Evaluation of Oxidative Stress Involvement in Breast Cancer carcinogenesis

ABSTRACT

Background: According to GLOBOCAN estimates, breast cancer was found to be the most often diagnosed cancer in women worldwide, (11.7 %) and the fourth leading cause of cancer mortality (6.9 %). The present study was aimed to evaluate the involvement of oxidative stress on breast cancer carcinogenesis in Egyptian population.

Methods: Lipid peroxidation as evidenced by malondialdehyde (MDA) and nitric oxide (NO) stress as well as the status of the antioxidants superoxide dismutase (SOD) and total antioxidant capacity (TAC) were estimated in serum of 163 breast cancer patients. Correlations between oxidant/ antioxidant profile and different prognostic variables in BC patients were estimated.

Results: Lipid peroxidation in BC was enhanced in response to cancer stage and tumor size ($p < 0.01$). Similarly, NO was increase in response to NPI, Her2/neu and cancer stage ($p < 0.02$). Inversely in antioxidant, SOD was decrease in response to Her2/neu only ($p < 0.002$). While, TAC was increase in response to cancer stage and tumor size ($p < 0.01$). We found that oxidant/antioxidant status was dependent on NPI, Her2/neu, cancer stage and tumor size of BC patients.

Conclusion: The results of our study have shown higher oxidant stress production and decreased SOD activity support the oxidative stress hypothesis in breast carcinogenesis.

Keywords: Breast cancer, Oxidant Stress, Antioxidant, carcinogenesis.

1. INTRODUCTION

Breast cancer (BC), consider the persistent diagnosing type of cancer worldwide which growing with more than two million new cases each year reflecting over (11.7%) of all lived diagnosed cancer. It represent the primarily cause of women death with more than (6.9%) of total cancer deaths. The death rate in female BC was found more in transitioning countries when compared to transitioned countries (15.0 to 12.8 per 100,000 cases), **[1].** In Egypt, breast cancer represents the highest incidence female's cancer types; with more than (32%) and a three-fold increase is predicted by 2050 as recently reported by the National Cancer Institute (NCI), Egypt **[2].** Comparing to USA and other Western societies, Egypt show lower incidence in BC, while Egyptian BC patients shows higher mortality rate. BC is the second-leading cause of death from cancer in Egyptian women. Egyptian BC patients with no family history of BC shows 85% of all diagnosed BC. This may explained by the genetic mutations that happen as a result of the aging or life style with a tendency to occur in younger age groups with advanced stages **[3- 5].**

BC develops due to complex interactions between genetic and different risk factors. Different classical pathological markers was used to conform patients clinical character like tumor size, as well as estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (Her2) statuses. The high-risk patients should be identified at the earliest stage by applying a novel diagnostic and therapeutic regimen **[6- 9].**

The balance between oxidative damage and antioxidant protection is the main challenge in lived cells. Inadequate antioxidant scavenging or excess oxygen-free- radical formation

creates a condition known as oxidative stress. Excess generation of oxygen free radicals can cause oxidative damage to biomolecules resulting in lipid peroxidation, mutagenesis, and carcinogenesis. There have been enormous advances and developments in the knowledge of the mechanism and factors involved in breast carcinogenesis. The precise mechanisms of oxidative stress being induced in breast cancer cells are still not exactly understood and documented. There are only few reports on the oxidant–antioxidant profile in breast cancer patients **[10- 13].**

ROS and RNS can contribute also to oxidative damage to lipids and nucleic acids. Lipid hydroperoxides, malondialdehyde (MDA) are useful biomarkers of oxidative stress to lipids. Therefore, in conditions characterized by an imbalance between ROS/RNS levels and antioxidants, oxidative cell injury may occur and trigger oxidation of lipids, proteins, and DNA. Different molecular mechanisms are involved such as alterations of mitochondria and peroxisome, increased activity of metabolic transduction pathways, and transcriptional cellular receptor signaling. The cell redox potential affects transcription factors that regulate the expression of genes responsible for proliferation, apoptosis, angiogenesis, and production of cytokines **[14- 16].**

Cancer cells are usually submitted to higher ROS levels that further stimulate a malignant phenotype, promoting sustained cell proliferation, cell survival, angiogenesis, metastasis and inflammation. Therefore, it is considered an established source of carcinogenesis. In breast cancer, ROS and oxidative stress are involved in DNA damage, which can inhibit or induce transcription, signal transduction pathways, replication errors, genomic instability and activation of oncogenes. There are several risk factors in breast cancer associated with ROS-induction, such as aging, menopause, genetic predisposition or estrogens, which results in DNA damage and chromosomal aberrations, and hence, supporting the development and progression of the disease. Provided that the regulation of oxidative stress and the maintenance of REDOX homeostasis are important factors in both tumor development and response to anticancer therapies, targeting REDOX regulation is emerging as a promising strategy for the treatment of breast cancer **[10, 12, 13, 17].**

We therefore try to evaluate the extent of lipid oxidant, peroxidation (MDA) and nitric oxide (NO) as well as the status of the antioxidants superoxide dismutase (SOD) and total antioxidant capacity (TAC) in the involvement of breast cancer carcinogenesis.

2. MATERIAL AND METHODS

2.1. Ethical declaration:

The patients were admitted to Mansoura University Oncology Center Hospitals, Mansoura, Egypt, over the years 2019 and 2020. The protocol approval was allowed by the Institutional Review Board (IRB) at Mansoura University before starting the study. All methods were performed in accordance to the guidelines and regulations proposed in the 1975 Declaration of Helsinki. Informed consent letter was obtained from all the participants. All the patient related data including biological samples were anonymized to ensure confidentiality.

2.2. Patients recruited:

BC female patients 163 the median age = 52.7 years, (age range = 27– 80 years). BC patients are classified by different grading systems which influence the prognosis and different factors for histo-pathological diagnosis. Histological appearance is usually used to classify BC which is derived from the lobules or epithelium lining the ducts and these cancers are classified according grade, stage, node status and metastasis as well operation type **[18].** For each patient, tumor size, as well as ER, PR and Her2 statuses were detected by which the BC group was further correlate these separate individual prognostic factors to the ACE I/D polymorphism genotypes. BC patients group have been recently diagnosed as having breast cancer with no chemo/radiotherapy involvements. NPI, the mandatory Nottingham prognostic index accurately predicts survival in BC patients **[19]** was calculated for each BC patient. Three prognostic groups were cut-off points separated. They were (NPI of < 3.4) represent the good prognostic index (GPI), (NPI of 3.41–5.4) was performed as the moderate

prognostic index (MPI) and finally the (NPI of > 5.41) were illustrating the poor prognostic index (PPI). The equation used in NPI quantitation is:

NPI= (0.2 X tumor size) + Node status + Grade status.

2.3. Determination of Oxidative/Antioxidant Status:

Plain tubes were used to collect blood samples. Serum was collected after spin at 2500 g for 9 min at RT. Oxcidant/Antioxidant parameters (MAD, NO, SOD and TAC) were done according to the instructions of the manufacture (Bio-diagnostic, Giza, Egypt).

2.3.1. Lipid Peroxide (Malondialdehyde, nmol / ml) Cat. No. MD 25 29

Thiobarbituric acid (TBA) reacts with malondialdehyde (MDA) in acidic medium at temperature of 95°C for 30 min to form thiobarbituric acid reactive product the absorbance of the resultant pink product can be measured at 534 nm.

2.3.2. Nitric Oxide Assay (NO, µmol/ L) Cat. No. NO 25 33

In acid medium and in the presence of nitrite the formed nitrous acid diazotise sulphanilamide and the product is coupled with N-(1–naphthyl) ethylenediamine. The resulting azo dye has a bright reddish – purple color which can be measured at 540 nm. It depend on the addition of Griess Reagents which convert nitrite into a deep purple azo compound, photometric measurement of the absorbance due to this azo-chromophore accurately determines NO2 - concentration.

2.3.3. Superoxide Dismutase (SOD, U/ ml) cat. No. SD 25 21

This assay relies on the ability of the enzyme to inhibit the phenazine methosulphate-mediated reduction of nitroblue- tetrazolium dye. The amount of SOD present in cellular and extracellular environments is crucial for the prevention of diseases linked to oxidative stress. Measure the increase in absorbance at 560 nm. The amount of SOD present in cellular and extracellular environments is crucial for the prevention of diseases linked to oxidative stress.

2.3.4. Total Antioxidant Capacity (TAC, mM/ L) Cat. No. TA 25 13

The determination of the anti-oxidative capacity is performed by the reaction of antioxidants in the sample with a defined amount of exogenously provide hydrogen peroxide (H2O2). The antioxidants in the sample eliminate a certain amount of the provided hydrogen peroxide. The residual H2O2 is determined calorimetrically by an enzymatic reaction which involves the conversion of 3, 5, dichloro–2– hydroxy benzene sulphonate to a colored product. The absorbance of the resultant product can be measured at 505 nm.

2.4. Statistical Analysis

Frequency tables and statistical analyses were calculated with SPSS for Windows 21.0 (SPSS, Chicago, IL, USA). The data for analyses are expressed as mean SEM. Statistical comparisons were performed by Student's t-test. Pearson correlation test was used in correlation between all parameters. A value of $p < 0.05$ was considered statistically significant.

3. RESULTS

3.1. Demographic, distribution of prognostic parameters BC patients:

Demographic, clinico-pathological data and biomarker parameters of the study participants which have been gathered from patients' medical records were shown in **(Table 1).** Different features listed in the table represent the number and percentage of each parameter in relation to the BC patients. among these features the predominant cancer stage was stage II (67.5%), node status was N0 (34.4%), cancer grade was grade II (71.2%), tumor size was ≥ 2 cm- 5 cm (74.2%), NPI was >3.4- 5.4 (76.7%), positive ER was (79.8%), positive PR was (76.1%), negative Her2/neu expression was (54.6%), negative metastasis was (85.3%) and left operated breast was (61.3%).

3.2. Correlation of NPI with prognostic parameters in BC patients:

Regarding NPI, the correlation among different prognostic parameters in BC patients was listed in **(Table 2).** The significant increase in NPI has been noted in positive Her2/neu expression marker (p= 0.05) as well as positive metastasis (p= 0.01) when compared to the negative ones. NPI also show a significant increase as the cancer stage and tumor size increase (p< 0.0001). NPI show no significant differences in response to neither ER nor PR.

3.3. Correlations of Oxidant/ Antioxidant profile to different prognostic parameters in BC patients:

The correlations between oxidant/antioxidant profile (Mean ± SEM) and different prognostic parameters in breast cancer are presented in **Table 3.** MDA shows a significant increase as the cancer stage and tumor size increase (p = 0.01, 0.05 respectively). NO shows a significant increase as the NPI and cancer stage increase (p= 0.04, 0.02 respectively) as well as positive Her2/neu expression marker (p= 0.02). SOD shows a significant decrease in positive Her2/neu expression marker (p= 0.002). TAC shows a significant increase as the cancer stage and tumor size increase (p= 0.01).

3.4. The interrelationships of Oxidant/Antioxidant status in response to NPI, Oxidative Stress and Antioxidant in BC patients:

The interrelationships of oxidant/antioxidant status in response to NPI **(Table 4),** oxidative stress **(Table 5)** and antioxidant **(Table 6)** were calculated using Pearson correlation test. We determined similar data existed in literature.

3.5. Diagnostic Performance of Serum oxidant/antioxidant status to different prognostic parameters in BC patients:

Figure 1, depicts the results of the ROC curve analysis which was used to explore the discrimination ability of serum oxidant/antioxidant status with response to different prognostic parameters in BC patients. Case Processing Summary revealed that larger values of the test result variable(s) indicate stronger evidence for a positive actual state. The test result variable(s): MDA, NO, SOD, TAC and NPI have at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased. **Table 7,** presents the values of area under the curve of serum levels of MDA, NO, SOD, TAC and calculated NPI as well as Sensitivity and Specificity using ROC curve in response to different prognostic markers. The significant AUC was a result of data analysis of the ROC curve in response to Her2/new (AUC = 0.398, p= 0.02 for NO; AUC = 0. 619, p = 0.009 for SOD and AUC = 0. 412, p= 0.05 for NPI). The rest of markers give no significant difference in the AUC except in metastasis with NPI where it was (AUC = 0.347, p= 0.01). SOD shows the highest sensitivity and specificity over 90% in response to different prognostic markers.

Table 1: Characteristic frequency of tumor different prognostic factors in BC patients (163 Patients).

Variables	Patients # (%)	Variables	Patients # (%)
Cancer Stage		**Node Status**	
T1	26 (15.9)	**N0**	56 (34.4)
T2	110 (67.5)	**N1**	42 (25.7)
T3	21 (12.9)	**N2**	40 (24.5)
T4	6 (3.7)	**N3**	25 (15.4)
Overall grade		**Tumor size**	
G1	3 (1.8)	**<2cm**	14 (8.6)
G2	116 (71.2)	**2- 5cm**	121 (74.2)
G3	44 (27)	**>5 cm**	28 (17.2)
NPI		**Operation Type**	

>2.4- 3.4	5 (3.1)	**Lt MRM**	100 (61.3)
>3.4- 5.4	125 (76.7)	**Rt MRM**	63 (38.7)
>5.4	33 (20.2)		
ER		**PR**	
Negative	33 (20.2)	**Negative**	39 (23.9)
Positive	130 (79.8)	**Positive**	124 (76.1)
Her2/neu		**Metastasis**	
Negative	89 (54.6)	**Negative**	139 (85.3)
Positive	74 (45.4)	**Positive**	24 (14.7)

Table 2: Correlations between NPI and different prognostic factors in BC patients.

Variables	**Mean ± SEM**	**P**	**Variables**	**Mean ± SEM**	**P**
Her2/neu			**Metastasis**		
Negative	4.57 ± 0.08		**Negative**	4.62	
Positive	4.82 ± 0.09	0.052	**Positive**	5.07	0.012
ER			**PR**		
Negative	4.61 ± 0.14		**Negative**	4.77 ± 0.14	
Positive	4.71 ± 0.07	0.56	**Positive**	4.66 ± 0.07	0.47
Cancer Stage			**Tumor size**		
T1	4.05 ± 0.12		**<2cm**	4.10 ± 0.19	
T2	4.66 ± 0.07	0.000	**2- 5cm**	4.62 ± 0.07	0.01
T3	5.34 ± 0.13	0.000	**>5 cm**	5.29 ± 0.14	0.000
P1	T2 VS T3	0.000	**P1**	2cm- 5cm VS	0. 000

Table 3: Correlations between Oxidant / Antioxidant profile and different prognostic factors in BC patients.

Variables	MDA	NO	SOD	TAC
NPI	**Mean ± SE**			
3.4-5.4	10.67 ± 0.82	12.68 ± 1.06	10.22 ± 0.26	3.33 ± 0.34
>5.4	10.78 ± 1.84	17.49 ± 2.09	11.11 ± 0.49	2.73 ± 0.83
P	0.95	0.04	0.11	0.45
Her2/neu				
Negative	10.25 ± 1.03	11.63 ± 1.22	11.02 ± 0.29	3.51 ± 0.5
Positive	11.32 ± 1.09	15.79 ± 1.40	9.58 ± 0.34	2.86 ± .36
P	0.47	0.02	0.002	0.296
Cancer Stage				
T1	9.25 ± 1.69	12.60 ± 2.32	10.53 ± 0.52	2.99 ± 0.67
T2	10.16 ± 0.83	13.32 ± 1.14	10.45 ± 0.28	2.92 ± 0.31
T3	15.99 ± 2.91	16.82 ± 2.71	9.39 ± 0.74	5.20 ± 1.65
(T1><T2) P	0.63	0.78	0.90	0.92
(T1><T3) P	0.04	0.02	0.20	0.01
(T2><T3) P	0.01	0.02	0.14	0.02
Tumor size				
<2cm	7.35 ± 2.01	11.33 ± 2.59	9.56 ± 0.67	2.53 ± 0.86
2- 5cm	10.35 ± 0.79	12.98 ± 1.12	10.62 ± 0.25	3.12 ± .30
>5 cm	14.11 ± 2.02	± 2.03 16.93	± 0.65 9.65	± 1.26 3.99
P	0.013	0.10	0.15	0.53
P1	0.03	0.10	0.92	0.34
P2	0.05	0.09	0.12	0.01
ER				
Negative	10.15 ±1.61	14.15 ± 2.01	10.11 ± 0.56	2.86 ± 0.5
Positive	10.89 ± 0.84	13.36 ± 1.05	10.43 ± 0.25	3.31 ± 0.38
P	0.69	0.72	0.57	0.49
PR				
Negative	10.52 ± 1.42	14.61 ± 1.88	9.89 ± 0.44	2.56 ± 0.42
Positive	10.81 ± 0.87	13.18 ± 1.07	10.51 ± 0.27	3.42 ± 0.39
P	0.86	0.51	0.23	0.141
Metastasis				
Negative	10.78 ± 0.80	13.23 ± 1.0	10.22 ± 0.25	3.36 ± 0.36
Positive	10.49 ± 1.98	15.19 ± 2.57	11.21 ± 0.46	2.36 ± 0.64
P	.892	.459	.070	.178

P= **<2cm vs 2- 5cm,** P1= **<2cm vs >5 cm,** P2= **<2- 5cm vs >5 cm.**

Table 4: Correlations between Oxidant and Antioxidant in response to NPI prognostic value in BC patients.

Oxidant/Antioxidant		NO	SOD	TAC
MDA	Correlation	-.110-	-.281-	.547
	Sig. (2-tailed)	.165	.000	.000
NO	Correlation		-.267-	-.255-
	Sig. (2-tailed)		.001	.001
SOD	Correlation			-.022-
	Sig. (2-tailed)			.785

Table 5: Correlations between Oxidant and Antioxidants in response to Oxidative Stress (MDA and NO) in BC patients.

MDA					NO		
SOD	**TAC**	**NO**	**Oxidant/Antioxidant**		**SOD**	**TAC**	**MDA**
.104	.009	.138	**NPI**	Correlation	.136	.050	.026
.190	.909	.080		Sig. (2-tailed)	.084	.531	.746
	.165	-.293-	**SOD**	Correlation		-.088-	-.317-
	.036	.000		Sig. (2-tailed)		.264	.000
		-.231-	**TAC**	Correlation			.541
		.003		Sig. (2-tailed)			.000

Table 6: Correlations between Oxidant and Antioxidants in response to Antioxidants (SOD and TAC) in BC patients.

SOD					TAC		
MDA	**NO**	**TAC**	**Oxidant/Antioxidant**		**MDA**	**NO**	**SOD**
.039	.166	.015	**NPI**	Correlation	.004	.144	.097
.620	.034	.846		Sig. (2-tailed)	.960	.067	.221
	-.190-	.564	**MDA**	Correlation		.037	-.320-
	.015	.000		Sig. (2-tailed)		.640	.000
		-.264-	**NO**	Correlation			-.263-
		.001		Sig. (2-tailed)			.001

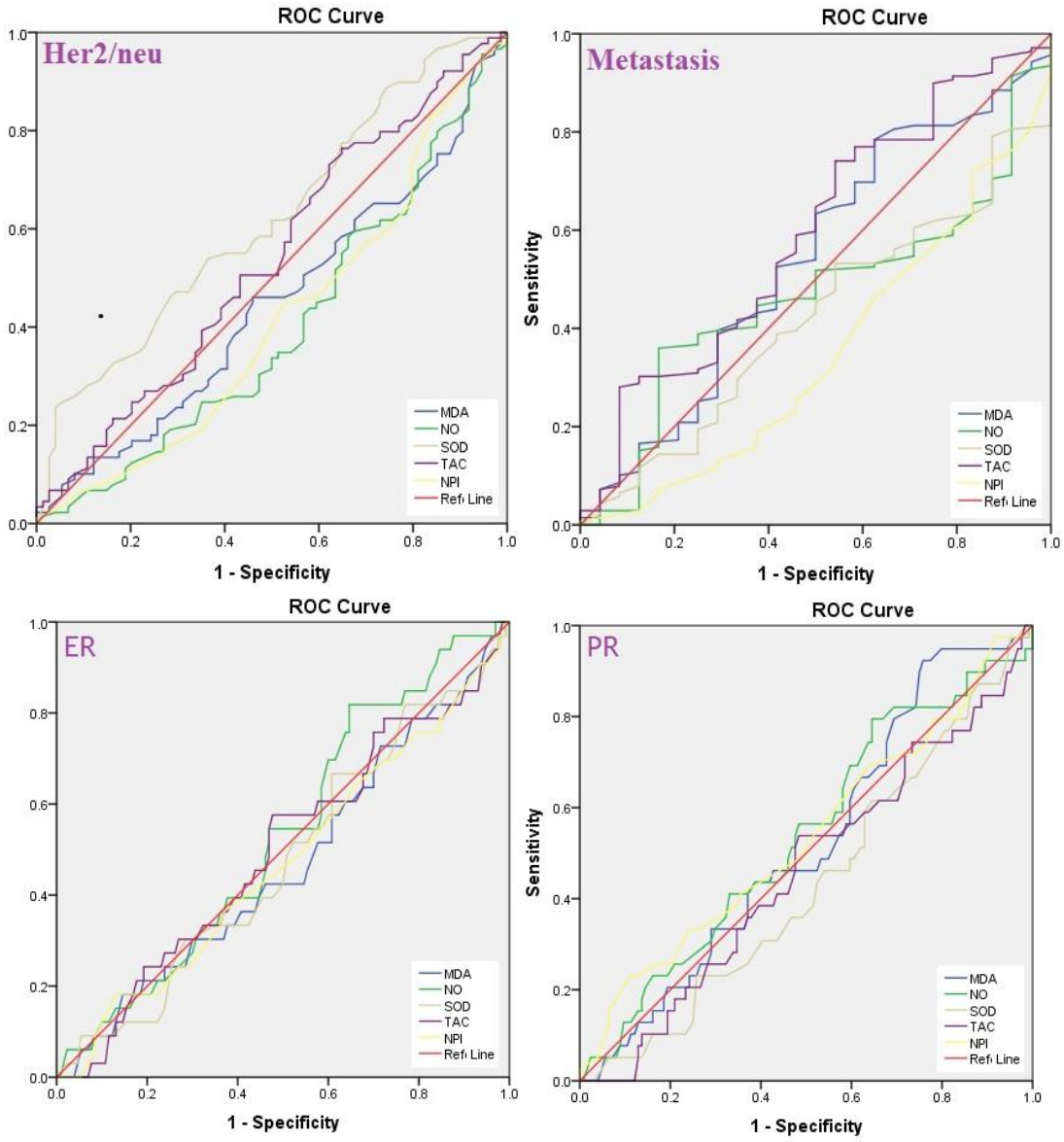

Figure 1: ROC curve for Oxidant and Antioxidants in response to different prognostic markers in Breast Cancer Patients.

Table 7: AUC, Sensitivity and Specificity of Oxidant and Antioxidants parameters in response to different prognostic factors in BC patients using ROC curve.

Parameters	Sensitivity %	Specificity %	AUC	CI^{95}	P
MDA					
ER	60.8	57.6	0.532	0.420- 0.643	0.576
PR	67.7	69.2	0.479	0.381- 0.578	0.694
Her2	56.8	50.6	0.554	0.466- 0.643	0.235
Met.	62.5	71.9	0.460	0.332- 0.588	0.535
NO					
ER	63.8	57.8	0.469	0.365- 0.574	0.587
PR	85.8	89.7	0.463	0.360- 0.567	0.491
Her2	86.5	80.9	0.602	0.514- 0.689	**0.026**
Met.	91.7	84.2	0.536	0.425- 0.646	0.577
SOD					
ER	94.6	90.9	0.525	0.415- 0.635	0.663
PR	92.7	87.2	0.568	0.468- 0.668	0.202
Her2	91.9	98.9	0.381	0.295- 0.467	**0.009**
Met.	100	89.9	0.582	0.470- 0.693	0.203
TAC					
ER	86.9	81.8	0.505	0.393- 0.617	0.931
PR	87.1	82.1	0.541	0.438- 0.644	0.438
Her2	85.1	89.9	0.463	0.373- 0.552	0.413
Met.	79.2	90.6	0.409	0.285- 0.533	0.155

AUC = Area under the ROC curve, CI=Confidence Interval, P=Significance level.

4. DISCUSSION

Breast cancer is known as complex and multifactorial disease, the initiation and development of the disease may results from the interaction between environmental and genetic factors. It is now well established that breast cancer is the most persistent diagnosed cancer worldwide and considered a chief reason of cancer mortality among females worldwide **[1].** In Egypt, the dispersal of BC is growing and it remains a major health problem of the country with no solution. It constitutes 33% of female cancer cases and more than 22,000 new cases diagnosed each year **[20].** This is expected to rise exponentially over the next years given the enlarging population. A three-fold increase is predicted by 2050 as recently reported by the National Cancer Institute (NCI), Egypt **[2].** Thus, it requires improved methods of screening, diagnosis, and treatment. In this disease, risk factors are multifactorial including obesity, delayed menopause, history of benign breast disease, genetics, and early menarche. These factors compromise all cellular mechanisms including cell proliferation, pathways of gene expression regulation, and apoptosis **[21, 22].**

In the present study, the Nottingham Prognostic Index NPI, was calculated to each case and correlated to different prognostic parameters in BC patients **[8],** where it shows significant increase in positive Her2/neu expression as well as positive metastasis ones. NPI also show a significant increase as the cancer stage and tumor size and no change in response to neither ER nor PR. It seems that we are the first to correlate this index to different prognostic parameters in BC patients.

Oxidative stress is a state of disequilibrium between pro-oxidant and antioxidant. Under normal physiological conditions, oxidants are neutralized by an enzymatic and non-enzymatic antioxidant defense system. If the free radicals are incompletely eliminated by antioxidants, they will cause an accumulation of ROS **[11- 13].** Some studies have reported higher serum MDA levels in breast cancer patients **[23- 25],** while some have reported lower levels **[22, 26].** Our findings supported the common observation that breast malignancies are related to an increased level of MDA as compared to healthy subjects (data not shown). Since our patients group was formed from newly diagnosed breast cancer patients, the increased level of oxidative factors can be a feature of the early stages of cancer progression. Increased MDA level in the serum of breast carcinoma was found to be significantly increased with the increase of tumor stage and tumor size. In this study, NO shows also significant increased with the increase of tumor stage and tumor size as well as predictive index NPI increase and with positive Her2/ neu expression.

The increase in serum lipid peroxidation in breast cancer seen in the present study was associated with enhanced antioxidant capacities. Increased generation of oxygen free radicals can induce TAC but not SOD, in concomitant to our findings, Gupta et al., **[21]** shows decreased SOD activity in breast cancer patients. An increase in SOD activities due to overexpression has been reported **[23].** In our study, SOD activities were found significantly lower in positive Her2/ neu expressed patients and not changed in response to other predictive markers, while the activity of TAC is significantly increased with the increase of tumor stage and tumor size.

Our finding showed that serum level of MDA was positively linked with the NO, SOD and TAC but not with NPI. Our findings could support the potential diagnostic value of MDA and NO in BC. To the best of our knowledge, this is the first study to report the sensitivity and specificity for MDA, NO, SOD and TAC in response to different prognostic factors in BC patients; previous studies focused only on one or few oxidant/antioxidant biomarkers to evaluate oxidative stress status in BC **[22, 23].** Despite supporting the association of the studied biomarkers with the occurrence and progression of BC, these reports were insufficient to reflect the true status of oxidative stress in those patients or reveal the potential clinical value of the studied biomarkers for the diagnosis or prediction of BC. Moreover, the studied panel of oxidation/antioxidant biomarkers may potentiate each other in amplifying their biological effects **[23].** Expected mechanisms for the increase of oxidative stress in breast cancer were supposed to induce genetic changes in antioxidant enzymes, estrogen treatment, increase of reactive oxygen species generation, as well as decrease in antioxidant system **[27].** There

are considerable facts documented the effects of free radicals, oxidative damage, and lipid peroxidation in initiation and development of cancer types such as breast cancer. The best method to evaluate oxidative stress is to measure the compounds obtained by the reaction of oxidants with biomolecules as a biomarker which is clinically important in evaluation and identifying cancers **[10, 21, 27, 28].**

The analysis of the receiver operating characteristic curve (ROC) for oxidant/ antioxidant was plotted and the area under each curve (AUC) was calculated. A better diagnostic value has been detected when the area under the ROC curve is large. Derouiche et al., **[22]** used the ROC curve to evaluate the diagnostic value of MDA, GSH and catalase for breast cancer. This study showed that oxidative stress markers NO and SOD in serum, have a significant correlation with breast cancer marker Her2/ neu expression, which can serve as a sensitive indicator of a cancer diagnosis. The high sensitivity and specificity of NO and SOD in serum is a very sensitive marker to oxidative stress. In the current study, ROC curve analysis of oxidant/ antioxidant revealed that SOD and NO have potential diagnostic value in BC patients, where it shows a highly sensitivity and specificity. Previously, the performance characteristics of other oxidative stress biomarkers revealed that oxidant stress had a better BC diagnostic value than total antioxidant status **[29].**

5. CONCLUSION

In conclusion, the findings of the present study show that patients with breast cancer are more exposed to oxidative stress with higher free radical production increased oxidative stress as evidenced by an increase in oxidant markers MDA and NO and a decrease in antioxidant marker SOD. This oxidative stress is related to Her2/neu expression marker of breast cancer. This study is confirming the importance of preventing oxidative stress to prevent the development/progression of breast cancer where oxidative stress plays an important role. Further understanding of tumor biology from the standpoint of reactive oxygen species may be helpful for establishing a new strategy for cancer therapy.

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DEFINITIONS, ACRONYMS, ABBREVIATIONS

Abbreviations:

BC- Breast Cancer, Her2- Human epidermal growth factor receptor 2, ER- estrogen receptor, PR- progesterone receptor, IRB- Institutional Review Board, NPI- the Nottingham Prognostic Index, MDA- malondialdehyde, NO- nitric oxide, SOD- superoxide dismutase, TAC- total antioxidant capacity, ROS- reactive oxygen species, RNS- reactive nitrogen species, NCI- National Cancer Institute, REDOX- reduction- oxidation, ROC- receiver operating characteristic curve, AUC- area under each curve.