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# The Role of Acetylcholinesterase, Paraoxonase, and Oxidative Stress in Breast Tumors

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## Abstract

**Background:** Oxidative stress is associated with the development of a large variety of malignancies.
**Objectives:** This study was conducted to evaluate the acetylcholinesterase (AChE) and arylesterase (ARE) activities, malondialdehyde (MDA), and total antioxidant capacity (TAC) plasma levels in two groups of patient with breast cancer and benign breast diseases compared to healthy volunteers.
**Methods:** The present study was composed of two groups of patients with malignant breast tumors (MBT) and benign breast diseases (BBD), and a control group (CON). Enzyme activities and antioxidants markers were measured, using spectrophotometry.
**Results:** In both case groups, MBT and BBD, ARE was found to show lower activity compared to CON group ($P = 0.004$ and $P = 0.014$, respectively). Lower activity of AChE was found in both MBT and BBD compared to CON subjects ($P = 0.003$ and $P = 0.034$, respectively). The mean plasma levels of MDA in both groups of patients MBT and BBD were higher than those in CON ($P < 0.001$ for both comparisons). No significant differences were detected between groups regarding the mean levels of TAC.
**Conclusions:** The results obtained from the current study indicate that healthy subjects show a different redox status than patients with MBT and BBD. Our data suggest that erythrocyte AChE may be considered as an indicator of oxidative stress along with other factors in patients with breast tumors. Thus, consuming antioxidant supplements can be helpful for the prevention of breast diseases.

*Keywords:* Acetylcholinesterase, Arylesterase, Breast Cancer, Malondialdehyde, Oxidative Stress, Total Antioxidant Capacity

## 1. Background

Accounting for relatively one-fourth of the total cancers among Iranian women, breast cancer is a major cause of gynecologic cancer deaths (1). It is a multifactorial disease, whose etiology is dependent on genetics, lifestyle, and environmental factors (2, 3). Both exogenous and endogenous factors can bring about oxidative stress in the human body. Oxidative stress can be considered as an important parameter in the incidence of cancer and is associated with various aspects of cancer, including carcinogenesis, tumor-bearing state, treatment, and prevention (4). Overall, the disturbance in the balance of reactive oxygen species (ROS) production and antioxidants capacity leads to oxidative stress, which is related to the occurrence of oxidative injuries such as damage to DNA, protein, and lipid (5). Generation of high levels of ROS results in the initiation of lipid peroxidation of polyunsaturated fatty acids (PUFAs) in erythrocyte membranes. Malondialdehyde (MDA), as a secondary product of lipid peroxidation, is a potential biomarker of oxidative stress (6).

It has been well established that some enzymes can act as antioxidants in the regulation of the redox status. An important antioxidant enzyme, human serum paraoxonase1 (PON1), has been studied in inflammatory and oxidative stress-related diseases such as cancer (7, 8). PON1 is a 43 - 45 kDa glycoprotein that is basically synthesized by

the liver and secreted into the blood within high-density lipoprotein-cholesterol (HDL-c) (9). In accordance with the substrate type, PON1 fulfills different activities, namely, paraoxonase, arylesterase (ARE), and lactonase, which hydrolyzes organophosphorus insecticides, phenyl acetate, and lactone, respectively (10). PON1 activity has been reported to be reduced in patients suffering from lung, colorectal, breast, and oral cancers (11, 12).

An accumulating body of evidence has highlighted the active involvement of cholinergic system in oxidative stress (13, 14). According to substrate type, cholinesterases are grouped into two categories, namely, acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). The former hydrolyzes the neurotransmitter acetylcholine (ACh) likely seen in red blood cells, and the latter, which utilizes butyrylcholine as a substrate, is found in plasma (15). In spite of the key role that AChE plays in cholinergic systems, shreds of experimental evidence support that disruption in gene expression, structural alteration, and multiple activities of AChE could occur in different cancers (16).

Total antioxidant capacity (TAC) is considered as a significant protective factor employed to assess the redox state of the body. Several research groups have demonstrated lower levels of serum TAC in various types of cancer that can indicate oxidative stress or increased susceptibility to oxidative damage in patients (17, 18).

## 2. Objectives

The incidence rate of breast cancer and BBDs is experiencing a significantly growing trend in Kerman province, particularly among young women. However, the causes or risk factors of these breast diseases are not well understood. Given the environmental susceptibilities and nutritional patterns, we supposed that the wide presence of oxidant substances might be the possible cause of the spread of these diseases. On the other hand, according to the authors' knowledge and the literature review, few studies have been conducted regarding the roles and relationships between oxidative stress markers and breast tumors. Therefore, the present study was carried out with the aim of evaluating the association of AChE and ARE activities as well as MDA and TAC levels in patients with breast tumors.

## 3. Methods

Acetylthiocholine iodide, 5,5-dithio-bis-2-nitrobenzoic acid (DTNB), Hyamine 1622, and Phenyl acetate were purchased from Sigma-Aldrich (St. Louis, USA). Lipid peroxidation (MDA) (CAT NO: NS-15022) and TAC assay kit (CAT NO: NS-15012) were obtained from Navandsalamat Company (Urmia, Iran).

### 3.1. Subjects

The current case-control study was conducted at Shahid Bahonar Hospital, Kerman, Iran (June 2015 to October 2016). The study group included 27 women newly diagnosed with malignant breast tumor (MBT), 36 patients with benign breast diseases (BBD), and 27 healthy individuals. Diagnosis of MBT and BBD was established by clinical and pathologic examinations. Tumor node metastasis (TNM) staging system was utilized to determine the stage of patients with MBT. Inclusion criteria included patients with no previous history of chemotherapy, radiotherapy, and pathological surgery. None of the patients was taking regular antioxidant supplements and they had no prior history of smoking and alcohol consumption. Based on clinical history and physical examination, all control subjects were non-cancerous and without any acute and chronic diseases. Healthy subjects were not taking antioxidant supplements and were not smoking or consuming alcohol. Written informed consent was signed by all participants. The research was performed according to the principles of the revised Declaration of Helsinki, a statement of ethical principles to provide instruction to physicians and other participants, who work in medical research on human subjects and approved by the Ethics Committee of Kerman University of Medical Sciences (code No.: IR. KMU. REC.1394.315).

### 3.2. Sampling

Blood samples were collected from each subject into lithium heparin tubes and cells were separated from plasma by centrifugation (3000 rpm). Packed erythrocytes were prepared after washing 3 times with normal saline (19). Hemolysates and plasma were stored at -80°C until assayed.

### 3.3. Malondialdehyde Measurement

Plasma MDA concentrations were measured by lipid peroxidation commercial kit. The method used in this kit is based on a reaction of MDA with thiobarbituric acid (TBA) in accordance with the method of Buege and Aust (20). Then, the produced color was measured by spectrophotometer at 532 nm, which is proportional to the MDA levels.

### 3.4. Arylesterase Activity Assay

Arylesterase activity was measured, using phenyl acetate substrate as described by Ayub et al. (21). The reaction mixture consisted of 2 mM substrate (phenyl acetate),

2 mM $CaCl_2$, and 10 $\mu$L of plasma in 100 mM Tris-HCl (pH 8.0). The mixture was incubated for 3 min at 37°C while the rate of phenol production was monitored at 270 nm.

### 3.5. Erythrocyte Acetylcholinesterase Activity Assay

The estimation of AChE activity for the erythrocytes was carried out according to a modified Ellman's assay (22). In a nutshell, 100 $\mu$L of packed erythrocytes was diluted with distilled water to 6 mL. Then, 100 $\mu$L of diluted samples were incubated with reaction buffer (0.28 mmol DTNB, 3.2 mmol Acetylthiocholine iodide, and 20 $\mu$m quinidine sulfate) at 37°C for 10 min. Finally, 1 mL of Hyamine® 1622 was added to the mixture to stop the reaction. The enzyme activity was evaluated by the production rate of thiocholine when acetylthiocholine reacts with Ellman's reagent. The absorbance of the colored product was recorded at 440 nm, using a spectrophotometer.

### 3.6. Measurement of Plasma Total Antioxidant Capacity Levels

Plasma TAC levels were determined based on Benzie and Strain method (23) by TAC commercial kit (CAT NO: NS-15012, Navandsalamat Company, Urmia, Iran). This kit measures TAC in terms of the ferric reducing ability of plasma (FRAP). Accordingly, ferric is reduced to ferrous ion at low pH and a ferrous-tripyridyltriazine complex is formed. The produced complex has a max absorbance at 593 nm, which is proportional to the TAC level.

### 3.7. Statistical Analysis

All continuous variable data were presented as mean $\pm$ standard deviation (mean $\pm$ SD) and categorical variables were presented as numbers (percentages). Kolmogorov-Smirnov test was used to determine the distribution of data. The differences among groups were explored, using one-way Analysis of Variance (ANOVA)/Kruskal-Wallis with post-hoc Tukey/Mann-Whitney U tests as well as Chi-square/Fisher's exact tests. Correlations between continuous variables were evaluated, using Pearson and Spearman' rho correlation coefficient. The statistical analyses were carried out using SPSS software version 23.0 for Windows (SPSS Inc., Chicago, IL). P values $< 0.05$ were regarded as statistically significant.

## 4. Results

Demographic and clinical characteristics of the study population are summarized in Table 1. No significant differences were observed in age, BMI, stress, residence, menopause status, and clinical stages of the three groups ($P > 0.05$ for all comparisons). Based on the World Health Organization (WHO) classification (24), participants' BMI were as follow: (1) Regarding the MBT group, a number of 2, 13, and 12 patients were underweight, normal weight, and overweight, respectively; (2) in BBD cases, a number of 5, 23, and 8 patients were underweight, normal weight, and overweight, respectively; (3) with regard to CON group, 1, 18, and 8 subjects were underweight, normal weight, and overweight, respectively. It is noteworthy that there were no obese subjects in the present study.

Table 2 illustrates the comparison of ARE and AChE activities as well as the mean plasma levels of TAC and MDA among MBT, BBD, and healthy subjects. In both MBT (62.67 $\pm$ 14.57 U/mL) and BBD (67.04 $\pm$ 21.86 U/mL) groups, ARE was found to show lower activity than that of CON group (80.57 $\pm$ 18.59 U/mL) ($P = 0.004$ and $P = 0.014$, respectively). Likewise, AChE had lower activity in both MBT (4.48 $\pm$ 1.46 U/mL) and BBD (4.91 $\pm$ 1.24 U/mL) groups compared to CON group (5.87 $\pm$ 1.78 U/mL) ($P = 0.003$ and $P = 0.034$, respectively). Furthermore, the mean plasma levels of MDA in both MBT (20.63 $\pm$ 3.17 nM/mL) and BBD (18.17 $\pm$ 3.17 nM/mL) groups were higher than those in CON group (5.63 $\pm$ 2.68 nM/mL) ($P < 0.001$ for both comparisons). However, we did not find significant differences in the mean levels of TAC within three studied groups ($P = 0.429$). It is noteworthy that in all comparisons, no significant differences were found between MBT and BBD groups ($P > 0.05$ for all comparisons).

As shown in Table 3, no significant differences were identified between measured parameters and different stages among MBT women ($P > 0.05$ for all comparisons).

Table 4 illustrates the significant positive correlation between BMI and MDA among the MBT ($r = 0.72$, $P < 0.001$), BBD ($r = 0.55$, $P < 0.001$) and CON ($r = 493$, $P = 0.009$) groups. Moreover, ARE was shown to have a significant inverse correlation with MDA ($r = -0.38$, $P = 0.02$) and a positive correlation with AChE ($r = 0.48$, $P = 0.01$) in BBD and CON groups, respectively. It was found that AChE negatively correlated with MDA in CON group ($r = -0.44$, $P = 0.021$).

## 5. Discussion

There is a consensus that imbalance in redox homeostasis is implicated in carcinogenic processes. The oxidative stress may be caused by an overproduction of ROS or insufficient antioxidant capacity (25). Therefore, we determined the enzymatic activities of ARE and AChE along with MDA and TAC levels in the patients with breast tumors, indicating the lower activities of ARE and AChE and higher levels of MDA in patients with MBT and BBD compared to healthy individuals.

It is well understood that AChE functions as the catalytic hydrolysis of cholinergic neurotransmitters for terminating transmission at cholinergic synapses. However,

**Table 1.** Comparison of Clinical and Demographic Characteristics Among MBT, BBD, and Control Groups[a]

| | MBT (n = 27) | BBD (n = 36) | CON (n = 27) | P Value[b] |
|---|---|---|---|---|
| **Age (y)** | 49.48 ± 7.25 | 47.30 ± 6.11 | 45.74 ± 4.26 | 0.077[c] |
| **BMI (kg/m²)** | 23.50 ± 2.93 | 22.47 ± 2.67 | 22.89 ± 2.57 | 0.277[d] |
| **Stress** | | | | 0.090[e] |
| Yes | 19 (70) | 28 (78) | 14 (52) | |
| No | 8 (30) | 8 (22) | 13 (48) | |
| **Residence** | | | | 0.468[e] |
| Urban | 6 (22) | 8 (22) | 3 (11) | |
| Rural | 21 (78) | 28 (78) | 24 (89) | |
| **Menopause** | | | | 0.922[e] |
| Yes | 24 (89) | 32 (89) | 23 (85) | |
| No | 3 (11) | 4 (11) | 4 (15) | |
| **Stages** | | | | |
| I | 2 (8) | | | |
| II | 17 (63) | | | |
| III | 8 (29) | | | |

Abbreviations: BBD, benign breast diseases; BMI, body mass index; CON, control; MBT, malignant breast tumor.
[a] Continuous and categorical values are expressed as mean ± SD and number (percentage), respectively.
[b] Significant at < 0.05 levels. No significant differences were observed regarding the demographic and clinical parameters of the three studied groups.
[c] One-way ANOVA followed by post-hoc Tukey test.
[d] Kruskal-Wallis test.
[e] Chi-square/Fisher exact test.

**Table 2.** Comparison of ARE and AChE Activities, TAC, and MDA Levels Among MBT, BBD, and Control Groups[a]

| Parameters | Groups | | | | | | P Value[b] |
|---|---|---|---|---|---|---|---|
| | MBT (n = 27) | | BBD (n = 36) | | CON (n = 27) | | |
| | Mean ± SD | Median (IQR) | Mean ± SD | Median (IQR) | Mean ± SD | Median (IQR) | |
| **ARE** | 62.67 ± 14.57[c] | 59.10 (21.05) | 67.04 ± 21.86[c] | 61.42 (23.90) | 80.57 ± 18.59 | 78.20 (27.51) | 0.002[d] |
| **AChE** | 4.48 ± 1.46[c] | 4.41 (2.16) | 4.91 ± 1.24[c] | 4.80 (1.45) | 5.87 ± 1.78 | 6.30 (2.80) | 0.003[e] |
| **TAC** | 0.78 ± 0.17 | 0.75 (0.19) | 0.76 ± 0.21 | 0.73 (0.35) | 0.85 ± 0.26 | 0.82 (0.38) | 0.429[d] |
| **MDA** | 20.63 ± 3.17[c] | 21.00 (3.85) | 18.17 ± 3.18[c] | 18.75 (4.29) | 5.64 ± 2.68 | 6.30 (4.45) | < 0.001[d] |

Abbreviations: AChE, acetylcholinesterase; ARE, arylesterase; BBD, benign breast diseases; CON, control; IQR, interquartile range; MBT, malignant breast tumor; MDA, malondialdehyde; SD, standard deviation; TAC, total antioxidant capacity.
[a] Table 2 depicts higher activity of ARE in both MBT and BBD compared to CON group (P = 0.004 and P = 0.014, respectively). Likewise, AChE had higher activity in both MBT and BBD compared to CON group (P = 0.003 and P = 0.034, respectively). Moreover, a higher level of MDA was found in both MBT and BBD compared to CON group (P < 0.001 for both comparisons). However, no significant differences were observed regarding the TAC within 3 studied groups.
[b] Significant at < 0.05 levels.
[c] Significant differences with CON group (P < 0.05).
[d] Kruskal-Wallis followed by post-hoc Mann-Whitney U-test.
[e] One-way ANOVA followed by post-hoc Tukey test.

growing evidence has suggested the involvement of AChE in pivotal processes of carcinogenesis and tumor progression, which could be due to cancer-mediated alterations either in AChE activity levels or in the protein levels or both (26, 27). Based on the present data, we have shown a significant decrease in the activity of erythrocytic AChE in MBT and BBD samples as compared to their respective controls. In agreement with the current results, but in different samples of cancerous patients, previous studies have presented a large reduction in the AChE activity in human liver tumor samples, bronchial aspirates from patients with lung cancer, and metastasis-bearing nodes of patients with breast cancer (26-28). On the other hand, some studies demonstrated the contribution of AChE in oxidative stress, such as ROS-mediated inhibition of erythrocytic AChE (29) and reduction in AChE activity due to the high levels of

**Table 3.** Comparison of AChE and ARE Activities, TAC, and MDA Plasma Levels Among Different Stages in MBT Group[a]

| Parameters | Cancer Stages in MBT Group | | | P Value[b] |
|---|---|---|---|---|
| | Stage I (n = 2) | Stage II (n = 17) | Stage III (n = 8) | |
| ARE | 66.93 ± 11.07 | 60.71 ± 14.18 | 65.78 ± 16.93 | 0.674 |
| AChE | 3.72 ± 3.07 | 4.34 ± 1.07 | 4.96 ± 1.88 | 0.482 |
| TAC | 0.75 ± 0.00 | 0.80 ± 0.16 | 0.74 ± 0.21 | 0.685 |
| MDA | 23.27 ± 0.88 | 20.35 ± 2.26 | 20.55 ± 4.84 | 0.486 |

Abbreviations: AChE, acetylcholinesterase; ARE, arylesterase; MBT, malignant breast tumor; MDA, malondialdehyde; TAC, total antioxidant capacity.
[a] Values are expressed as mean ± SD.
[b] One-way ANOVA followed by post-hoc Tukey test was applied. Significant at < 0.05 levels. As shown in Table 3, no significant differences were observed between measured parameters and different stages among MBT women.

**Table 4.** Correlations Among Age, BMI, ARE, AChE, TAC, and MDA Within MBT, BBD, and Control Groups[a]

| | Groups | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | MBT | | | | | BBD | | | | | CON | | | | |
| | BMI | ARE | AChE | TAC | MDA | BMI | ARE | AChE | TAC | MDA | BMI | ARE | AChE | TAC | MDA |
| Age | 0.071 | -0.303 | 0.074 | 0.259 | 0.010 | 0.428[b] | -0.128 | 0.091 | 0.087 | 0.135 | 0.534[b] | -0.251 | -0.086 | 0.215 | 0.098 |
| BMI | | 0.285 | 0.117 | 0.156 | 0.729[b] | | -0.169 | 0.228 | -0.093 | 0.555[b] | | -0.325 | -0.051 | 0.215 | 0.493[b] |
| ARE | | | 0.356 | 0.533 | 0.258 | | | -0.200 | -0.157 | -0.386[c] | | | 0.489[b] | -0.159 | -0.204 |
| AChE | | | | 0.384 | 0.246 | | | | 0.256 | -0.070 | | | | -0.369 | -0.442[c] |
| TAC | | | | | 0.040 | | | | | -0.047 | | | | | 0.528 |

Abbreviations: AChE, acetylcholinesterase; ARE, arylesterase; BBD, benign breast diseases; BMI, body mass index; CON, control; MBT, malignant breast tumor; MDA, malondialdehyde; TAC, total antioxidant capacity.
[a] Table 4 illustrates the significant positive correlation between BMI and MDA among the MBT (r = 0.72, P < 0.001), BBD (r = 0.55, P < 0.001) and CON (r = 493, P = 0.009) groups. Moreover, ARE showed a significant negative correlation (r = -0.38, P = 0.02) with MDA and a positive correlation (r = 0.48, P = 0.01) with AChE in BBD and CON groups, respectively. AChE was negatively correlated with MDA in CON group (r = -0.44, P = 0.021).
[b] Correlation is significant at the 0.01 level (2-tailed).
[c] Correlation is significant at the 0.05 level (2-tailed). Spearman correlation test was used.

oxidative stress during aging (14). Moreover, Sun et al., following of an in vitro study, suggested that AChE is involved in oxidative stress by influencing on some signaling pathways such as peroxisome proliferator-activated receptor gamma coactivator 1$\alpha$ (PGC-1$\alpha$) and forkhead box subfamily O3a (FoxO3a). Acetylcholinesterase, acting on PCG-1$\alpha$ and FoxO3a signaling pathway, activates superoxide dismutase (SOD), which is an important enzyme in antioxidant defense against ROS, thereby decreasing oxidative stress (30). Furthermore, several studies have documented the decreased activity of SOD in patients with breast cancer malignancy (31, 32). According to the result of this study, we suggest that low activity AChE probably lessens SOD activity in those patients. Additionally, findings also revealed that there is some correlation between AChE activity and other oxidative stress factors. AChE activity was negatively correlated with plasma MDA levels, an important oxidative stress biomarker, and also a positive correlation with ARE activity, a key antioxidant enzyme in control group. Therefore, since the results of the present study are consistent with previous reports on the role of AChE in oxidative stress, erythrocytic AChE activity may be considered as a biomarker for studying oxidative stress in patients with breast diseases.

Paraoxonase 1 is a HDL-c-associated enzyme that is responsible for the protection of both HDL-c and low-density lipoprotein-cholesterol (LDL-c) from oxidative modifications (33). Overall, human PONs have different functions in the body such as inactivation of pro-oxidant and pro-inflammatory mediators, metabolism of certain drugs and xenobiotics, and regulation of cells proliferation (34). Three distinct activities have been attributed to PON1, which include paraoxonase, arylesterase, and lactonase (10). So far, a few studies have investigated PON1 activities in patients with breast cancer; we measured the plasma arylesterase activity of PON1 in subjects suffering from breast diseases. The results of ARE activity indicated significant changes in plasma ARE activity of the patients with MBT and BBD compared to healthy subjects, for whom the enzyme activity was less than the control. Bobin-Dubigeon et al. have reported that plasma ARE activity in patients with breast cancer was lower than the control group. Their

study confirmed the positive association between ARE hydrolysis rate and survival time, suggesting that lower ARE activity increases the death rate of patients (35). Based on the literature data, plasma ARE activity is generally lower in various types of cancers including lung, breast, and colorectal (12). The diminished ARE activity in both groups of patients, MBT and BBD, supports the involvement of oxidative stress not only in patients with MBT but also in BBD. Moreover, ARE activity had an inverse correlation with MDA in BBD group and a positive correlation with AChE in control group, a finding that indicates the association between oxidant and antioxidant factors and imbalance in redox homeostasis.

MDA arises from the decomposition of lipid peroxidation, which is increased in various cancers (36, 37). It is well accepted that lipid peroxides and their products can cause damage to macromolecules such as DNA and play an important role in the initiation and promotion of carcinogenesis (37). In the present study, it was found that plasma levels of MDA were increased among patients with MBT and BBD compared to healthy individuals. In a research carried out by Huang et al. (36), plasma MDA levels in patients with breast cancer were found to be notably higher than those in healthy ones. Moreover, Polat et al. (38) also documented higher plasma MDA levels in patients with both MBT and BBD in comparison with the controls. The elevated MDA levels in patients with cancer, observed in the current study, suggest a markedly increased level of oxidative stress in patients and support the previous findings (39, 40). Interestingly, the present study demonstrated that BMI has an influence on MDA levels in MBT, BBD, and control groups. This is in agreement with previous reports, which suggested that obesity promotes increased MDA levels in experimental models and obese human subjects (41, 42). Yesilbursa et al. (43) demonstrated that MDA level will decrease with weight loss. One possible mechanism that obesity induces oxidative stress is through the elevation of tumor necrosis factor-a (TNF-a) concentrations and TNF-a may stimulate generation of reactive oxygen species generation (44, 45). Therefore, the current study strongly confirms the role of obesity in the induction of oxidative stress by the increasing levels of lipid peroxidation.

The synergistic effect of plasma antioxidants provides more protection against free radicals than when the antioxidant is measured alone. In recent years, plasma TAC has been evaluated in different neoplastic conditions. The main compounds that contribute to plasma TAC are sulphydryl groups (mostly albumin), urate, ascorbate, alpha-tocopherol, and bilirubin. Therefore, TAC can reflect the degree of oxidative stress through assessing residual antioxidant capacity after the consumption of free radical (46-48). In the present study, plasma levels of TAC in both MBT and BBD patients were lower than those in the controls, though not statistically significant. In contrary, some studies have reported significant decreases in the mean level of TAC in the breast cancer in comparison to the healthy subjects and it may lead to inadequate ROS removal and oxidative stress (49).

The approximately same levels of TAC in MBT and BBD groups in comparison of control may be a consequence of high rate of ROS production in those patients and the antioxidant system might compensate for this elevated free radical levels. Also, depending on the type of ROS produced, different antioxidants are produced. Therefore, depending which antioxidants are evaluated, the plasma antioxidant status will be different. Additionally, no significant alteration in TAC levels in patients with MBT and BBD can be attributed to the fact that most of our patients are diagnosed in the early stages and that dramatic changes in TAC levels may occur in more advanced stages of the MBT and BBD.

### *5.1. Conclusions*

The deficiency of erythrocyte AChE and plasma ARE activities in patients with breast cancer may be due to increased utilization of scavenging plasma MDA along with sequestration by tumor cells. An interesting finding from the current research is that the significant alterations in enzyme antioxidants and MDA seen in patients with malignant tumor were also evident in patients with benign breast tumors, thereby placing them in a “high-risk” category. Thus, the determination of antioxidants enzymes activities and MDA level in blood circulation could be used as a marker, which might help to diagnosis and follow-up of patients with breast tumors. Also, consuming antioxidants can be helpful for the prevention of breast diseases.

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## Footnotes

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