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8-Hydroxy-2'-deoxyguanosine as a Discriminatory Biomarker For Early Detection of Breast Cancer

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ABSTRACT

Background: Breast cancer (BC) is one of the most prevalent and reported cancers among Saudi women. Detection of breast cancer in early invasive stage (stages I, II) has an advantage in treating patients than late invasive stage detection (stages III, IV). Tumor markers are used to aid in diagnosis, treatment monitoring and recurrence detection of malignant tumors. 8-hydroxy-2'-deoxyguanosine (8-OHdG) is a marker of nucleic damage due to oxidative stress.

Patients and Methods: We studied the blood levels of 8-OHdG in fifty women with benign breast tumor and fifty women with breast cancer and fifty healthy women as a control group.

Results: The concentrations of 8-OHdG were significantly increased in breast cancer group (55.2 ng/dl) compared with benign tumor group (30.2 ng/dl) in comparison with the healthy control group (9.08 ng/dl). The same pattern was observed with other diagnostic markers carcinoembryonic antigen (CEA) and cancer antigen 15-3 (CA 15-3). Significant positive correlations between 8-OHdG and both CEA (r=0.63, P<0.001) and CA15-3 (r=0.51, P<0.001) were noticed. The levels of 8-OHdG were significantly higher in stage I (81 ng/dl) comparing to stage II (51 ng/dl, P<0.05), stage III (38 ng/dl, P<0.01) and stage IV (19 ng/dl, P<0.001). In addition, serum 8-OHdG had high diagnostic performance in breast cancer (AUC= 0.86, sensitivity=82%, specificity= 80% at cutoff value 21.4 ng/ml). 8-OHdG is associated with breast cancer risk according to the logistic regression analysis.

Conclusion: We concluded that the significant increase of serum levels of 8-OHdG in breast cancer patients can be used as a potential non-invasive biomarker for early detection of breast cancer. However, large sample size from different stages and types of breast cancer should be included in any future study to confirm the present findings before translating the findings into routine clinical application.

***Key Words**:* biomarker; oxidative damage; DNA; 8-OHdG; breast cancer

Introduction

Breast cancer (BC) is considered as the most common cancer in the women population worldwide, and represented about 30% of all new cancer diagnosis in women. BC is known as an estrogens-dependent disease. It is characterized by high rate of mortality, so it considered as an aggressive malignant tumor (1). In Saudi Arabia, BC is one of the leading causes of cancer-related death that affects the health status and quality of life of saudi women. However, BC is unlike the prostatic cancer (PC) or the liver cancer (LC) that diagnosed by specific markers such as prostate specific antigen (PSA) and α-fetoprotein (AFP) respectively. Nowadays, there is no biomarker recommended for the early warning of breast cancer in clinical practice except for the invasive genetic test of BRCA1/2 mutation, that evaluate the risk of hereditary breast cancer (2).

Generally, the advanced invasive stages (III and IV) of BC have a poor prognosis even after performing the recommended treatment. However, the prognosis and the survival rate of BC are increasing in the early invasive stages (I and II) (3). Therefore, there is a demand for early diagnosis and detection of BC in order to improve the survival rate and the prognosis in treating the BC women.

Today, the screening and diagnosis of BC mainly depend on the result of the mammography. The high false positive results of mammography lead to the needs for further expensive and invasive diagnostic techniques such as magnetic resonance imaging (MRI) and needle biopsies. The cost and the mental stress of both MRI and fine needles aspiration of biopsies are high. Given that only a small percentage of the investigated women have cancer and the majority has only benign masses. A robust, accurate and non-invasive diagnostic test is urgently required to minimize the need of such expensive and invasive diagnostic tests for those women with benign tumors. Therefore, the screening of BC, especially the discrimination of early invasive stage BC from benign lesions, is urgently needed in clinical practice.

Immunoassay technique has important advantages being simple, inexpensive, and highly sensitive has attracted great attention in the field of diagnosis and screening of cancer. Several commonly used serum diagnostic biomarkers play an important role in the diagnosis of different types of cancer including BC such as CA15-3 and CEA. However, little attention has been paid to their ability to differentiate between breast cancer and benign breast lesions.

The increasing production rate of ROS leads to many modifications in nucleotide base of DNA. These oxidative modifications produce several base lesion substances (4). Guanine base has the lowest oxidation potential comparing to other bases. Therefore, the guanine residues are more susceptible to the free radical attack, resulting in the formation of 8-hydroxy-2'-deoxyguanosine (8-OHdG). 8-OHdG got greater attention by the scientific researchers and commonly selected as a biomarker of oxidative stress indicating the DNA damage. This DNA damage lesion (8-OHdG residues) produces transversion-mutation by pairing with adenine or cytosine in replication process (GC to TA) (5). This mutation type was considered the second major somatic mutations expressed in human cancers. Therefore, the presence of 8-OHdG in cells indicating the ability of mutagenesis and increase the possibility of carcinogenesis (5). Permanent oxidative stress lesions lead to cancer (6). Previously, 8-OHdG was greatly evaluated in animal models and human in both cells and tissues (6-8). 8-OHdG has been used widely in many studies not only as a biomarker for the measurement of endogenous oxidative DNA damage but also as a risk factor for many diseases including cancer (9)

The levels of 8-OHdG were highly determined in breast cancer cells and tissues compared to normal cell lines and tissue. Significantly higher levels of 8-OHdG in both cells and tissues of breast cancer were found compared to those of non-cancerous breast (6). Similarly, the blood levels of 8-OHdG in breast cancer patients increased comparing to healthy controls (8). These interesting evidences encouraged us to propose that 8-OHdG as a biomarker of DNA damage due to oxidative stress can be an effective discriminatory biomarker in the early detection and determination of the people at high risk of cancer for screening approach, treatment and prognosis of BC.

The common tumor markers; carcinoembryonic antigen (CEA) and cancer antigen 15-3 (CA15-3) have been given much attention in the recent years as a prognostic factor of BC (10). The levels of preoperative CEA and CA15-3 serve as a good confirmatory indicator for oncologist for the diagnosis and the selection of the proper treatment of BC (11, 12). In 2005, the European Group on Tumor Markers has recommended using the levels of both markers; CEA and CA15-3 in the evaluation of prognosis, the early detection, and treatment of BC patients (13). In 2007, the guidelines of the American Society of Clinical Oncology (ASCO) stated that "do not recommend the use of serum CA 15–3 and CEA for or screening,

diagnosis, staging, or routine surveillance of breast cancer patients after primary therapy" (14).

The previous work showed 8-OHdG levels was high urine samples of BC patients compared to control groups (15-17). In addition, other groups studied the role of 8-OHdG in breast cancer, and found that the levels were higher in BC patients (16). However, its diagnostic role at different stages of breast cancer has not been investigated previously; therefore, there is a rationality to assess the levels 8-OHdG in BC patients at different stages of the disease.

For early cancer initiation, several molecular modifications take place that assist cancer driving at initial stages. Among of these alterations is DNA damage, accumulation of DNA damage in combination with poor DNA repairing mechanism results in cancer cells formation. To explain why the levels of oxidative stress marker is low at later stages of breast cancer comparing to early stage of the cancer, one possible explanation is that at early stages of cancer patients could be exposed to high rate of endogenous and exogenous oxidative stress. The exogenous stress could be diminished at later stages of breast cancer

In this study, the blood levels of 8-hydroxy-2'-deoxyguanosine (8-OHdG) as biomarkers of DNA damage by oxidative stress combined with common tumor markers; carcinoembryonic antigen (CEA) and cancer antigen 15-3 (CA15-3) were evaluated in benign and malignant BC in comparison to their levels in normal healthy women. The levels of the studied parameters in different invasive stages of BC (I-IV) were investigated, in order to, distinguish the early invasive stages (I and II) of BC from benign tumor patients and to test (8-OHdG) as a biomarker for risk estimation, early screening and for further detection of breast cancer.

Materials and Methods

Subjects

This study included 50 female patients with benign breast mass and 50 female patients with malignant breast cancer mainly of postmenopausal age not receiving antitumor therapy (Table 1). Patients were selected and examined at the oncology clinic of King Abdallah Medical City, In Makkah, during the period between October 2014 and March 2017. The controls are 50 volunteer healthy women. Fasting blood sample was collected. Serum was

separated by centrifugation (3500-4000 rpm) of clotted samples and stored at -20 °C until analysis.

Ethics Statement

This study was carried out in accordance with the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the medical ethics committee of the Faculty of Medicine-Umm Al-Qura University and the medical ethics committee of King Abdallah Medical City, Makkah, KSA. Written informed consent was obtained from every participated patient.

Determination of serum levels of 8-OHdG

8-OHdG serum levels were determined by a competitive inhibition enzyme immunoassay kit, (EU2548, Wuhan Fine Biological Technology Co., Ltd. Wuhan, China) according to the provided assay procedure. (http://www.fn-test.com).

Determination of serum levels of carcinoembryonic antigen (CEA)

Serum levels of carcinoembryonic antigen (CEA) were determined by *an in vitro* enzyme-linked immunosorbent assay kit (SEA150Hu, cloud-clone crop, Houston, USA) according to the manufactory instructions and provided assay procedure. (http://cloud-clone.com).

Determination of Serum levels of cancer antigen 15-3 (CA15-3)

Serum levels of cancer antigen 15-3 (CA15-3) were determined by a solid phase *in vitro* enzyme-linked immunosorbent assay kit (MBS580044, MyBioSource.Com. San Diego, USA) according to manufactory instructions. (http://mybiosource.com).

Evaluation of diagnostic performance of serum 8-OHdG using ROC curve analysis

We applied to our data set the analysis of Receiver Operating Characteristic (ROC) curve. Accuracy was measured by the area under the ROC curve. An area of 1 represents a perfect test; an area of 0.5 represents a worthless test. A rough guide for classifying the accuracy of a diagnostic test is the traditional academic point system: 0.9-1 = excellent (A), 0.8-0.9 = good (B),0.7-0.8 = fair (C),0.6-0.7 = poor (D), and 0.5-0.6 = fail (F).

Relation between serum 8-OHdG and the risk of breast cancer (odd ratio)

We assumed that the high level of oxidative damage biomarker 8-OHdG is a risk factor for developing the breast cancer. This study was a case control design, so the estimated odd ratio

of breast cancer risk was calculated according to quartiles of serum 8-OHdG levels using binary logistic regression analysis.

Statistical analysis

The results were statistically processed by SPSS 24 software using parametric (Studen's t test) and nonparametric Spearman's correlation. The differences were considered significant at p value <0.05.

Results

The clinical and demographic characteristics of subjects:

Blood samples from diagnosed breast cancer patients were collected from all patients prior to any treatment. The diagnosis was confirmed by histopathology, clinical data as well as the medical records. The clinical details and demographic characteristics of both BC and Benign patients are summarized in Table 1. The BC and benign patients were age matched with control subjects. Out of 50 BC patients 6 (12%) patients were grade I, 31 (62%) were grade II, 11 (22%) were grade III, and 2 (4%) were grade IV (Table1). According to immunohistochemistry data estrogen-receptor-positive (ER+) sample were 35 (70%), progesterone-receptor- positive (PR+) were 28 (56%), and human epidermal growth factor receptor 2 positive (Her2+) were 14 (28%) (Table 1). Out of 50 benign patients with benign breast mass, 39 were diagnosed as fibroadenoma, while 11 patients were diagnosed as other types including granulomatous mastitis, papilloma, fibroglandular tissue, ductal ectasia…etc. (Table 1).

Serum levels of 8-OHdG (A), CA15-3 (B), and CEA

The serum level of 8-OHdG in BC was highly significantly increased in BC patients than in patient with benign lesion with the mean value of 55.21 ng/dl and 30.21 ng/dl (P<0.001), respectively (Table 2 and Fig. 1). In comparison with normal health control (9.08 ng/dl), the serum levels 8-OHdG in both BC and benign groups were significantly higher (P<0.001).

Interestingly, the mean value of the levels of other studied two parameters CEA and CA15-3 were sharply increased in BC group comparing to control group 472.56 ng/dl (P<.001) and 57.28 ng/dl (P<.001), respectively (Table 2 and Fig. 1). By contrast there were no significant difference between the levels of CEA (328.42 ng/dl) and CA15-3 (15.16 ng/dl) in benign group comparing to control group. There was a significant difference between BC and benign group in the levels of both parameters CEA and CA15-3 (P<0.001) as shown in figure.

Serum levels of 8-OHdG increased in early invasive of BC

The levels of 8-OHdG were significantly higher in stage I (81 ng/dl) comparing to stage II (51 ng/dl, $P<0.05$), stage III (38 ng/dl, $P<0.01$) and stage IV (19 ng/dl, $P<0.001$). While the levels of CA15-3 and CEA showed non-significant difference among the different invasive stages of BC (Fig. 2).

Changes of the levels of 8-OHdG in BC patients according to clinical presentations

The serum levels of 8-OHdG, CA15-3, and CEA in the BC patients with different clinical presentations; mass (A), pain (B), and discharge (C) were presented in Figure 3. The levels of 8-OHdG and CA15-3 were significantly lower in BC patients with pain ($P<0.01$) and discharge ($P<0.001$). However, in BC patients with mass significant increased levels of 8-OHdG and CA15-3 ($P<0.001$) were detected. There was non-significant difference in the levels of CEA among the different clinical observation for BC patients.

Association between predictive immunohistochemistry (IHC) and 8-OHdG

Serum levels of 8-OHdG, CA15-3, and CEA in the BC patients with different histopathology observations (A) positive estrogen receptors (ER), (B) positive progesterone receptors (PR), and (C) positive human epidermal growth factor receptor-2 (Her2/neu) are illustrated in figure 4.

Relation of 8-OHdG and family history of BC, Metastasis

The levels of 8-OHdG, CA15-3, and CEA were increased in BC patients with family history of BC and metastasis as well as they increased in the samples with invasive lobular carcinoma more than samples with invasive ductal carcinoma (Fig 5).

Correlations of 8-OHdG with CA15-3 and CEA

The studied marker 8-OHdG showed significant positive correlations with CEA ($r = 0.63$, $P<0.001$) and CA15-3 ($r = 0.51$, $P<0.001$) . Non-significant positive correlation between CEA and CA15-3 was observed ($r= 0.21$) as shown in figure 6.

Diagnostic performance of serum 8-OHdG for breast cancer

The analysis of ROC curve of 8-OHdG serum levels of studied subjects was applied, in order to know how well the 8-OHdG test discrimination between the samples with and without BC. Figure 7 shows the area under the ROC curve. Significant area under the curve (AUC) was observed from data analysis of ROC curve (0.86, $P<0.001$). The sensitivity (82%) and specificity (80%) were selected at cutoff value of 8-OHdG equal to 21.4 ng/ml (Table 3).

Serum 8-OHdG and the risk of breast cancer

The estimated odd ratio of breast cancer risk was calculated according to quartiles of serum 8-OHdG levels using binary logistic regression analysis. Table 4 show the significant increase by ~ 74 times in the highest quartile group (high risk) of 8-HOdG levels compared to the lowest quartile group (low risk). Odd ratio was 74.1($P<0.001$)

223 **Table (1): The clinical and demographic characteristics of patients participating in the study**

Clinicopathological Factors	Characters	Benign	Malignant
Nationality	*Saudi*	47 (94%)	33 (67.3%)
	Non-Saudi	3 (6%)	16 (32.7%)
Marital Status	*Married*	30 (60%)	36 (72%)
	Single	19 (38%)	9 (18%)
	Divorced	1 (2%)	2 (4%)
	Widowed	-	3 (6%)
Parity	*Parity*	25 (50%)	35 (70%)
	Non-Parity	25 (50%)	15 3(0%)
Lactation (In past)	*Lactation*	27 (54%)	34 (68%)
	No-lactation	23 (46%)	16 (32%)
Menstrual phase (in present)	*Pre-Menopause*	48 (96%)	29 (58%)
	Post-Menopause	2 (4%)	21 (42%)
Oral contraceptive (past or present)	*OCP*	11 (22%)	20 (40%)
	No-OCP	39 (78%)	30 (60%)
Family history of BC	*Family history of BC*	6 (12%)	7 (14%)
	No- Family history of BC	44 (88%)	43 (86%)
Medical History of CD (e.g. HTN, DM, Asthma, Hypothyroid)	*History of CD*	9 (18%)	21 (42%)
	No-history of CD	41 (82%)	29 (58%)
Clinical observation 1	*Mass*	41 (82%)	48 (96%)
	No-Mass	9 (18%)	2 (4%)
Clinical observation 2	*Pain*	19 (38%)	9 (18%)
	No-Pain	31 (62%)	41 (82%)
Clinical observation 3	*Discharge*	4 (8%)	2 (4%)
	No-Discharge	46 (92%)	48 (96%)
Side of complained	*Right Breast*	27 (54%)	22 (44%)
	Left Breast	17 (34)	28 (56%)
	Both side	6 (12%)	-
Benign Types	*Fibroadenoma*	39 (78%)	-
	Others	11 (22%)	-
Cancer types	*Invasive/Infiltrating Ductal Carcinoma*	-	47 (94%)
	Invasive Lobular Carcinoma	-	3 (6%)
Cancer grade	*Stage I*		6 (12%)
	Stage II		31 (62%)
	Stage III		11 (22%)
	Stage IV		2 (4%)
Immunohistochemistry (IHC)	*Estrogen receptors: ER*		35 (70%)
	Progesterone receptors: PR		28 (56%)
	Human epidermal growth factor receptor-2: Her2		14 (28%)
Metastasis	*Metastasis*		21 (42%)
	No- Metastasis		29 (58%)

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226 **Table (2): Serum levels of studied biomarkers in normal, benign, and malignant groups of patients.**

		Normal	Benign	Malignant
8-OHdG (ng/ml)	**Mean ± SE**	9.08 ± 0.93	30.19 ± 4.24	55.21 ± 5.85
	Range	1.20 – 20.30	8.4 – 87.9	11 – 133.2
	n	38	29	38
CEA (ng/ml)	**Mean ± SE**	314.55 ± 15.67	328.42 ± 25.27	472.56 ± 44.96
	Range	148 – 494	107 – 780	137 – 990
	n	40	38	39
CA15-3 (U/ml)	**Mean ± SE**	14.35 ± 1.07	15.16 ± 0.91	57.28 ± 8.89
	Range	3.3 – 24.7	4 – 29	17.1 – 170
	n	29	44	32

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228 **Table (3): Diagnostic data of serum levels of 8-OHdG using ROC curve**

AUC	SE	Asymptotic Significance	Asymptotic 95% Confidence Interval		Cutoff value ng/ml	Sensitivity (%)	Specificity (%)
			Lower	Upper			
0.86	0.03	0.001	0.79	0.93	21.50	82%	80%

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232 **Table (4): Analysis of binary logistic regression analysis of serum 8-OHdG and the risk of breast cancer.**

Odd ratio (OR)	Significance	95% Confidence Interval (CI)	
		Lower	Upper
74.1	0.001	8.97	613.56

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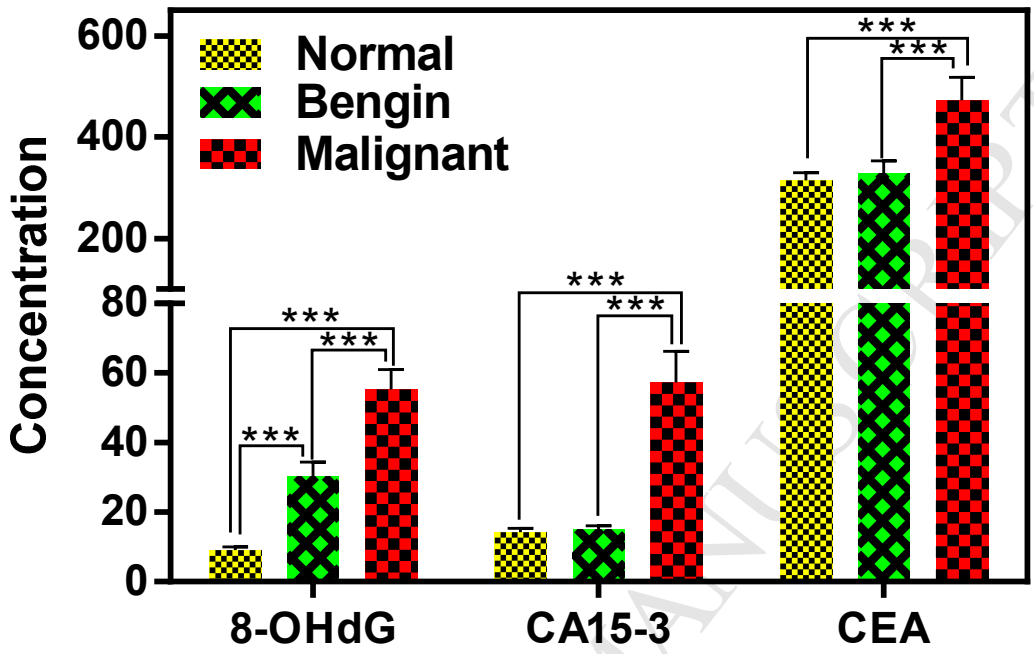
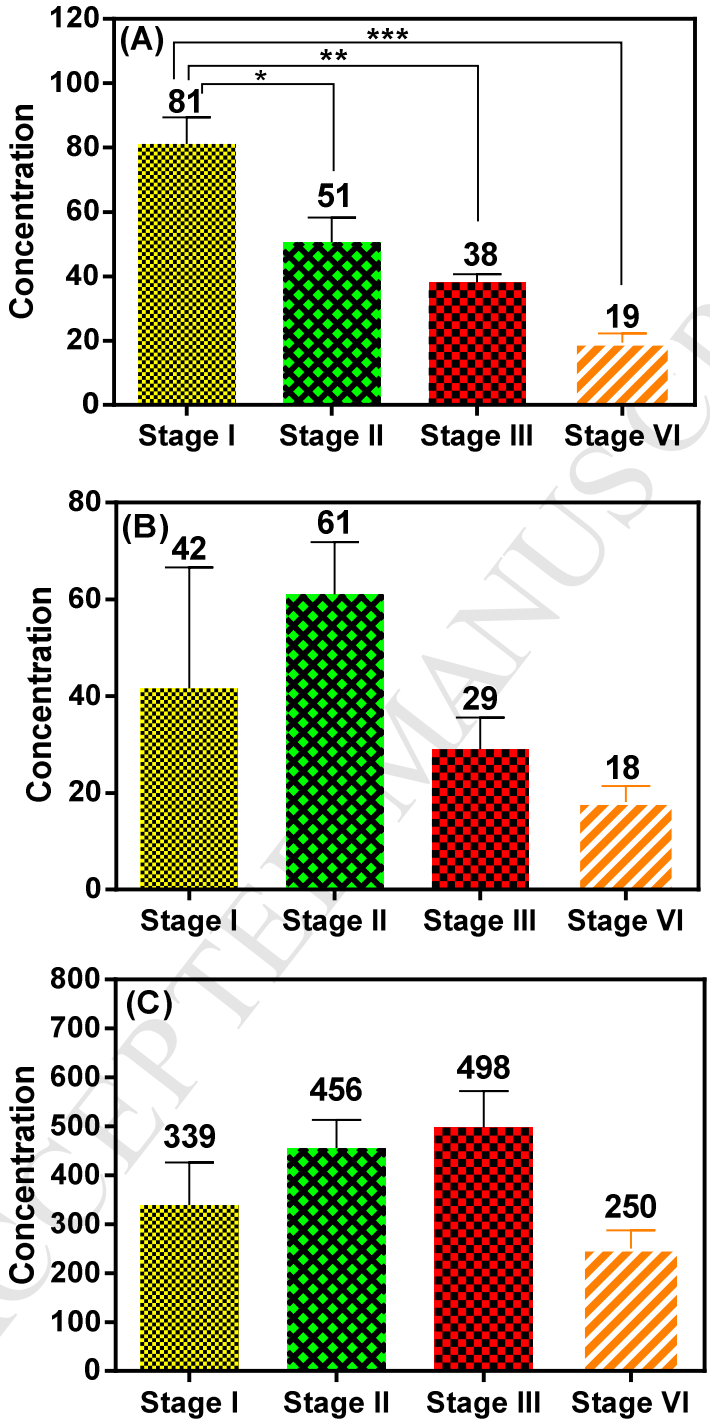


Figure 1: Serum levels of 8-OHdG, CA15-3, and CEA in comparison of benign and BC with normal group.



245

246 **Figure 2:** Serum levels of 8-OHdG (A), CA15-3 (B), and CEA (C) in different invasive
247 stages of BC.

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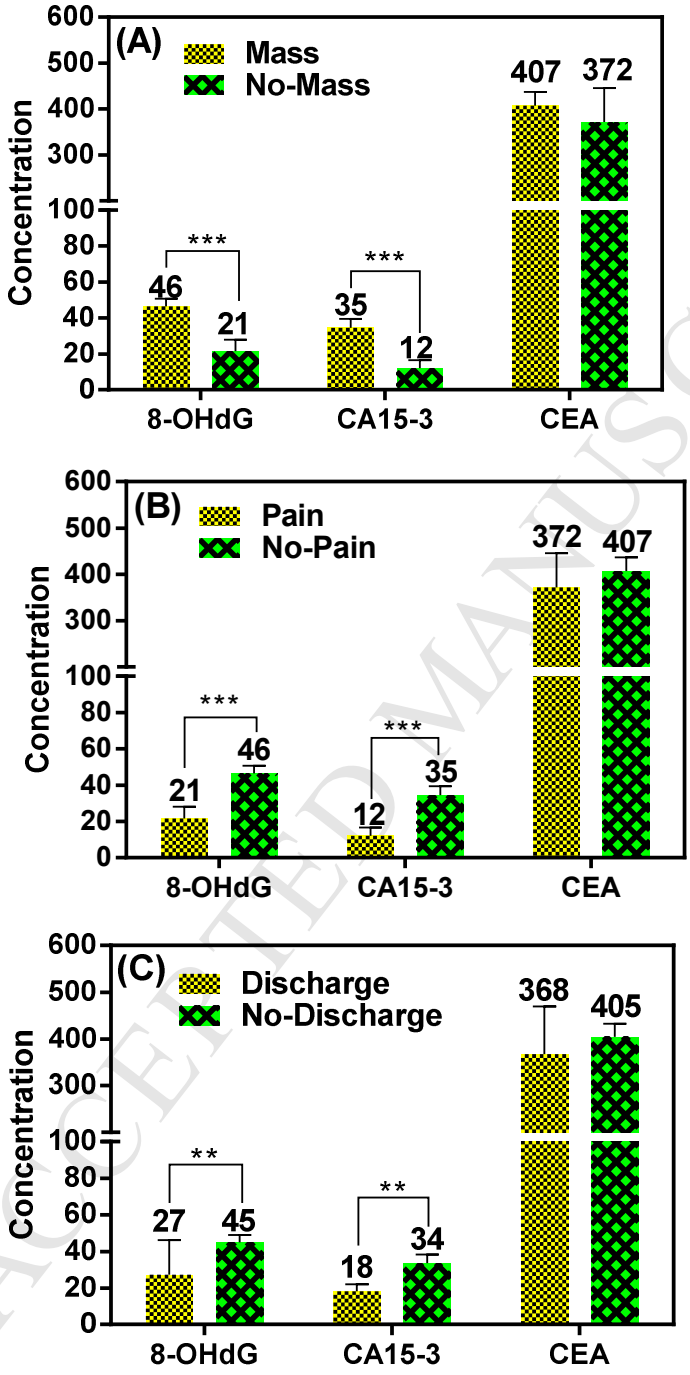

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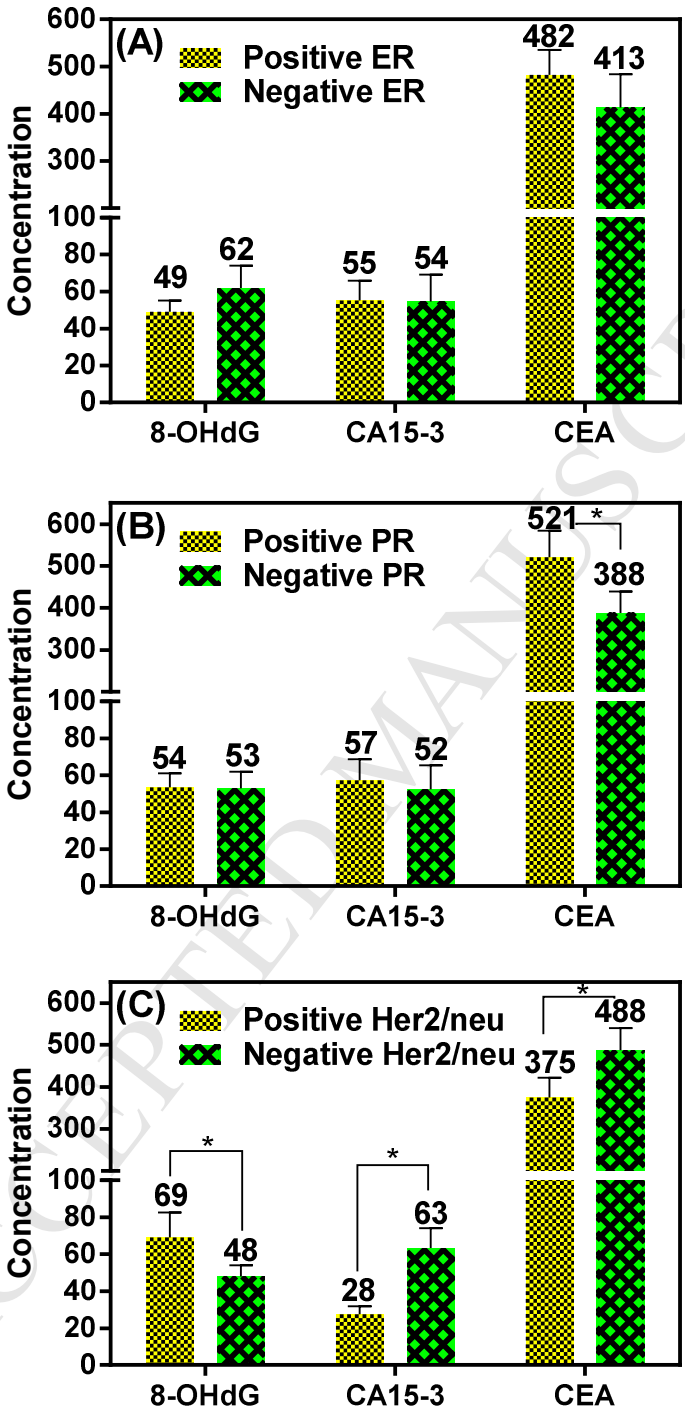
250 **Figure 3:** Serum levels of 8-OHdG, CA15-3, and CEA in the BC patients with different
251 clinical presentations mass (A), pain (B), and discharge (C).



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253 **Figure 4:** Serum levels of 8-OHdG, CA15-3, and CEA in the BC patients with different

254 histopathology observations

255 (A) positive estrogen receptors (ER), (B) positive progesterone receptors (PR), and (C)
256 positive human epidermal growth factor receptor-2 (Her2/neu).

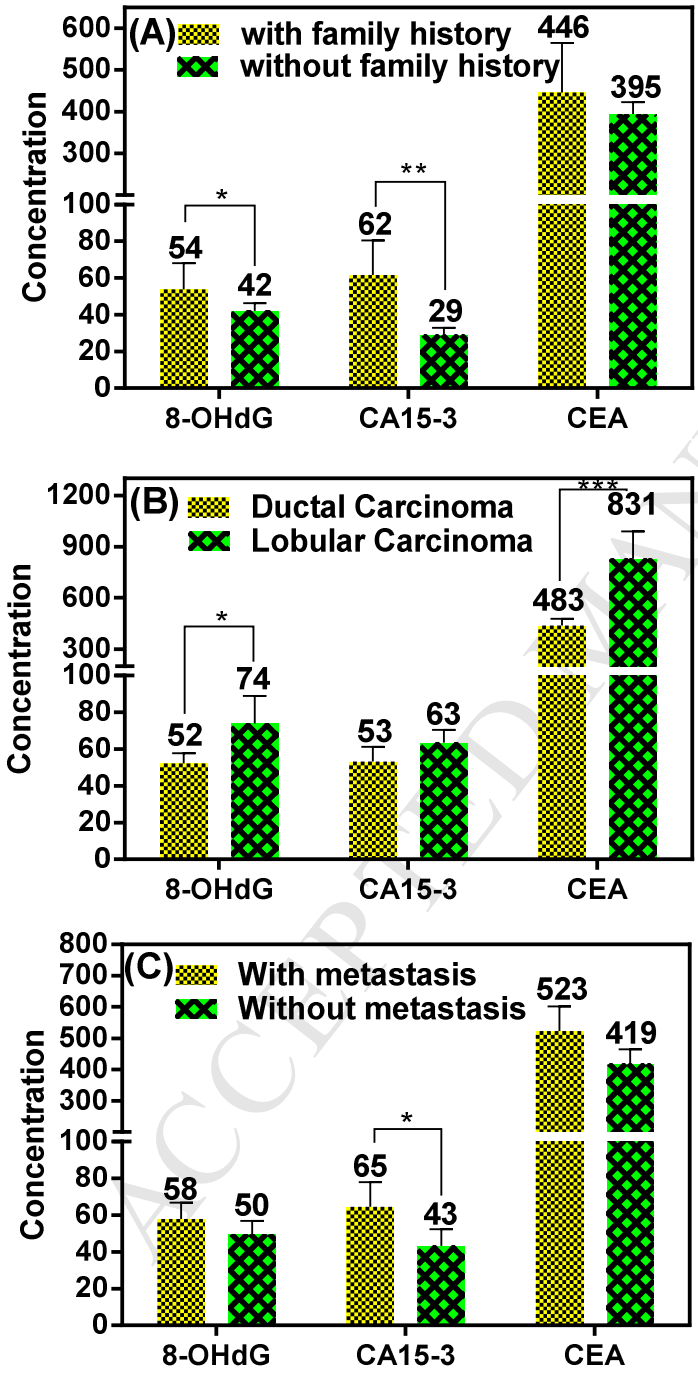

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Figure 5: Serum levels of 8-OHdG, CA15-3, and CEA in the BC patients with and without family history of BC (A), with different BC types either invasive/infiltrating ductal carcinoma or invasive lobular carcinoma (B), and in patients with and without metastasis.

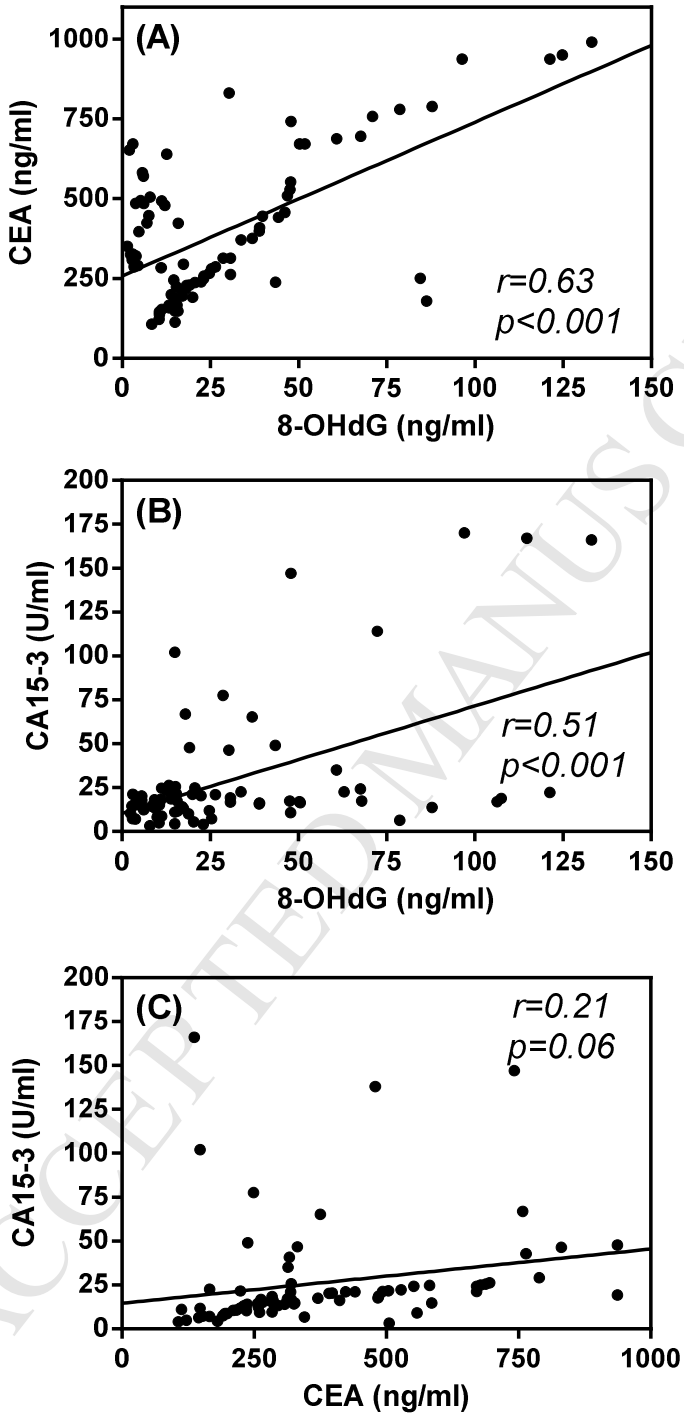

Figure 6: Correlation between the serum levels of 8-OHdG, CA15-3, and CEA.

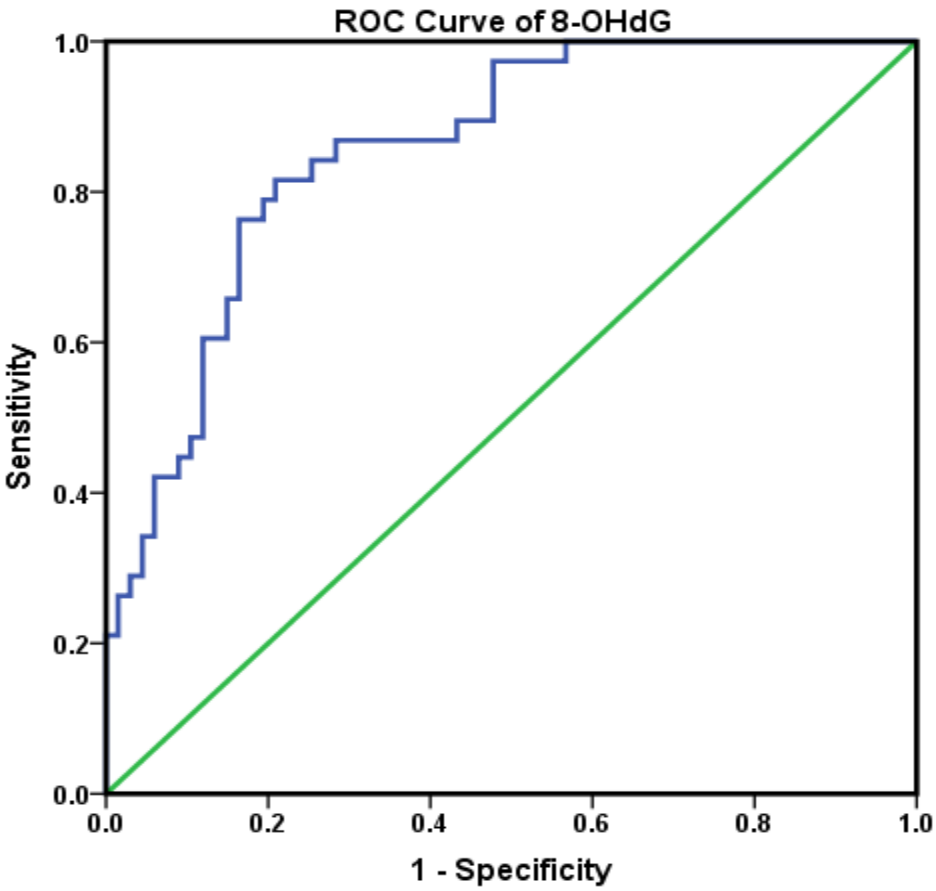

Figure 7: The Receiver Operating Characteristic (ROC) curve of serum levels of 8-OHdG of studied subjects.

ROC is a plot of the sensitivity (true positive rate) at y-axis against the 1-specificity (false positive rate) at x-axis for the different possible cut-points of 8-OHdG diagnostic test. When blue curve is closer to follows the left-hand border and then the top border of the ROC space then the test is more accurate. The area under the curve =0.86 is indicating the good accuracy of 8-OHdG test.

Discussion

Oxidative stress has been considered as a cause and/or a reason for breast cancer. The extensive damage of DNA leads to the production of oxidative stress at normal physiological conditions (18). One of the most prominent product of oxidative DNA damage is 8-Hydroxy-2′-deoxyguanosine (8-OHdG), which was recently used as reliable and sensitive marker of oxidative stress and carcinogenesis, is found in high levels in biological fluids of several cancer patients (19).

In this study, the levels of 8-OHdG were high in BC compared to the benign lesion and healthy control groups, which is compatible with previous published studies (15-17, 20). , 8-OHdG was higher in pre-operative BC patients following the postoperative than normal controls (21). Additionally, the levels of 8-OHdG were found to be elevated in breast cancer patients compared to healthy controls (15). Kuo, et al., found that the urine levels of 8-OHdG were significantly higher in patients with BC compared to control group that supported our findings (15). Berstein et al, reported that the serum levels of 8-OHdG increased in patients with BC. The presence of diabetes mellitus (DM) was significantly elevated the levels of 8-OHdG in BC group comparing to BC patients without DM (16). Therefore, it would be important taking in account the chronic diseases status such as DM, hypertension and osteoporosis during measuring oxidative stress biomarkers in cancer patients.

Our study observed a significant gradual decrease in the 8-OHdG levels in invasive BC stages from stage I to stage IV, while no significant differences were observed in CA15-3 and CEA levels. These results agreed with previous reports that found that expression of 8-OHdG in breast tissues decreased with each stage of breast carcinoma (6, 15). Recently, Guo et al., 2017 have reported that the benign lesion and early stage breast cancer could be differentiated by detection of 8-OHdG (22). Furthermore, the levels of 8-OHdG significantly decreased in the invasive breast carcinomas, compared to non-invasive lesions in the patients of BC with different degrees of malignancy. These similar data support our results and indicate that 8-OHdG concentrations are strongly dependent on tumor type and stage (23).

In other cancer type, such as, lung cancer, it was reported that the levels of 8-OHdG decreased in advanced cancer stages comparing to the early stages. Yano et al. studied the urinary levels of 8-OHdG in lung cancer patients; they noticed that the average of 8-OHdG in

the late stages of the disease was significantly lower in patients in the early stage of the disease. Although the previous studies used urine for measurement of 8-OHdG, it supported our findings of the potential use of 8-OHdG in the diagnosis of cancer in early stages(24).

Our findings observed that the studied parameters 8-OHdG and CA15-3 significantly increased in BC patient with clinical observation of breast mass presence, while these results opposed in BC patients with pain or discharge. At the molecular levels, there are several studies that reported the expression of 8-OHdG is significantly difference in cancerous and non-cancerous breast tissues (6, 20). These data with our finding are supporting the hypothesis that oxidative DNA damage is an important risk factor for breast cancer. However, others observed no significant differences in 8-OHdG levels in cancerous versus noncancerous tissue (25, 26). One of the explanations of the contradicted data is the methodological problem that arises during isolation and extraction of the DNA from the samples includes oxidation and degradation of DNA content.

The levels of 8-OHdG, CA15-3, and CEA were significantly higher in BC patients with positive Her2/neu. There was no difference in the levels of these parameters in BC patient with positive ER and PR test. Previously, Sova et al., found that there was no significant association between 8-OHdG levels and BC patients who had negative ER, PR, Her2/neu (27). For example, our result might be helpful in confirmation of Her2/neu positive test, thus can determine which patients may get benefit from Her2/neu-targeted therapy such as: trastuzumab (Herceptin®); lapatinib (Tykerb®); pertuzumab (Perjeta®) and T-DM1 (Kadcyla®). These targeted treatments can improve survival rate in patients with Her2-positive invasive breast cancer. The average level 8-OHdG is slightly higher in breast cancer patients that had negative estrogen receptor. This could be used to aid the targeting therapy when using estrogen-targeting drugs in breast cancer patients such as Tamoxifen and/ or aromatase inhibitors (28). However, more work should be conducted and include large sample size to investigate the potential discrimination role of 8-OHdG in estrogen receptor status. Therefore, More genetic studies should be conducted to reveal the correlation between the biomarkers of breast cancer especially serum 8-OHdG and genetic background and activity of the previous targets in order to apply for a specific therapy that would ultimately give a better outcome.

In the current study, significant positive correlations between 8-OHdG and both studied biomarkers; CA15-3 and CEA were observed. Our results indicate that the pattern of 8-OHdG concentrations in malignant, benign, and normal samples has the similar fashion of the pattern of both established biomarkers. This similarity confirms that 8-OHdG is important oxidative biomarker which could be approved as a diagnostic tool for breast cancer. However, large-scale study that includes more patients in different stages of BC would be important before starting any clinical trial to evaluate the use 8-OHdG in the diagnosis of BC in early stages.

Ductal carcinoma in situ DCIS is a non-invasive type of breast cancer (29). In our study, we have not assessed 8-OHdG in DCIS patients due the lack of samples for the study. Therefore, future work should include larger sample that includes patients with DCIS to clarify if 8-OHdG is high in this group of patients.

Strategies for prevention of accumulation of oxidative stress should be considered, in order to protect the highly risk groups of women from breast cancer. Consuming natural products that are highly content of antioxidants constituents would balance the potential harms of oxidative stress. For example, Cruciferous vegetable intake reduced the levels of oxidative stress in postmenopausal women and women with history of breast cancer (30). Furthermore, Lycopene (carotenoid) in tomatoes showed to be an antioxidant against that is balance the effects of free radicals and hence diminishes oxidative stress (31, 32). Green tea polyphenols consumption diminished 8-OHdG urinary levels in individuals who at high risk of liver cancer (33). Garlic also diminished 8-OHdG levels in brain and plasma of rats that exposed to moderate levels of radiation (34). The previous examples showed the protective effects of some natural products against oxidative stress; therefore, management of accumulation of oxidative stress would be a protective barrier in front of malignant transformation at highly risk group.

Conclusions

We can conclude that the increased levels of serum 8-OHdG in breast cancer patients compared to the benign lesion and healthy controls may have a significant effect in the BC development and might help as a potential biomarker for assessing individuals with high risk of breast cancer. 8-OHdG could use as a confirmatory and/or surrogate marker for breast

cancer. This could decrease the false positive or false negative during breast cancer diagnosis. The increasing levels of 8-OHdG with other routine biomarkers could be considered as a promising discriminatory biomarker of early detection and diagnosis of malignant of breast cancer and distinguishing malignant from benign lesion. However, large sample size from different stages and types of breast cancer should be included in any future study to confirm the present results before translating the findings into routine clinical application.

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Conflicts of Interest

All authors declare no conflicts of interest from any person or organization in the subject matter or materials discussed in this manuscript.

Author Contributions

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520