-Value	ALT,(U/L)	P-Value	ALP,(U/L)	P-Value
Control	23.16±5.90		22.60±6.24		72.13±8.68	
Patients after treatment	39.77±21.70	≤0.001	37.37±20.95	≤0.001	63.65±12.10	>0.05
Before treatment. Stage I	19.80±2.68	≤0.001	25.20±5.76	0.00	67.0±8.0	≤0.01
Before treatment Stage II	29.80±3.34	≤0.01	50.20±7.62	0.00	87.0±4.0	≤001
Before treatment Stage III	58.0±5.47	≤0.01	76.40±11.61	≤0.01	106.0±8.27	≤0.01
Before treatment Stage IV	91.20±4.08	≤0.01	102.4±11.34	≤0.01	129.80±6.18	≤0.01

The activity of AST was increased highly significantly (P≤ 0.001) in breast cancer patients, serum after treatment (39.77±21.70U/L) compared to that in healthy controls (23.16±5.90U/L) It was shown that the levels of AST enzyme in the blood serum of stage (I, II ,III ,IV) Breast, Cancer Patients Were, much higher than the, Levels ,of Control (19.80±2.68 ,29.80±3.34 ,58±5.47 ,91.20±4.08 respectively) increased highly significantly (P≤ 0.01).as shown in Figure (3)

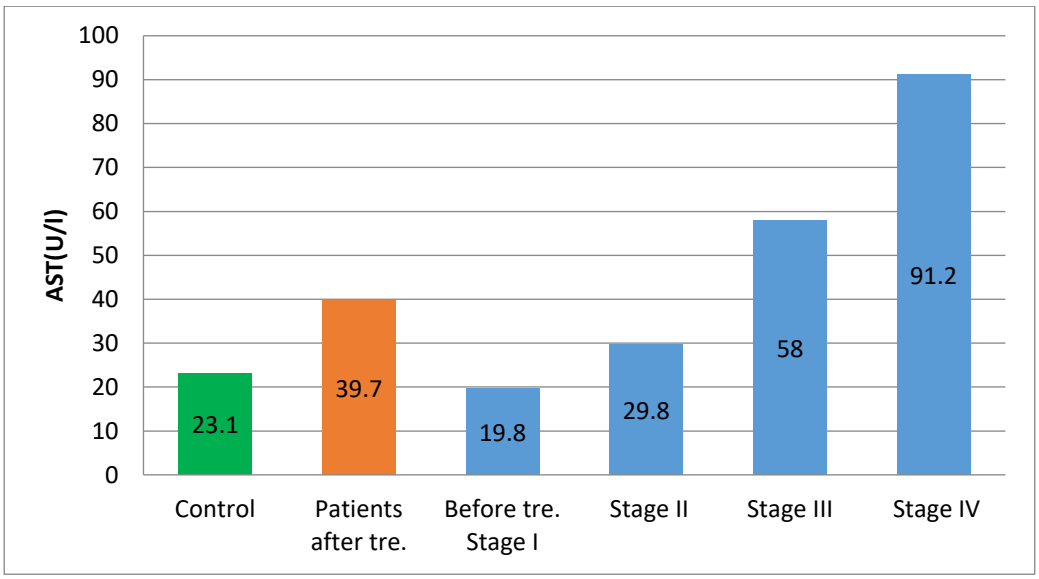

Figure 3: The, mean and SD of AST in control and patients

The activity of ALT was increased highly, significantly (P ≤ 0.01) in Breast, Cancer, patients, serum after treatment (37.37±20U/L) compared to that in healthy controls (22.6±6.24U/L)

This study may not be consistent with many studies, and it was found that ALT enzyme levels in the serum of stage (I, II, III, IV) Breast, Cancer patients were significantly higher, than those of control levels (25.20 ± 5.76, 50.20 ± 7.62, 76.40 ± 11.61, 102.4 ± 11.34) respectively where (P≤ 0.01) as shown in the figure (4).

The activity of (AST, ALT) enzymes was selected to study and evaluate the effectiveness of liver function performance, as the liver is the organ responsible for cleaning the body from many waste and toxic substances (Clarke & Clarke, 1977).

Also, the results of this study It is identical to what was reached by (Al-Hashemi et al., 2022), did not agree with what was reached by (Mahmoud 2019), which indicated a decrease in the level of AST enzymes. (ALT) in the serum of breast cancer patients compared to the control group [15]

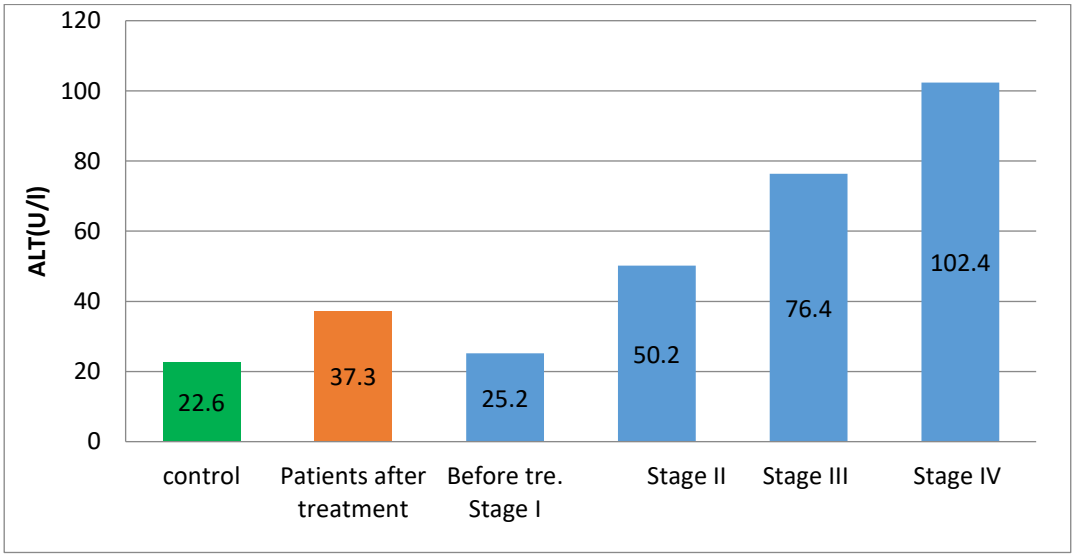

Figure 4: The mean and SD of ALT in control and patients

The activity of ALP was non- significantly (P >0.05) in breast; cancer, Patients, serum after treatment (63.65±12.10U/L) compared to that in healthy controls (72.13±8.68U/L)

It was shown that the levels of ALP enzyme in the blood serum of stage (I, II ,III ,IV) breast/ cancer; patients were much higher, than the levels of Control (67.0±8.0 , 87. 0±4.0 ,106±8.27 ,129.80±6.18 respectively) increased highly significantly (P≤ 0.01).as shown in Figure (5)

Our study found a significant positive association between ALP level and malignancy. Moreover, malignancy is mainly an independent variable that can be determined by ALP. This result can be explained by the fact that cancer cells may metastasize and settle in the bone, liver, intestine and brain where ALP enzyme is expressed leading to an elevation of the total ALP level. There is strong evidence to suggest that ALP is a potential tumor marker in bladder and breast cancer.

The ALP level increased significantly in breast cancer, especially after menopause. An explanation for this may be because bone damage in postmenopausal women leads to an increased level of ALP. And it showed an increased difference between ALP levels in breast cancer patients. These data indicate that there may be a possible hormonal influence on ALP activity. Moreover, patients with metastatic breast cancer have been shown to have a significantly higher ALP level than patients with non-metastatic breast cancer. These data are consistent with previous studies that suggested that measurement of ALP is an accurate and reliable marker[16].

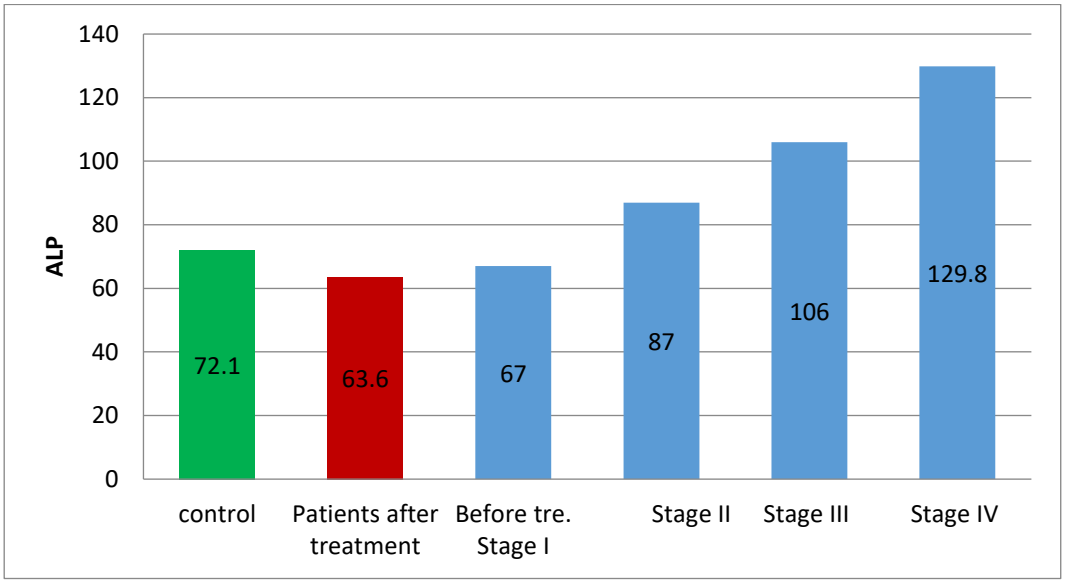

Figure 5: The; mean and SD of ALP in control and patients

The activity of enzymes (urea, creatinine) was studied on 120 patients with breast cancer (20) before treatment, (100) after treatment and 30 as healthy controls, and it is shown in the Table (4)

Table 4: The level of creatinine and urea in patients after treatment and patients before treatment and control

Patients groups	creatinine (mg/dl)	P-Value	Urea (mg/dl)	P-Value
Control	0.686±0.135		32.133±5.270	
Patients after treatment	0.693±0.132	≤ 0.01	39.893±9.626	>0.05
Before treatment. Stage I	0.820±0.083	>0.05	41.40±2.408	0.05
Before treatment Stage II	0.980±0.130	≤ 0.01	49.20±4.764	≤ 0.01
Before treatment Stage III	1.600±0.291	≤ 0.01	62.40±7.127	≤ 0.01
Before treatment Stage IV	2.100±0.212	≤ 0.01	82.80±8.167	≤ 0.01

The urea results indicate that there was non significantly (P >0.05) in breast cancer patients, serum after treatment (39.89±9.62mg/dl) compared to that in healthy controls (32.13±5.27mg/dl)

It was shown that the levels of urea in the blood serum of stage (II ,III ,IV) breast cancer patients were much higher than the levels of control (49.20±4.76, 62.4±7.127,82.8±8.16 respectively) increased highly significantly (P≤ 0.01).as shown in Figure(6).

The results creatinine indicate that there is high significantly (P ≤0.01) in breast cancer patients, serum after treatment (0.693±0.132mg/dl) compared to that in healthy controls (0.686±0.135mg/dl).

It was shown that the levels of creatinine in the blood serum of stage (II ,III ,IV) breast cancer patients were higher than the levels of control (0.980±0.130, 1.600±0.291,2.100±0.212 respectively) increased highly significantly (P≤ 0.01).as shown in Figure(7).

Analysis of a group of patients showed a significant rise in blood creatinine and a decrease in creatinine clearance. Patients with impaired renal function had a rise in creatinine [17].

The results of this study were consistent with the results of (Jessica et al., 2017), which indicated that the level of urea and creatinine was higher in patients with breast cancer compared to patients with breast cancer the control group [15].

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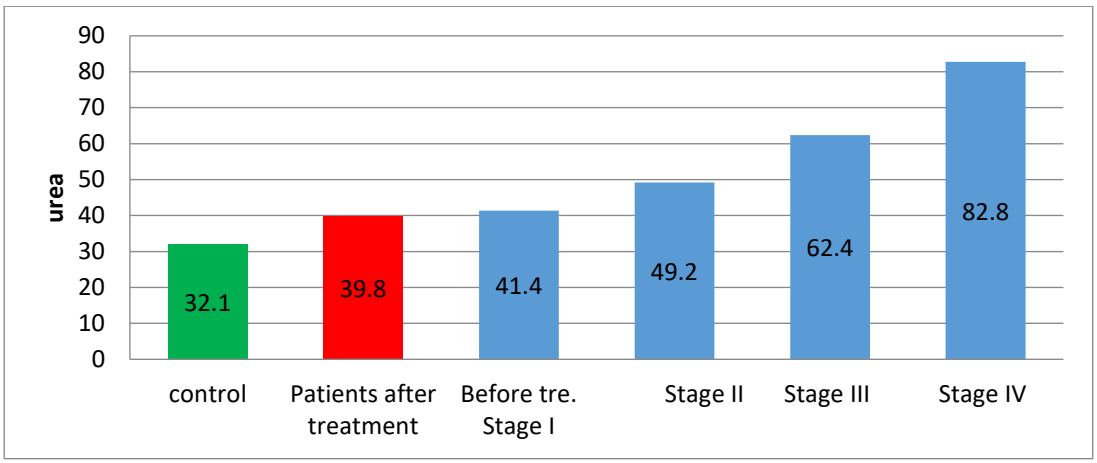

Figure 6: The mean and SD of urea in control and patients

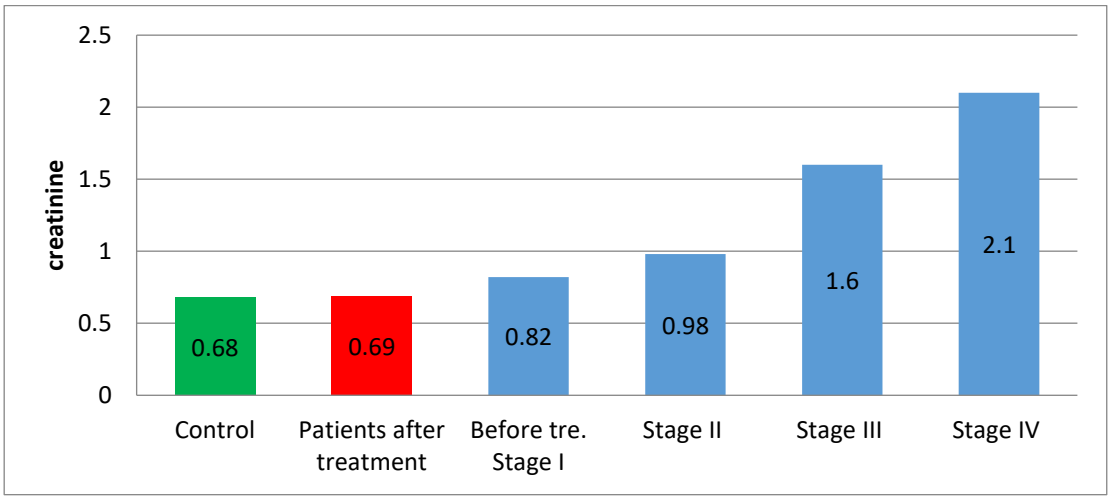

Figure 7: The mean and SD of creatinine in control and patients

The activity of zinc was studied on 120 patients with breast cancer (20) before treatment, (100) after treatment and 30 as healthy controls, and it is shown in the Table (5)

Table 5: The level of zinc in patients after treatment and patients before treatment and control

Patients groups	zinc (mg/dl)	P-Value
Control	110.53±11.17	
Patients after treatment	111.04±10.56	≤0.01
Before treatment. Stage I	75.46±2.03	0.04
Before treatment Stage II	67.72±1.88	≤0.01
Before treatment Stage III	58.70±6.50	≤0.01
Before treatment Stage IV	52.48±1.90	≤ 0.01

The results indicate that there is high significantly (P ≤0.01) in breast cancer patients serum after treatment (111.04±10.56mg/dl) compared to that in healthy controls (110.53±11.17mg/dl). It was shown that the levels of zinc in the blood serum of stage (II ,III ,IV) breast cancer patients were lower than the levels of control (67.72±1.88 ,58.70±6.50 ,52.48±1.90 respectively) increased highly significantly (P≤ 0.01).as shown in Figure(8)

Comparative studies of cancerous and non-cancerous breast tissue from the same patient showed that the latter contained elevated levels of zinc[18] Zinc concentration levels decreased in blood samples of breast cancer patients, while it was elevated in tumor tissues[19]. Demonstrated that zinc is involved in the invasive behavior of breast cancer cells. Zinc depletion within invasive breast cancer cells caused similar defects in the migratory activity of these cells. Breast cancer tissues have a high absorption of zinc. Data indicates that more advanced breast cancers can import more zinc into the cytosol than serum. This is consistent with previous observations of a high level of zinc in advanced breast cancer tissues and a similarly low level in serum. It is possible that highly metastatic and advanced breast cancers take up more zinc, and thus acquire more migratory activities[20].

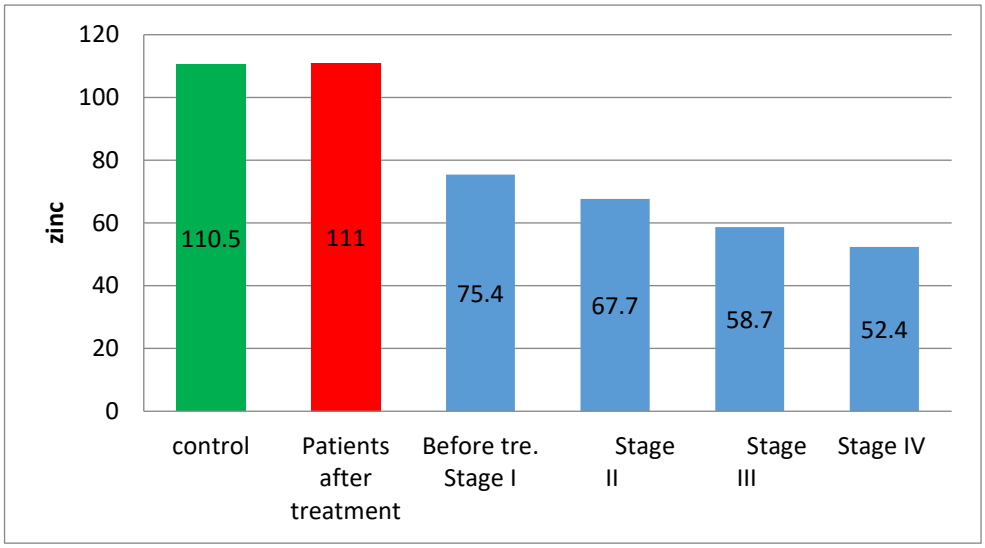

Figure 8: The mean and SD of zinc in control and patients

Conclusions

This study was conducted for breast cancer patients before taking treatment and after taking (1-12) dose of chemotherapy according to the patients' medical history. These measurements were conducted before and after taking the treatment once only.

This study is consistent with several studies that found that blood amylase levels in pre-treatment stage (I, II, III, IV) breast cancer patients were higher than control levels. High levels of AST, ALT, ALP, CA15.3, blood urea and creatinine before treatment and low levels after treatment, low zinc concentration before treatment and high zinc levels after treatment are associated with breast cancer.

Acknowledgment

I would, like to give my thanks to all the patients who were admitted to the hospital, as well as the authors.

Thanks and appreciation to the Department of Chemistry, College of Science, Diyala University

The authors thank Al-Amal Hospital in Baghdad, Iraq, for their support in carrying out this work.

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