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Evaluation of the Total Oxidant Status to the Antioxidant Capacity Ratio as a Valuable Biomarker in Breast Cancer Patients

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Abstract

Background: The oxidative balance is a state of equilibrium between oxidants and antioxidants disrupted in various disorders, including BC. This study aimed to assess this equilibrium in breast cancer (BC) patients by looking at the oxidant-to-antioxidant ratio.

Methods: This case-control study comprised 40 women patients with breast cancer and 30 age-matched healthy individuals. The oxidation-reduction colorimetric technique was used to determine serum levels of total oxidant status (TOS) and total antioxidant capacity (TAC). The oxidant-to-antioxidant balance was estimated using the TOS- to- TAC ratio (TOS/TAC).

Results: The mean TOS in healthy individuals was 8.40±2.06 µmol/L, while in BC patients it was 13.31±2.16 µmol/L (P< 0.001). The mean serum level of TAC was 1.43±0.21 mmol/L in healthy individuals and 1.19±0.15 mmol/L in BC patients (P< 0.001). The mean serum TOS/TAC was 6.01±0.32 in the healthy individuals and 11.42±0.41 in the BC patients (P< 0.0001). There were direct correlations between TAC and estrogen receptor (r=0.339, P=0.038). The TOS/TAC level has a sensitivity of 100% and specificity of 83.33%, distinguishing patients with BC from healthy controls (P< 0.001). A significant trend of increasing risk with rising TOS/TAC levels was also seen [OR=3.62, (95 % CI 1.79, 7.35)].

Conclusions: In breast cancer, the serum TOS to TAC ratio can better diagnose oxidative equilibrium than either component alone.

Keywords: Antioxidants, Biomarkers, Breast Cancer, Oxidants, Oxidative stress.

Introduction

Biological markers are dynamic and potent tools for studying diseases and pathways, with clinical applications ranging from disease screening, diagnosis, prognosis, progression, and treatment outcomes. They also encompass concepts for epidemiological, observational, and analytical research (1).

Breast cancer (BC) is one of the most prevalent malignancies among women. According to the International Agency for Research on Cancer in December 2020 (2), BC has now overtaken lung carcinoma as the most commonly diagnosed cancer in the world.

In 2020, there were 2.3 million new cases of BC diagnosed and 685,000 deaths worldwide. By the end of 2020, 7.8 million women were alive who had been diagnosed with BC in the previous five years, making it the most common cancer worldwide (3, 4).

Oxidative stress contributes to macromolecular oxidative damage, tissue protein denaturation, DNA damage, and lipid

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peroxidation, disrupting normal metabolic function, all of which contribute to the occurrence and progression of cancer (5, 6). Reactive oxygen species (ROS), the principal active oxidants mediating oxidative stress, play a role in the incidence and progression of cancer by inducing DNA damage and genetic alterations, inhibiting apoptosis, and promoting tumor cell proliferation, invasion, and metastasis (7). Identifying biomarkers that influence oxidative stress status can be helpful for prognosis and risk assessment in breast cancer patients.

As a result, total oxidant status (TOS) is commonly used to assess the body's overall oxidation state, while the total antioxidant capacity (TAC) is used to assess the body's overall antioxidant status (8, 9). Additionally, the ratio of TOS to TAC is considered a more accurate biomarker of oxidative stress. The TOS/TAC ratio can reveal an imbalance between oxidation and antioxidants when TAC and TOS are measured comprehensively (10).

While oxidative stress is defined as an imbalance in oxidant and antioxidant content, most previous studies examined oxidant and antioxidant content separately rather than as a whole (11). We reasoned that the oxidant-to-antioxidant ratio, rather than each component separately, would be a more helpful representation of the oxidative equilibrium because a higher oxidant level may benefit the disease state only if it outweighs the neutralizing power of antioxidants. Therefore, in this study, we investigated the clinical importance of the serum TOS/TAC ratio in patients with BC compared to healthy controls.

Healthy subjects and cancer patients

The study was approved by the Ethics Committee of the Medical University Jundishapur (code: IR. AJUMS.REC.1399.065). Before participating in the study, written consent was obtained from patients. Breast cancer patients referred to a specialized oncology clinic in Ahvaz, Iran, were evaluated in a case-control study. Patients who met the required criteria were included in this study. The tumor node metastasis (TNM) system was used to determine the stage of breast cancer (12).

Patients with any concurrent disorders that affect patients' oxidant status or antioxidant capacities, such as chronic inflammatory disorders, type 2 diabetes, infectious diseases, cardiovascular impairments, renal failure, and neurological disorders, were excluded from the study. Meanwhile, patients receiving antioxidant supplements, including Zinc, vitamin E, and C, were also excluded. A total of 40 patients were found to be eligible for this study. As for the control group, 30 healthy subjects, age-matched, were included in the study. Exclusion criteria were also considered for selecting healthy individuals for the control group.

Materials and Methods

Sample collection and biochemical tests

A total of 5 ml of blood was obtained from each eligible individual. The serum samples were kept at -80 °C for subsequent tests after being centrifuged for 15 minutes at 3000 rpm. Total Antioxidant Capacity (TAC) was measured using a method developed by Erel (8). The assessment is based on the ability of antioxidants to promote the reduction of ABTS+ to ABTS [2,2'-azino-bis-(3-ethyl-benzothiazoline-6-sulphonate)] in the sample. The TAC level was measured based on the change in ABTS+. The TAC was presented as mmol Trolox Eq/L.

Serum TOS was measured using Erel's TOS colorimetric method (7), which relies on the oxidation of ferrous ions to ferric ions in the presence of various oxidative species in an acidic medium. Xylenol orange was used as an indicator reflecting the increase of ferric ion to determine the TOS level. The assay was calibrated with a hydrogen peroxide (H2O2) standard. The results of TOS were expressed in umol/L H2O2 equivalent (umol/L H2O2).

By assessing each sample's TOS and TAC, the TOS/TAC ratio was calculated and considered as an estimation/predictive value of the oxidant/antioxidant equilibrium.

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Statistical analysis

The statistical analysis was performed using SPSS version 24. The mean ± SD, or number and percentage, were used to describe the descriptive data. The independent T-test and Mann-Whitney U Test were used to compare mean values between groups. The connections between categorical variables were assessed using a chi-square test.

Potential correlations were assessed using Pearson's or Spearman's correlation coefficients. Continuous variables like age and BMI were categorized using the median split method. The area under the curve (AUC) was calculated after the receiver operating characteristic (ROC) curve was drawn, and the power of TOS/TAC in discriminating patients with BC from healthy people was calculated (AUC). The ideal cut-off value was determined to be the most significant point of the Youden index. The odds ratio was calculated using conditional logistic regression models with a 95% confidence interval (95% CI). $P < 0.05$ was used as the significant level.

Results

The control group included 30 healthy women with a mean age of 44.1 ± 8.6 years and a mean BMI of 25.84 ± 3.7. There were 40 BC patients in the case group, with a mean age of 45.7 ± 8.3 years and a mean BMI of 29.08 ± 5.25 kg/m2. The BC Stage I, II, III, and IV were found in 10 (25%), 12 (30%), 14 (35%), and 4 (10%) of patients, respectively. Table 1 shows more details of the demographic and clinical characteristics of the enrolled patients.

Table 1. The demographic and clinical characteristics of the patients with breast cancer.

Variable	BC Patients(n=40)
Age (year)	45.7± 8.3
BMI (kg/m^2)	29.08± 5.2
Clinical stage	
Stage I	10 (%25)
Stage II	12 (%30)
Stage III	14 (%35)
Stage IV	4 (%10)
Lymph node involvement	
Yes	28 (%70)
No	12 (%30)
Her-2	
Positive	14 (%35)
Negative	26 (%65)
Estrogen receptor	
Positive	22 (%55)
Negative	18 (%45)
Progesterone receptor	
Positive	19 (%47.5)
Negative	21 (52.5)
Menopausal status	
Premenopausal	32 (%80)
Postmenopausal	8 (%20)
Pregnancy history	
Yes	37 (%92.5)
No	3 (%7.5)
Education	
High school or lower	31 (%77.5)
College or above	9 (%22.5)
Family history	
Positive	4 (%10)
Negative	36 (%90)

BC: breast cancer. The data are represented by mean ± SD or number (%).

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Table 2 shows the statistical relationship between TOS, TAC, and TOS/TAC with the clinical-demographic parameters of the patients. There were direct correlations between TAC and estrogen receptor (r=0.339, P=0.038). It was proved that there is a significant correlation between TAC and TOS, and its ratio TOS/TAC (r = -0.717, P < 0.001, r= 0.851, P < 0.0001).

The AUC of 0.96 was obtained using the TOS/TAC ROC curve (Fig. 1), to discriminate patients from healthy participants at the appropriate cut-off value of 7.6. On the other hand, sensitivity of 100% and specificity of 83.33% (95% CI, 0.9169 to 1; P < 0.0001). At this cut-off value, 100% of BC patients and only two healthy individuals were recognized as above the cut-off level. The values of the odds ratio and confidence interval for serum levels of oxidative stress TOS/TAC were 3.62, 1.79-7.35, respectively (P < 0.001).

Table 2. The statistical relationship between TOS, TAC, and TOS/TAC and clinical-demographic characteristics of BC patients.

Variable	TAC	P value	TOS	P value	TOS/TAC	P value
Age (years)						
<45	1.21 ± 0.177	0.44	13.28 ±2.32	0.92	10.6 ± 0.029	0.25
>45	1.17 ± 0.138		13.35 ± 2.01		11.72 ± 0.022	
BMI (kg/m^2)						
<29.15	1.19 ± 0.151	0.79	13.33 ± 2.26	0.93	11.42±0.025	0.30
>29.15	1.19 ± 0.180		13.28 ± 1.99		10.65 ±0.029	
Clinical stage						
Stage I	1.20 ± 0.156		14.43 ± 0.9		12.34 ± 0.03	
Stage II	1.16 ±0.174	0.79	13.11± 0.5	0.40	11.53 ± 0.02	0.54
Stage III	1.20 ± 0.144		13.09 ± 0.6		11.091 ± 0.02	
Stage IV	1.27 ± 0.233		12.35 ± 0.8		9.95 ± 0.01	
ER						
Positive	1.20 ± 0.157	0.84	13.94 ± 2.54	0.03*	11.94 ±0.019	0.11
Negative	1.21 ± 0.167		12.48 ±1.09		10.57 ± 0.02	
Her-2						
Positive	1.18 ± 0.162	0.64	12.60 ± 1.1	0.11	10.87 ± 0.02	0.39
Negative	1.21 ± 0.161		13.74 ± 2.4		11.64 ± 0.02	
PR						
Positive	1.20 ± 0.14	0.94	13.71±0.58	0.27	11.63 ± 0.02	0.53
Negative	1.20 ± 0.17		12.94 ± 1.6		11.08 ± 0.027	
Lymph node involvement						
Yes	1.24 ± 0.038	0.04*	13.37 ± 0.419	0.87	11.12 ± 0.620	0.45
No	1.14 ± 0.03		13.26 ± 0.554		11.76 ± 0.541	

BC: breast cancer; ER: estrogen receptor; PR: progesterone receptor; TOS: total oxidant status; TAC: total antioxidant capacity.

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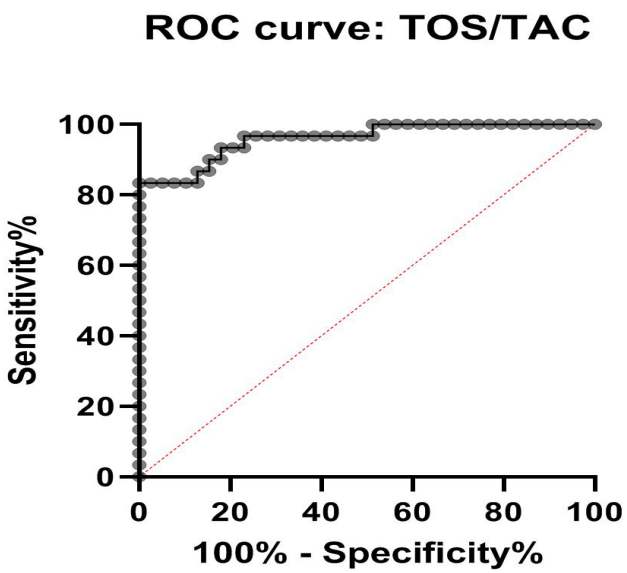

Fig. 1. Receiver operating characteristic (ROC) curve analysis of the TOS/TAC in breast cancer diagnosis. The oxidant-to-antioxidant balance was estimated using the TOS- to- TAC ratio (TOS/TAC). TOS: Serum levels of total oxidant status, TAC: Total antioxidant capacity.

Discussion

The serum TOC, TAS, and TAS/TOC levels were used to determine oxidative stress in BC patients in this investigation. The serum TAS level in BC patients was substantially greater than in healthy controls, according to our findings. The serum TAC level in healthy control participants was substantially greater. However, TOS/TAC was considerably higher in patients with BC, and it would be preferred to evaluate each element individually. The TOS/TAC showed 100% sensitivity and specificity in the diagnosis of BC from the healthy control group is 83.33%. In all patients with BC, the TOS/TAC level was higher than the cut-off value.

Some researchers have confirmed changes in oxidative stress indicators between cancer patients and healthy subjects (13).

Feng et al. (14) investigated serum oxidative stress markers in healthy people and people with breast cancer and benign breast tumors. TAS levels in patients with benign and malignant tumors were much lower than in healthy controls. Patients with benign and malignant tumors had greater serum levels of TOS and OSI than healthy controls.

Sener et al. (15) discovered that plasma levels of TAS in patients with breast cancer, including those in stages II and III, were significantly lower than in healthy controls.

In breast cancer patients, Ray and Husain (16) found higher plasma total cholesterol, total triglyceride, and LDL-cholesterol but lower HDL-cholesterol and vitamins C and E.

Several other researchers have examined the oxidant and antioxidant levels in cancer patients (11, 17). The oxidative equilibrium's components (oxidants and antioxidants) were examined separately rather than in combination in most studies (11, 18, 19).

The TOS/TAC value, according to our data, could be a potential marker for identifying BC patients from healthy people. Because the TOS/TAC value has a higher sensitivity in grade I BC, it could be used as a screening indicator for BC early identification. However, if oxidative stress is a treatment target, it could be applied as a disease marker for monitoring and evaluating the therapeutic effect (20). Manipulation of patients' TOS/TAC ratios toward TAC dominance could be considered a therapeutic option for disease effect deceleration as a clinical implication of these findings.

To that end, patients' TOS/TAC ratios should be monitored regularly, and if a

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downward trend in this equilibrium is detected, antioxidant therapy may be indicated. Regardless of these potential consequences, it is worth noting that the subjects in this study were recruited using stringent criteria to rule out any underlying disease that can impair oxidative equilibrium. Several investigations have found a relationship between estrogen-induced breast cancer and oxidative stress (20). In addition, oxidative DNA damage has been reported to increase in breast cancer tissue compared to normal breast and is strongly associated with estrogen receptor (ER) status (21, 22). Changes in the oxidant/antioxidant status of patients with breast cancer before and after surgery, including increased MDA and decreased blood glutathione levels, have been reported (23). The mean TOS concentration was significantly different regarding the estrogen receptor expression. It was observed towards higher TOS in the ER-positive subgroup than in the ER-negative one. The above findings may suggest that oxidative stress might be related to ER expression, and thus there is a need for further investigation.

Our study has several flaws that should be highlighted. Because various confounding variables, such as age and body mass index, can influence an individual's oxidant and antioxidant status (24), when determining oxidant/antioxidant state, the effect of these variables must be taken into account.

While oxidative stress markers like TAC have been linked to the risk of breast cancer in some research (25, 26), the link between TOS/TAC and the risk of breast cancer has yet to be examined. The current study found that elevated levels of serum TOS/TAC can dramatically potentiate breast cells for malignancy, indicating that the assayed biomarkers are linked to the risk of breast carcinogenesis.

Despite this, the study's main limitation was the small number of individuals, which prevented a multivariate data analysis. To achieve a more substantial consensus on the importance of the oxidant to antioxidant ratio, further research with larger sample sizes is warranted.

The findings of this study showed that the BC patients had a considerably higher serum TOS/TAC value than the healthy individuals. These findings confirm that a ratio based on serum total oxidant to antioxidant is a better indicator of oxidative stress in BC cases than measuring each ingredient separately.

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Conflict of Interest

The authors declare no conflict of interest.

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