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Prolidase Specificity as a diagnostic marker for breast cancer

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ABSTRACT

The study was carried out to evaluate the activity of prolidase-PD (as a diagnostic marker) and some antioxidant parameters in sera of patients with breast cancer. Ninety serum samples were collected from women(60 samples from women with breast cancer-BC and 30 samples from healthy women as a control group) with ages ranging between 30-71 years. The samples were divided into three groups: Control group C, First group G1 for the newly diagnosed cases with BC, and Second group-G2 for breast cancer after undergoing chemotherapy.

The study includes the determination activity of serum enzymes (PD and catalase-CAT) and the concentration of glutathione-GSH, ceruloplasmin-Crp, and Malondialdehyde-MDA. The results of the present study indicate that the activity of PD, CAT, Crp, and also GSH and MDA significantly elevated ($P \leq 0.05$) in the sera of the patients' group(G1 and G2) as compared with the control group.

Otherwise, the sensitivity rate for PD,MDA and Crp were 88.89%, 100% and 89.86% respectively in the group newly diagnosed with BC. While in the group after treatment the higher sensitivity rate were shown to MDA with 100% as an indicator for the treatment response and outstanding value for MDA (AUC=1.0) in the patients' groups Vs control. From all the results we can conclude that the high level of prolidase activity was associated with an increased risk of breast cancer, especially with high oxidative stress.

Keywords: *Prolidase, Glutathione, Catalase, ceruloplasmin, Malondialdehyde*

INTRODUCTION

Breast cancer-BC is one of the most common malignant tumor-between women (1). Every year approximately 2.3 million new cases of breast cancer are diagnosed worldwide(2), and the first cause of death as compared with other types of malignant tumor cancer(3). The causes of breast cancer have not yet been confirmed but many researchers establish several risk factors which may be conducive to the development of the disease which include: gender, old age, family history, hormonal effect, the density of breast tissue, pregnancy, breastfeeding, genetic mutation, type of nutrition and other lifestyle-related(2,4-9). BC are invasive lobular carcinoma-ILC and invasive ductal carcinoma-IBC, In which ILC is the 2nd most common subtype of BC after IBC representing 10-15% among BC cases(10). The early detection of breast cancer raised the survival average of females(11), so many researchers were interested to report several macromolecules as diagnostic tools for BC such as circular RNA, fascin-1, Metastasis-associated in colon cancer-1, glutathione-GSH, malondialdehyde-MDA...etc. The identification of these biochemical parameters may be helpful in the early detection of the disease(12- 15).

The main constituent of the extracellular matrix is collagen, which is important for cellular interaction and extracellular matrix proteins, and also plays an important role in the differentiation, regulation of cellular gene expression, growth, and also in tumorigenicity and invasiveness(16,17). So, any changes in the metabolism of collagen may potentially affect the motility and metabolism of the cells. Under in vivo states, cancer is recognized by the crash of tissue organization and invasiveness. So, any changes in the metabolism of collagen may potentially affect the motility and metabolism of the cells. Under in vivo states, cancer is recognized by the crash of tissue organization and invasiveness. Actually, the progression of the tumor is enhanced by the breakdown of extracellular matrix proteins. The critical event in the metastasis and progression of cancer is the excretion of matrix metalloproteinases, which are answerable for the degradation of the extracellular matrix(18). The expression by tumor cells to some collagenases may subscribe to the metastatic process by altering extracellular matrix cell interactions as well as breaking down extracellular matrix barriers(19). The metallo-proteinases of the matrix are contributed remodeling of the extracellular matrix and the breakthrough of tumors and normal cells via tissue barriers(20,21). In spite of extracellular collagenases beginning collagen breakdown, the final step for collagen degradation is interposed by prolidase(18).

Prolidase is a cytosolic metalloproteinase that hydrolyzes the C-terminal of hydroxyproline and proline in imidodipeptides, also necessary in the metabolism of protein, matrix remodeling, collagen turnover, and collagen recycling(22).

The current study aimed to evaluate the activity of prolidase and oxidative stress state in sera of women with breast cancer .

SUBJECTS AND METHODS

Study design: Ninety serum samples were collected in the present study , 30 samplesfrom women with BC and 30 samplesfrom healthy womenas a control group, with ages ranging between 30-71 years. The samples were collected from Saladin oncology center and Medical oncology specialist,Oncology-Teaching Hospital Baghdad Medical Cityfrom the period between 1/11/2021 to 1/3/2022. The collected samples were divided into three groups:

Control group-C: which includes 30 serum samples for healthy women.

First group-G1: which includes 30 serum samples for the newly diagnosed cases with BC.

Second group-G2: which includes 30 serum samples for BC after undergoing chemotherapy.

Methods: The study includes the determination of the activity of prolidase and catalase according to the methods of(23,24) respectively, and also the determination of the concentration of GSH, MDA and ceruloplasmin-Crp according to the methods of(25-27) respectively, while the determination of Carcinoma Antigen 15-3 (CA 15-3) was done according to the standard method provided with cobas e 601 kits (28)

Statistical analysis: The statistical analysis of the obtaining data was analyzed by using Duncan's Multiple Range test by the SPSS program at probability($p \leq 0.05$), and also calculating

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Receiver operating characteristic- ROC for the parameters under investigation for the patients and control groups.

RESULTS AND DISCUSSION

The results of the present study were summarized in table(1).

TABLE 1: Mean±SD of prolidase,catalase activity and CA15-13, GSH and MDA levels in sera of groups under investigation

Parameters	Control group	First group	Second group
PD (IU/L)	1.717±0.787 c	7.476±2.308 a	3.383±1.303b
CAT(K/ml)	0.286±0.152c	0.716±0.196b	1.257±0.269a
Crp(g/L)	0.162±0.009c	0.896±0.123a	0.445±0.065b
GSH(mol/L)	21.080±6.103b	42.763±11.998a	43.643±11.512a
MDA(mol/L)	4.755±1.347c	21.022±6.191b	55.490±15.717a

The results indicate that the activity of prolidase, catalase,ceruloplasmin, and also glutathione and malondialdehyde significantly elevated(P≤0.05) in sera of patients group(G1 and G2) as compared with the control group, Fig1,2,3,4,5.

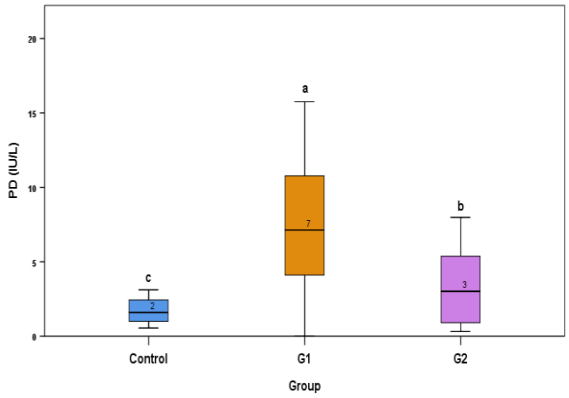

FIG. 1: Prolidase activity in sera of patients and control groups

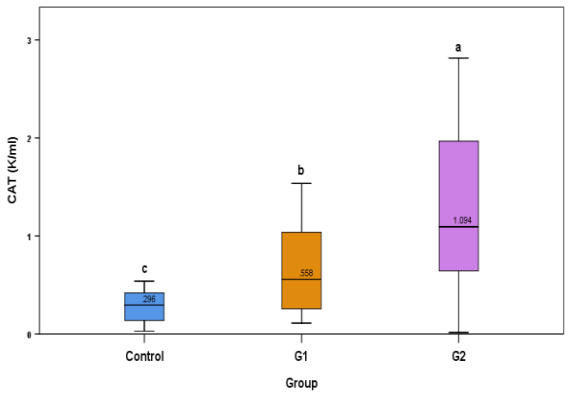

FIG. 2: Catalase activity in sera of patients and control groups

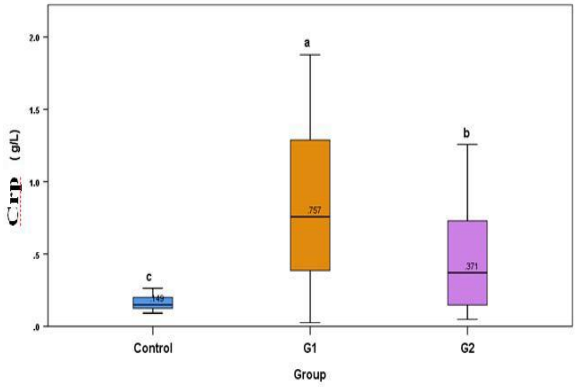

FIG. 3: Crp concentration in sera of patients and control groups

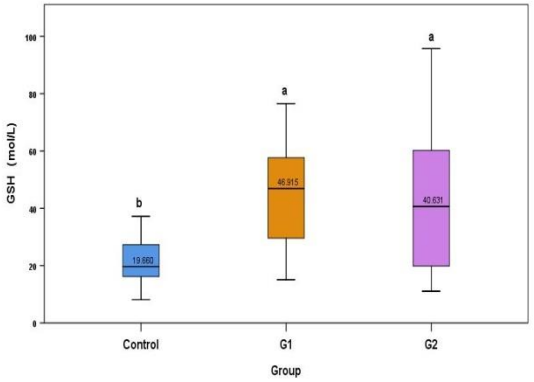

FIG. 4: GSH concentration in sera of patients and control groups

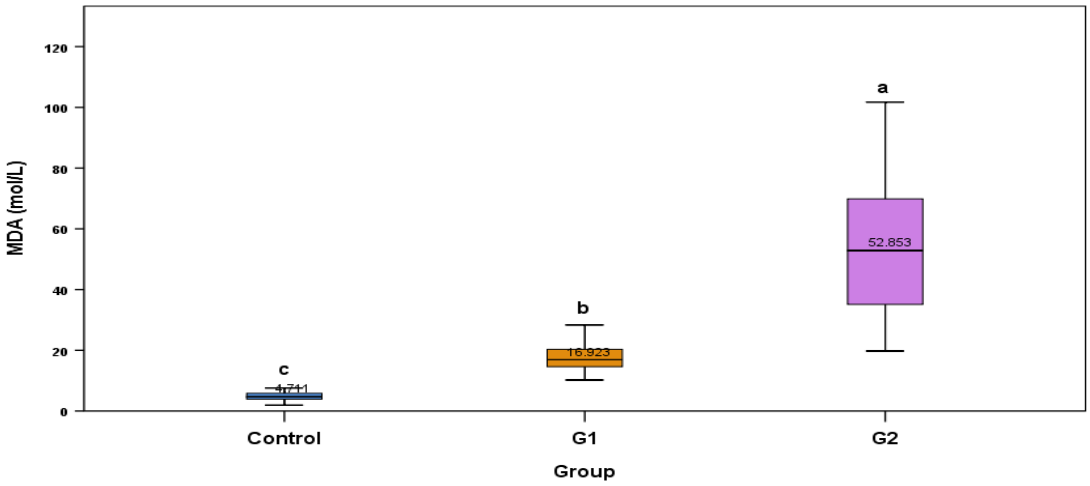

FIG. 5: GSH concentration in sera of patients and control groups

Prolidase is an enzyme that plays a role in the metabolism of proteins. It is found in the liver, kidneys, and other organs and tissues. Recent research has suggested that prolidase may be involved in the development of breast cancer(29-30). A study published in the journal Cancer Research found that high levels of prolidase activity were associated with an increased risk of breast cancer in postmenopausal women. The researchers also found that women with higher levels of prolidase activity had a greater risk of developing more aggressive forms of breast cancer(29).

The results of the present study for prolidase agreed with the finding of Abusoglu, et al(31), indicating that the activity of PD significantly increased in patients with breast cancer as compared with the control group, and then decreased gradually progressed after the treatment stages. Prolidase activity has been associated with many types of cancer, in which the activity significantly increased in the sera of patients with prostate cancer(32) and also in epithelial ovarian cancer(33).

Antioxidants are compounds that can help protect cells from damage caused by free radicals. Free radicals are molecules that have an unpaired electron, which makes them highly reactive and can cause damage to cells. Antioxidants can neutralize free radicals and help prevent cell damage(34).Studies have shown that antioxidants may play a role in cancer prevention and treatment. Antioxidants can reduce oxidative stress, which is an imbalance between the production of free radicals and the body's ability to neutralize them. Oxidative stress has been linked to the development of cancer, so reducing it may help reduce the risk of cancer. In addition, antioxidants may help reduce inflammation, which is another factor that has been linked to cancer development(35-36).

Kadam, and Abhang(37) indicate that the level of glutathione significantly decreased in sera of patients with the second stage of BC after surgery and before chemotherapy, and greater decreased after undergoing treatment. Glutathione in its reduced form plays an important role in preserving and protecting the cell from the effect of free radicals, especially reactive oxygen species. It also has an important role in renewing other antioxidants and protecting the cell from the risk of oxidative stress(38).

The results of the current study indicate that the activity of CAT significantly elevated in the patients group (G1 and G2). In recent years, researchers have begun to explore the potential role of catalase in cancer prevention and treatment. In which that catalase can help protect cells from damage caused by oxidative stress, which is believed to be a major factor in the development of cancer. This protection may be due to its ability to reduce the amount of hydrogen peroxide present in cells, which can lead to DNA damage and cell death. Additionally, catalase has been found to reduce inflammation, which is also thought to play a role in cancer development(39), the increase in GSH level in the present study may be due to the life style of patients with BC.

Abdul-Barry et al,(40) found that Crp significantly increased in malignant tumors and also in breast cancer(41), and this finding agrees with the finding of the present study. The elevation in Crp in non-metastatic BC was lower than in metastatic cancer due to the extra-hepatic production of Crp to reduce oxidative stress(42).

Malondialdehyde is an indicator of oxidative stress and it's an important factor in an increased risk of breast cancer. Studies have shown that exposure to MDA increases the risk of developing breast cancer, in which the free radicals cause the oxidation of many biochemical molecules, especially carbohydrates, proteins, fats and damage DNA and interfere with the normal functioning of cells. It can also cause changes in hormones, which can lead to an increased risk of breast cancer(43,44).

Otherwise, the study includes the calculation of the sensitivity and specificity of parameters under investigation for the three groups by using the ROC curve, the results obtained were summarized in table 2.

TABLE 2: The AUC, Cutoff, sensitivity and Specificity of group 1 and control group

Control vs New diagnoses (G1)					
Parameter	AUC	Cutoff	Sensitivity %	Specificity %	p value
PD	0.8494	>1.874	88.89%	55.17%	<0.0001
GSH	0.5357	>3.79	67.86%	46.67%	0.6406
CAT	0.7062	>0.231	70.37%	53.33%	0.0076
Crp	0.9187	>0.283	89.86%	96.43%	<0.0001
MDA	1.0	>7.148	100%	96.43%	<0.0001

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The results obtained from table 2 showed that the high sensitivity rate for PD, MDA and Crp was 88.89%, 100% and 89.86% respectively in groups newly diagnosed with BC. While in the group after treatment the higher sensitivity rate was shown to MDA with 100% as an indicator for the treatment response. While the Area Under Curve-AUC for the parameters under investigation showed outstanding value for MDA (AUC=1.0); Crp (AUC=0.9187), and excellent value for PD(AUC=0.8494) in the G1 Vs control group, while in G2 Vs control the AUC value was outstanding value for MDA (AUC=1.0) and excellent value for CAT(AUC=0.8419), table 3.

TABLE 3: The AUC, Cutoff, sensitivity and Specificity of group 2 and control group

Control vs Treated group (G2)					
Parameter	AUC	Cutoff	Sensitivity %	Specificity %	p value
PD	0.6237	>1.959	68.97%	62.07%	0.1058
GSH	0.6518	>4.19	78.57%	40%	0.0473
CAT	0.8419	>0.434	80.65%	83.33%	<0.0001
Crp	0.5498	>0.146	61.26%	53.57%	0.5139
MDA	1	>7.184	100%	96.43%	<0.0001

CONCLUSION

From the results of the present study, we can conclude that the high level of prolidase activity was associated with an increased risk of breast cancer, especially with high oxidative stress.

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