Thiol-disulphide Homeostasıs and Ischemia-modified Albumın Level and its Relatıonshıp with Clınicopathological Features of Breast Cancer

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ABSTRACT

Objective: To investigate the status of thiol-disulphide homeostasis and ischemic-modified albumin and their association with clinicopathological parameters in breast cancer.

Study Design: A cross-sectional descriptive study.

Place and Duration of Study: Department of Internal Medicine and Department of Medical Oncology, Dicle University, Turkey, from April to September 2021.

Methodology: Forty treatment-*naive* female patients diagnosed with breast cancer who presented to the Oncology Clinic of the hospital and 33 healthy women with no comorbidities were included. Serum levels of native thiol (NT), disulphide (Ds), total thiol (TT), IMA (ischemic modified albumin) at diagnosis and disulphide/native thiol (Ds/NT), disulphide/total thiol (Ds/TT), and the ratios of native thiol/total thiol (NT/TT) were analysed by the colorimetric method.

Results: Median age at diagnosis was 44 (29-70) years. The majority of patients had stage II-III disease (77.5%). Mean serum levels of TT were significantly lower in breast cancer patients (462.45 ± 100.2 µmol/L) compared to healthy controls (507.28 ± 75.72 µmol/L) (p=0.038). Mean serum levels of Ds were significantly lower in breast cancer patients (20.25 ± 5.94 µmol/L) compared to healthy controls (22.99 ± 3.56 µmol/L) (p=0.018). Meanwhile, mean IMA levels were significantly higher in breast cancer patients (0.81 ± 0.05 µmol/L) compared to healthy controls (0.73 ± 0.19 µmol/L) (p=0.016). NT and TT levels showed a moderate correlation with the percentage of fat mass and body mass index (BMI), and a weak correlation with age (p<0.05). Univariate and multivariate analyses examining the association between thiol-disulphide levels and patient clinical characteristics demonstrated that NT and TT levels had a statistically significant relationship with body mass index and menopausal status (p<0.05), with lower levels of NT and TT in postmenopausal patients and patients with high BMI.

Conclusion: Decreased TT and Ds levels and increased IMA levels were determined in patients diagnosed with breast cancer compared to the healthy control group. Thiol-disulphide levels were observed to be associated with clinical characteristics such as menopausal status and BMI.

Key Words: *Breast cancer, Thiol, Ischemic modified albumin.*

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INTRODUCTION

Breast cancer is one of the most common types of cancer worldwide.[1] The aetiology of breast cancer involves various factors such as environmental, hormonal, and hereditary factors. Several mechanisms are responsible for the pathogenesis of breast cancer. One of these is oxidative stress.

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Increased production of free oxidative radicals or impairment in the antioxidant mechanism results in oxidative stress. Free oxygen radicals (ROS) formed due to oxidative stress are involved in the occurrence of cancer by affecting lipids, proteins, and cellular components such as DNA.

Oxidative stress plays an important role in promoting the proliferation and metastases of cancer, which can be represented by ischemia-modified albumin (IMA) and thiols.[2] IMA has been presented as a new oxidative stress marker due to mainly on ischemic conditions.[3] Thiols are important antioxidant agents in organisms and contain a sulfhydryl (-SH) group, which serves as a substrate for antioxidant enzymes.[3,4] More than 90% of glutathione is found within the cell in a reduced state and it is the most abundant thiol compound in the body. The thiol that is

found most abundantly in the plasma is albumin, of which 75% is in a reduced state.[5,6] It was suggested that the disruption of thiol-disulphide (T-Ds) homeostasis, which functions as a component of the antioxidant system, and reduced levels of the oxidoreductase enzyme influence bacterial virulence, facilitating the occurrence of infections. T-Ds homeostasis was shown to be impaired in several diseases such as ankylosing spondylitis, inflammatory bowel diseases, renal failure, and malignancies, the courses of which involve chronic inflammation.[7-10] Experimental studies showed lower total thiol levels in breast cancer patients than the control group.[11,12]

A growing amount of literature suggests that an abnormal TD balance may be a factor underlying the pathogenesis of diverse malignant states including endometrial, cervical, colon, lung and breast cancers.[13-16] Also, the severity of tissue inflammation and oxidative stress is directly associated with the prognosis of cancer.[17] However, there is a limited number of studies investigating the relationship between T-Ds levels and clinicopathological characteristics in cancer. In this study, the objective was to investigate the status of T-Ds homeostasis in breast cancer and ischemic-modified albumin and their association with clinicopathological parameters.

METHODOLOGY

This study included 40 newly diagnosed female patients with breast cancer who presented to the Department of Medical Oncology, Dicle University, between April and September 2021 and 33 healthy female participants with no comorbidities. Clinical data and demographic characteristics (age, height, weight, habits, comorbidity, performance status, family history, and tumour stage) of the patients at diagnosis and prior to the treatment were recorded prospectively. Five mL (milliliters) of venous blood sample was collected into a lab tube after an 8-hour fast at the initial evaluation from the healthy individuals and prior to chemotherapy from the patients in order to analyse T-Ds levels and IMA. Blood samples were centrifuged at 1500 rpm for 10 minutes to separate the serum and the plasma from the blood. Serum samples were transferred into Eppendorf tubes and stored at -80°C. Tanita measurements (bioelectrical impedance analysis, by Bodystat quad scan 400 multi-frequency equipment) were performed to determine the fat mass percentages of the patient group, while only thiol-disulphide (T-Ds) was analysed in healthy individuals.

This study included female patients older than 18 years who were newly diagnosed with breast cancer of any stage and had histopathologically confirmed diagnoses, newly diagnosed patients who had not been operated for breast cancer at the time of the collection of blood for T-Ds analysis and who had not received any treatment for breast cancer, and patients who did not have serious comorbid diseases and had good performance status (ECOG PS ≤2). The healthy participants were adult women older than 18 years with no acute or chronic diseases (such as active infections or chronic infections, medication use, hypertension, chronic renal failure, liver diseases, and diabetes) or history of cancer. The control group was kept similar to the patient group in terms of age, gender, history of smoking, and use of other substances, as well as their demographic data. The patients with any systemic and/or infectious disease, endocrinopathies, other malignancies, inflammatory conditions and/or medication use were excluded.

Serum levels of native thiol (NT), total thiol (TT) and disulphide (Ds) (μmol/L) were analysed using the method developed by Erel and Neşelioglu. In this method, firstly, the dynamic disulphide bonds are reduced by sodium borohydrate to extract free functional thiol groups. Unused sodium borohydrate is removed from the environment using formaldehyde to prevent the additional reduction of DTNB (5,5'-dithiobis-2-nitrobenzoic acid). The TT levels were calculated by the spectrophotometric measurement of the chromogen compound it creates with the modified Elmann's reagent at a wavelength of 415 nm. NT levels are measured using a modified Elmann's reagent. Half of the difference between TT and NT levels equals the amount of disulphide bonds. Using these measurements, ratios of Ds/NT, Ds/TT, and NT/TT (%) are computed.[4] NT, TT, Ds, Ds/NT, Ds/TT, NT/TT, and ischemic modified albumin (IMA) levels were analysed for both groups.

Data analysis was conducted using the SPSS 26 (Statistical Package for the Social Sciences) statistics software. Whether the data from the groups conformed to normal distribution was checked using the Kolmogorov Smirnov. Mean and standard deviation values were specified for normally distributed parameters. Meanwhile, median, minimum and maximum values were specified for non-normally distributed parameters. In univariate analysis; independent t-test, ANOVA, and linear regression were performed. In multivariate analysis; multiple regression was performed using the Backward method. $p<0.05$ was considered significant for all tests. Spearman's rho was preferred in the analysis of correlations between the variables.

This study was approved by the Dicle University Faculty of Medicine Non-Invasional Clinical Research Ethics Committee (approval number: 249/2021). Signed consent forms were obtained from the patient and control groups included in the study.

RESULTS

This study included 40 females from the patient group with breast cancer and 33 females from the healthy control group. The median patient age at diagnosis was 44 (29-70) years. All patients had invasive ductal carcinoma histology. Of the patients, 77.5% (n=31) had stage II-III disease Subtyping classified 6 patients (15%) as luminal A, 14 (35%) as luminal B, 16 (40%) as HER2 positive, and 4 (10%) as triple negative. Thirty (75%) patients had no concomitant diseases; 6 (15%) patients had hypertension, 2 (5%) patients had asthma, and 2 (5%) patients had hypothyroidism. These patients had normal thyroid levels with hormone replacement therapy. Of these patients, 27 (67.5%) were premenopausal while 13 (32.5%) were postmenopausal. The clinicopathological characteristics of the patients are presented in Table I.

Table I: General characteristics of the patients.

Parameters	n (%)	Parameters	n (%)
Age (median, range)	44 (29-70)	Tumor localisation	
Family history		Right	13 (32.5)
Absent	27 (67.5)	Left	25 (62.5)
Present	13 (32.5)	Bilateral	2 (5)
Comorbidity		Clinical stage	
Absent	30 (75)	1	2 (5)
Present	10 (25)	2	20 (50)
Menopausal status		3	11(27.5)
Premenopause	27 (67.5)	4	7 (17.5)
Postmenopause	13 (32.5)	Subtype	
ECOG performance score		Luminal A	6 (5)
0	30 (75)	Luminal B	14 (35)
≥1	10 (25)	HER2 positive	16 (40)
Smoking history		Triple negative	4 (10)
Absent	25 (62.5)		
Present	15 (37.5)		

	Control Group		Patient Group		
	Mean	St. Dev	Mean	St. Dev	P
Age	45.37	10.02	40.27	11.8	0.05
Native Thiol (µmol/L)	421.94	96.87	461.3	74.87	0.06
Total Thiol (µmol/L)	462.45	100.2	507.28	75.72	0.038
Disulphide (µmol/L)	20.25	5.94	22.99	3.56	0.018
Disulphide/Native Thiol Ratio (%)	5.00	1.78	5.18	1.69	0.671
Disulphide/Total Thiol Ratio (%)	4.50	1.46	4.65	1.26	0.638
Native Thiol/Total Thiol Ratio (%)	90.99	2.92	90.68	2.52	0.638
IMA (µmol/L)	0.81	0.05	0.73	0.19	0.016

HER2; Human epidermal growth factor receptor, ECOG; Eastern cooperative oncology group, IMA; Ischemic modified albumin, st. Dev; Standard deviation.

The patient and control groups were compared with respect to age, NT, TT, Ds, Ds/NT, Ds/TT, NT/TT ratios, and IMA levels. While the mean TT (p=0.038) and Ds levels (p=0.018) were higher in the control group with statistical significance, the mean IMA level was significantly higher in the patient group when compared to the control group (p=0.016). There were no significant differences between breast cancer patients and healthy controls in terms of age, other thiol parameters (Table I).

The correlations between laboratory parameters and the clinicopathological factors in breast cancer patients were investigated. Spearman correlation analysis determined a moderate negative correlation between NT and the percentage of fat mass (p=0.013, r=-0.389), a moderate negative correlation between NT and BMI (p=0.009, r= -0.410), and a weak negative correlation between age and NT (p=0.028, r= -0.257). TT was determined to have a moderate negative correlation with the percentage of fat mass (p=0.012, r= -0.392), a moderate negative correlation with BMI (p=0.008, r= -0.412), and a weak negative correlation with age (p=0.032, r= -0.251). No significant relationships were determined between Ds levels and the percentage of fat mass (p=0.388), BMI (p=0.403), or age (p=0.863). A moderate positive correlation was determined between IMA levels and the percentage of fat mass (p=0.049, r=0.313). Meanwhile, no significant relationship was determined between IMA levels and age (p=0.662) or BMI (p=0.099).

Multivariate and univariate analyses were performed in order to evaluate the relationship between the T-Ds and IMA levels of the patient group and their clinicopathological characteristics [menopausal status, smoking, stage, subtype, comorbidity, percentage of fat mass, BMI (kg/m$^{2)}$ and age]. TT had a significant relationship with menopause and BMI in multivariate analysis. TT levels were higher in premenopausal patients with statistical significance (487.58 *vs.* 410.24 µmol/L) (p=0.032). TT levels decreased as BMI increased, with statistical significance (unstandardized beta= -0.6452, p=0.013). NT showed a significant relationship with menopause and BMI in multivariate analysis. NT levels were higher in premenopausal patients with statistical significance (446.88 *vs.* 370.13 µmol/L) (p=0.026). NT levels decreased as BMI increased, with statistical significance (unstandardized beta= -6.173, p=0.014). Ds and IMA levels did not demonstrate significant relationships with any of the researched parameters (p>0.05). Also, in multivariate analysis, we could not find any relationship between other clinicopathological factors (age, stage, smoking, comorbidity, subtype, fat mass) and thiols / IMA (p>0.05). Results of univariate and multivariate analysis are presented in Table II.

DISCUSSION

In this study, the objective was to evaluate the status of T-Ds homeostasis in breast cancer and its association with other clinical parameters. There are few studies in the literature that have been conducted for this purpose.[4,18] The main results of the study showed low levels of TT and Ds compared to the control group. The secondary results of the study showed an increase in IMA in breast cancer patients compared to the control group.

Table II: Univariate and multivariate analysis results of investigated parameters.

Univariate analysis	N	Mean	Total thiol (µmol/L) St. Dev	p	Mean	Native thiol (µmol/L) St. Dev	p	Mean	Disulphide (µmol/L) St. Dev	p	Mean	IMA (µmol/L) St. Dev	p
Menopausal status				0.02*			0.017*			0.885*			0.329*
Premenopause	13	487.58	92.85		446.88	89.21		20.35	6.06		0.8	0.06	
Postmenopause	27	410.24	98.15		370.13	94.58		20.05	5.91		0.82	0.04	
Smoking				0.239*			0.235*			0.796*			0.477*
Absent	15	447.84	103		407.71	98.26		20.06	6.35		0.82	0.05	
Present	25	486.80	93.81		445.66	92.88		20.57	5.39		0.8	0.05	
Stage				0.213**			0.131**			0.361**			0.099**
1	2	449.85	5.444		399.55	12.65		25.15	3.6		0.81	0.01	
2	20	477.37	107.4		437.14	22.96		20.11	6.27		0.8	0.05	
3	11	483.20	68.39		446.5	19.41		18.35	5.58		0.8	0.03	
4	7	390.81	115.9		346.32	41.28		22.24	5.63		0.86	0.08	
Subtype				0.164**			0.238**			0.083**			0.672**
Luminal A	6	503.4	131.1		461.8	126.3		20.87	3.28		0.79	0.04	
Luminal B	14	488.6	95.2		444.5	95		22.02	5.16		0.81	0.05	
HER2	16	444.4	91.8		404.1	88.3		20.16	6.5		0.81	0.06	
Triple Negative	4	381.2	59.8		354.3	62.3		13.47	6.1		0.84	0.01	
Comorbidity				0.675**			0.597**			0.567**			0.665**
Absent	30	467.43	96.67		427.58	91.82		19.92	6.57		0.81	0.06	
Hypertension	6	418.45	133.8		375.41	131.1		21.51	2.82		0.83	0.04	
Asthma	2	479.95	124.6		430.55	130.6		24.7	2.96		0.84	0.07	
Hypothyroidism	2	502.2	May.23		468.35	6.29		16.92	0.53		0.79	0.03	
Fat mass (%)				0.012***			0.013***			0.39***			0.049***
BMI (kg/m^2)				0.008***			0.009***			0.40***			0.099***
Age				0.032***			0.028***			0.86***			0.66***
Multivariate analysis		**uns. Beta**	**P**	**A. R square**	**uns. Beta**	**P**	**A. R square**	**uns. Beta**	**P**	**A. R square**	**uns. Beta**	**P**	**A. R square**
Menopausal status		-66.968	0.032***	0.229	-66.825	0.026***	0.234						0.121
Smoking													
Stage													
Subtype								-1.986	0.067***	0.061	0.018	0.092***	
Comorbidity													
Fat mass (%)											0.002	0.061***	
BMI (kg/m^2)		-6.452	0.013***		-6.173	0.014***							
Age													

**T-test, **One way ANOVA test, ***Regression analysis, BMI; Body mass index, HER2; Human epidermal growth factor receptor, st. Dev; Standard deviation, IMA; Ischemic modified albumin, A; Adjusted. uns; Unstandardised.*

Reviewing previous studies on T-Ds in breast cancer; TT and NT levels of the patient and control groups were evaluated in a study by Eryilmaz *et al.* that included 37 participants diagnosed with breast cancer and 31 healthy participants, and no statistically significant difference was found between the two groups despite the detection of numerically lower thiol levels in the patient group.[16] In a similar study conducted by Ozdemir *et al.* that included 39 patients diagnosed with breast cancer and 41 healthy participants, no statistically significant difference was determined between the groups although lower levels of thiol were found in the patient group. However, the study did not determine any difference between the patient and control groups in terms of Ds levels.[19] Meanwhile, in a study by Kedzierska *et al.* that included 47 patients diagnosed with breast cancer and 55 healthy participants, the analysis of thiol compounds such as glutathione, cysteine, cysteinyl, glycine, and homocysteine revealed significantly lower levels of these compounds in the patient group compared to the control group.[12] In the comparison of the patient and control groups with respect to the ratios of NT/TT, Ds/NT and Ds/TT, the mean values of these ratios were found to be comparable between the two groups with no significant differences determined (p>0.05). Similar to the study, these ratios were also similar between the two groups in the study by Ozdemir *et al.*, and no significant differences were found.[19]

Several studies have evaluated thiol-disulfide balance in many cancer including breast, prostate, lung, skin and colon cancers. The results of these studies showed that the thiol-disulfide parameters (TT and NT) decreased or were at subnormal levels. In contrast, an increment in disulfide levels was detected in many cancer patients.[13,16,20] When T-Ds homeostasis is imbalanced, the paired system shifts to disulfide formation. But the authors found that serum disulfide levels tended to decrease. Decreased disulfide level might result from the rebound phenomenon to continue protective influence by neutralising oxidative stress.

IMA is the oxidative modified form of albumin and is a novel marker of ischemia. This marker of ischemia also increases under conditions of oxidative stress. It was studied as a sensitive biomarker in conditions such as DM, myocardial infarction, and peripheral vascular disease.[21] In the same study by Ozdemir *et al.*, IMA levels were found to be higher in the control group compared to the patient group, with a statistically significant difference between the two groups.[19] On the other hand, in this study, the mean of the patient group was found to be higher than that of the control group and a statistically significant difference was determined between the two groups (p=0.016). IMA has a structure that is sensitive to low temperatures. IMA levels may demonstrate an increase at low temperatures. Since blood samples collected from the patient and control groups in this study

were stored at -80 °C, it is possible that IMA levels were affected.

NT and TT levels were significantly lower in postmenopausal patients and patients with high BMI. The study by Eryilmaz *et al.* could not find any relationship between clinicopathological factors and T-Ds levels in breast cancer patients.[16] In a study conducted by Elmas *et al.* on obese children, a positive correlation was found between BMI and oxidant parameters such as the ratios of Ds/NT and Ds/TT. Meanwhile, a negative correlation was reported between BMI and antioxidant parameters such as NT, TT, and NT/TT ratio, which is consistent with this study.[22] Obesity is known to result in oxidative stress due to inflammation of adipose tissue. Compounds that involve a thiol group play an important role in defence against oxidative stress. Oxidative stress that appears in parallel to an increase in BMI and the percentage of fat mass may signal a breakdown of T-Ds homeostasis.

The lack of estrogens during the postmenopausal period contributes to decreasing defence against oxidative stress.[23] In a study by Kolesnikova *et al.* that compared the antioxidant capacities of 37 patients in the premenopausal period and 41 patients in the postmenopausal period, the total antioxidant activity in the blood was determined to be 26% lower in postmenopausal women compared to premenopausal women.[24] This study also determined lower levels of antioxidant parameters such as NT and TT in the postmenopausal period. Meanwhile, Ds and IMA levels were comparable between the premenopausal and postmenopausal groups.

Thiol disulfide parameters and IMA may be altered in aggressive clinical types of breast cancer with poor prognosis.[4] ER-positive breast cancers have an improved prognosis compared to other breast cancer subtypes. Oxidative stress can influence the arrangement and function of ER and PR. A relation between oxidative stress and advanced stage and poor performance status has been shown in cancer patients.[25] In one study of breast cancer and T-Ds homeostasis, only 16% of subjects were at stage I.[16] In the present study, the majority of patients had stage II-III disease (77.5%). The positive results of this study may have been due to the inclusion in that study of patients with advanced-stage disease, resulting in higher levels of oxidative stress. However, in multivariate analyses, there were no significant differences between histological subtypes or clinical stages of breast cancer in terms of T-Ds levels ($p>0.05$).

The limitations of this study include the small number of cases in the patient and control groups and its single-centre nature. There are few studies in the literature that have been conducted for this purpose. This makes this study important. Multicentre studies with a larger number of patients are warranted in order to demonstrate the clinical value of T-Ds homeostasis in breast cancer and its association with other clinical parameters.

CONCLUSION

The present results demonstrated that there was an imbalance of dynamic thiol-disulfide hemostasis, resulting in decreased TT and Ds levels and increased IMA levels in breast cancer patients. It was also shown that T-Ds levels were associated with clinical characteristics such as menopausal status and BMI. Based on the results of this study, it may be reasoned that T-Ds homeostasis is involved in the pathophysiology of breast cancer.

ETHICAL APPROVAL:
This study was approved by the Dicle University Faculty of Medicine Non-Invasional Clinical Research Ethics Committee (Approval No. 249/2021).

PATIENTS' CONSENT:
Signed consent forms were obtained from the patient and control groups included in the study.

COMPETING INTEREST:
No potential competing interest was reported by the authors.

AUTHORS' CONTRIBUTION:
UH: Conception and design of the study, writing of the manuscript
ZO: Conception and design of the study, writing of the manuscript
SE, AI: Data analysis and interpretation
IO: Acquisition of clinical data
SN: Laboratory analysis and interpretation
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