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

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Abstract

For early detection of malignant tumors, serum levels of 8-hydroxy-2'-deoxyguanosine were determined in 50 women with benign breast tumors, 50 women with breast cancer (BC), and 50 healthy women as a control group. 8-hydroxy-2'-deoxyguanosine levels were significantly increased in the BC group compared with the benign tumor and the healthy control groups; thus it can be used as a potential noninvasive biomarker for early detection of BC.

Background: Breast cancer (BC) is one of the most prevalent and reported cancers among Saudi women. Detection of BC in the early invasive stage (stages I, II) has an advantage in treating patients over detection in the late invasive stage (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 owing to oxidative stress. **Patients and Methods:** We studied the blood levels of 8-OHdG in 50 women with benign breast tumors, 50 women with BC, and 50 healthy women as a control group. **Results:** The concentrations of 8-OHdG were significantly increased in the BC group (55.2 ng/dL) compared with the benign tumor group (30.2 ng/dL) and with the healthy control group (9.08 ng/dL). The same pattern was observed with other diagnostic markers, including carcinoembryonic antigen and cancer antigen 15-3. Significant positive correlations between 8-OHdG and both carcinoembryonic antigen ($r = 0.63$; $P < .001$) and cancer antigen 15-3 ($r = 0.51$; $P < .001$) were noticed. The levels of 8-OHdG were significantly higher in stage I (81 ng/dL) compared with stage II (51 ng/dL; $P < .05$), stage III (38 ng/dL; $P < .01$), and stage IV (19 ng/dL; $P < .001$). In addition, serum 8-OHdG had a high diagnostic performance in BC (area under the curve, 0.86; sensitivity = 82%; specificity = 80% at cutoff value 21.4 ng/mL). 8-OHdG is associated with BC risk according to logistic regression analysis. **Conclusion:** We concluded that the significant increase of serum levels of 8-OHdG in patients with BC can be used as a potential noninvasive biomarker for early detection of BC. However, large sample sizes from different stages and types of BC should be included in any future study to confirm the present findings before translating the findings into routine clinical application.

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Introduction

Breast cancer (BC) is considered the most common cancer in the female population worldwide, and represents about 30% of all new cancer diagnoses in women. BC is known as an estrogen-dependent disease. It is characterized by a high rate of mortality and is considered 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 the quality of life of Saudi women. However, BC is unlike prostatic cancer or liver cancer, which are diagnosed by specific markers such as prostate specific antigen and α-fetoprotein,

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respectively. Currently, there is no biomarker recommended for the early warning of BC in clinical practice except for the invasive genetic test of the BRCA1/2 mutation, which evaluates the risk of hereditary BC.[2]

Generally, the advanced invasive stages (III and IV) of BC have a poor prognosis even after receiving 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 to improve the survival rate and the prognosis in treating the women with BC.

Currently, the screening and diagnosis of BC mainly depend on results of mammography. The high false-positive results of mammography leads to a need for further expensive and invasive diagnostic techniques such as magnetic resonance imaging and needle biopsies. The cost and the mental stress of both magnetic resonance imaging and fine needle 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 noninvasive 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,

Table 1 The Clinical and Demographic Characteristics of Patients Participating in the Study

Clinicopathologic Factors	Characteristic	Benign, n (%)	Malignant, n (%)
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 (past)	Lactation	27 (54)	34 (68)
	No lactation	23 (46)	16 (32)
Menstrual phase (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 (eg, 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	Right breast	27 (54)	22 (44)
	Left breast	17 (34)	28 (56)
	Both sides	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	Estrogen receptor		35 (70)
	Progesterone receptor		28 (56)
	Human epidermal growth factor receptor 2		14 (28)
Metastasis	Metastasis		21 (42)
	No metastasis		29 (58)

Abbreviations: BC = breast cancer; CD = chronic disease; DM = diabetes mellitus; HTN = hypertension; OCP = oral contraceptive pills.

especially the discrimination of early invasive stage BC from benign lesions, is urgently needed in clinical practice.

The immunoassay technique has the important advantages of being simple, inexpensive, and highly sensitive, and 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 cancer antigen (CA) 15-3 and carcinoembryonic antigen (CEA) in BC. However, little attention has been paid to their ability to differentiate between BC and benign breast lesions.

The increasing production rate of reactive oxygen species leads to many modifications in the nucleotide base of DNA. These oxidative modifications produce several base lesion substances.[4] Guanine base has the lowest oxidation potential compared with other bases. Therefore, the guanine residues are more susceptible to free radical attack, resulting in the formation of 8-hydroxy-2'-deoxyguanosine (8-OHdG). 8-OHdG has received greater attention from scientific researchers and is commonly selected as a biomarker of oxidative stress indicating DNA damage. This DNA damage lesion (8-OHdG residues) produces transversion-mutation by pairing with adenine or cytosine in the replication process (GC to TA).[5] This mutation type was considered the second major somatic mutation expressed in human cancers. Therefore, the presence of 8-OHdG in cells indicates the ability of mutagenesis and increases the possibility of carcinogenesis.[5] Permanent oxidative stress lesions lead to cancer.[6] Previously, 8-OHdG was greatly evaluated in animal and human models 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 BC cells and tissues compared with normal cell lines and tissue. Significantly higher levels of 8-OHdG in both cells and tissues of BC were found compared with those of noncancerous breast.[6] Similarly, the blood levels of 8-OHdG in patients with BC increased compared with healthy controls.[8] This interesting evidence encouraged us to propose that 8-OHdG as a biomarker of DNA damage owing to oxidative stress can be an effective discriminatory biomarker in the early detection and determination of people at a high risk of cancer to assist in the screening approach, treatment, and prognosis of BC.

The common tumor markers CEA and CA15-3 have been given much attention in recent years as prognostic factors of BC.[10] The levels of preoperative CEA and CA15-3 serve as a good confirmatory indicator for oncologists 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, early detection, and treatment of patients with BC.[13] In 2007, the guidelines of the American Society of Clinical Oncology (ASCO) stated that they "do not recommend the use of serum CA 15-3 and CEA for or screening, diagnosis, staging, or routine surveillance of patients with BC after primary therapy."[14]

Previous work has shown that 8-OHdG levels were high in urine samples of patients with BC compared with control groups.[15-17] In addition, other groups studied the role of 8-OHdG in BC and found that the levels were higher in patients with BC.[16] However, its diagnostic role at different stages of BC has not been investigated previously; therefore, there is a rationale to assess the levels of 8-OHdG in patients with BC at different stages of the disease.

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

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

Materials and Methods

Subjects

This study included 50 female patients with benign breast mass and 50 female patients with malignant BC mainly of postmenopausal age and 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

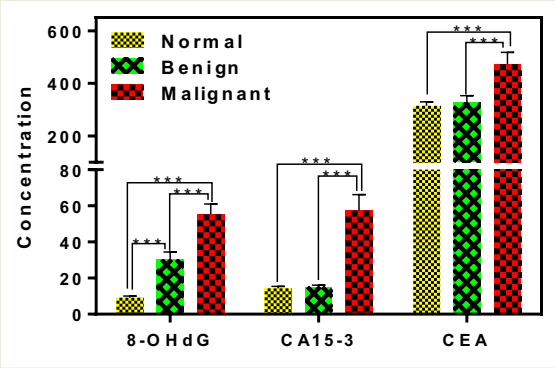
This study was carried out in accordance with the ethical guidelines of the 1975 Declaration of Helsinki and was approved by

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

Abbreviations: CA15-3 = cancer antigen 15-3; CEA = carcinoembryonic antigen; 8-OHdG = 8-hydroxy-2'-deoxyguanosine.

Figure 1 Serum Levels of 8-OHdG, CA15-3, and CEA Compared Between the Benign and Malignant BC Groups With the Normal Group. ***P < .001



Abbreviations: CA15-3 = cancer antigen 15-3; CEA = carcinoembryonic antigen; 8-OHdG = 8-hydroxy-2'-deoxyguanosine.

the medical ethics committee of the Faculty of Medicine at Umm Al-Qura University and the medical ethics committee of King Abdallah Medical City, Makkah, Kingdom of Saudi Arabia. Written informed consent was obtained from every participating 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 CEA

Serum levels of CEA were determined by an in vitro enzyme-linked immunosorbent assay kit (SEA150Hu, Cloud-Clone Corp, Houston, TX) according to the manufacturer's instructions and provided assay procedure (http://cloud-clone.com).

Determination of Serum Levels of CA15-3

Serum levels of CA15-3 were determined by a solid phase in vitro enzyme-linked immunosorbent assay kit (MBS580044, MyBioSource.Com, San Diego, CA) according to manufacturer's instructions (http://mybiosource.com).

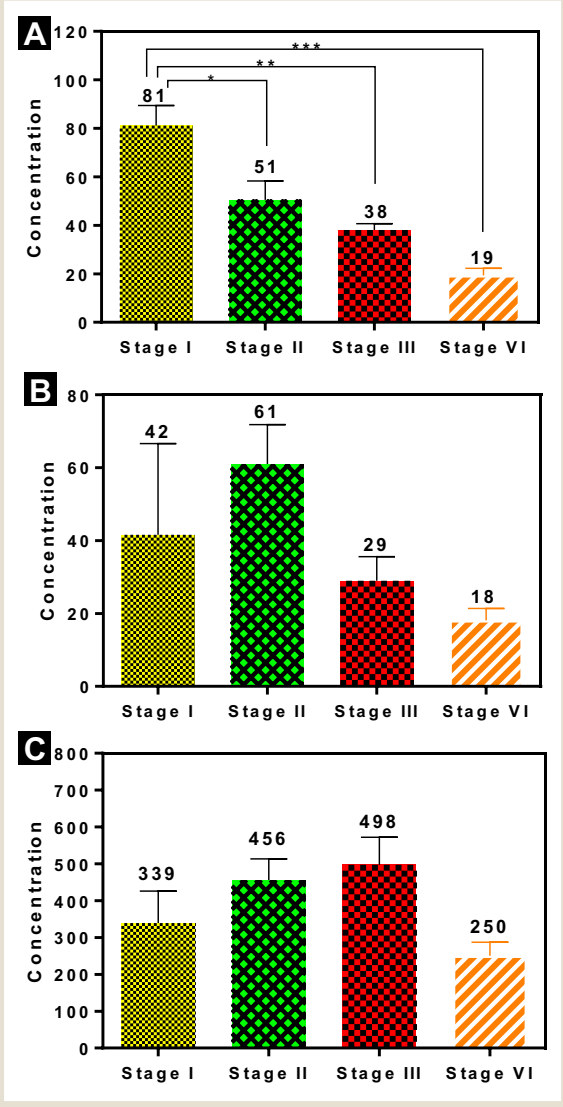
Evaluation of Diagnostic Performance of Serum 8-OHdG Using Receiver Operating Characteristic (ROC) Curve Analysis

We applied an analysis of the ROC curve to our data set. The 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 to 1 = excellent (A), 0.8 to 0.9 = good (B), 0.7 to 0.8 = fair (C), 0.6 to 0.7 = poor (D), and 0.5 to 0.6 = fail (F).

Relation Between Serum 8-OHdG and the Risk of BC (Odds Ratio [OR])

We assumed that a high level of the oxidative damage biomarker 8-OHdG is a risk factor for developing BC. This study was a case

Figure 2 Serum Levels of 8-OHdG (A), CA15-3 (B), and CEA (C) in Different Invasive Stages of Breast Cancer. *P < .05, **P < .01, and ***P < .001



Abbreviations: CA15-3 = cancer antigen 15-3; CEA = carcinoembryonic antigen; 8-OHdG = 8-hydroxy-2'-deoxyguanosine.

control design, so the estimated OR of BC risk was calculated according to quartiles of serum 8-OHdG levels using binary logistic regression analysis.

Statistical Analysis

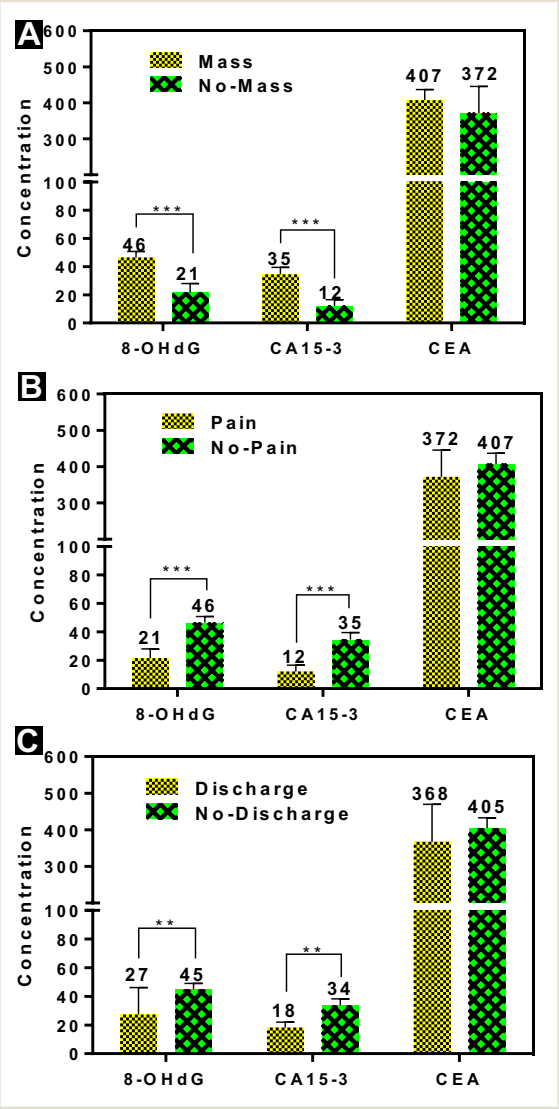
The results were statistically processed by SPSS 24 software using the parametric Student t test and the nonparametric Spearman correlation. The differences were considered significant at a P value < .05.

Results

Clinical and Demographic Characteristics of Subjects

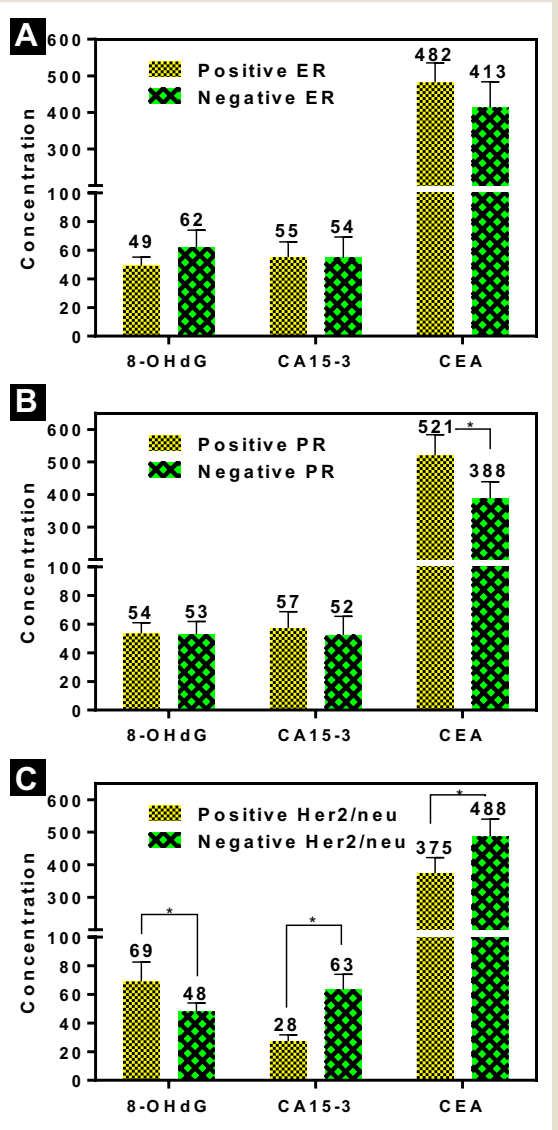
Blood samples were collected from all patients prior to any treatment. The diagnosis was confirmed by histopathology and clinical

Figure 3 Serum Levels of 8-OHdG, CA15-3, and CEA in Patients With Breast Cancer With Different Clinical Presentations: Mass (A), Pain (B), and Discharge (C). $^{**}P < .01$, and $^{***}P < .001$



Abbreviations: CA15-3 = cancer antigen 15-3; CEA = carcinoembryonic antigen; 8-OHdG = 8-hydroxy-2'-deoxyguanosine.

Figure 4 Serum Levels of 8-OHdG, CA15-3, and CEA in Patients With Breast Cancer With Different Histopathology Observations. A, ER-positive, B, PR-positive, and C, HER2/neu-positive. $^{*}P < .05$



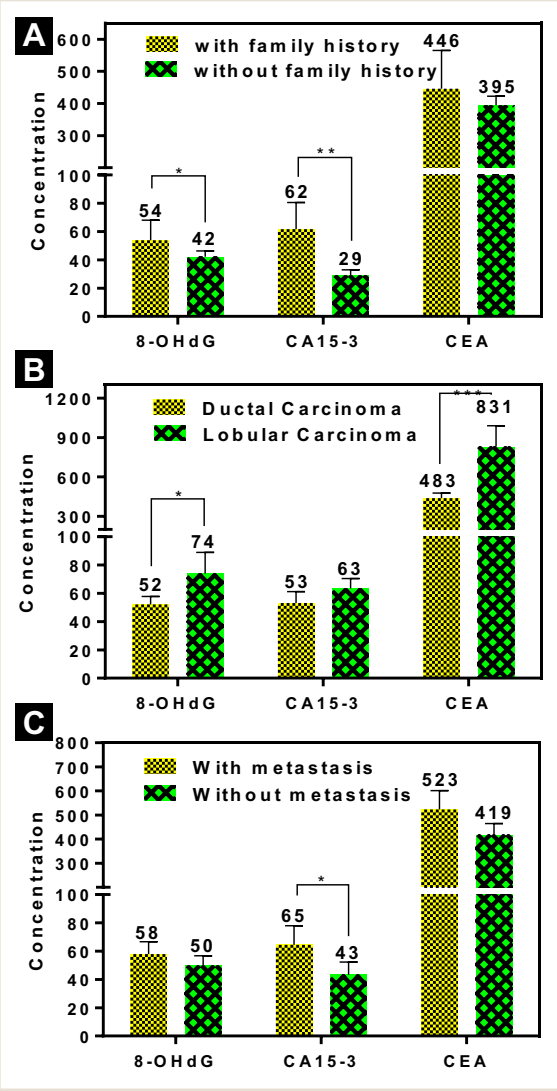
Abbreviations: CA15-3 = cancer antigen 15-3; CEA = carcinoembryonic antigen; ER = estrogen receptor; HER2 = human epidermal growth factor Receptor 2; 8-OHdG = 8-hydroxy-2'-deoxyguanosine; PR = progesterone receptor.

data, as well as medical records. The clinical details and demographic characteristics of both patients with BC and benign tumors are summarized in Table 1. The patients with BC and benign tumors were age-matched with control subjects. Of 50 patients with BC, 6 (12%) patients were grade I, 31 (62%) were grade II, 11 (22%) were grade III, and 2 (4%) were grade IV (Table 1). According to immunohistochemistry data, there were 35 (70%) patients who were estrogen receptor-positive (ER^+), 28 (56%) patients who were progesterone receptor-positive (PR^+), and 14 (28%) patients who were human epidermal growth factor receptor 2-positive ($HER2^+$) (Table 1). Of 50 patients with benign breast masses, 39 were diagnosed as fibroadenoma, whereas 11 patients were diagnosed as other types, including granulomatous mastitis, papilloma, fibroglandular tissue, ductal ectasia, and others (Table 1).

Serum Levels of 8-OHdG, CA15-3, and CEA

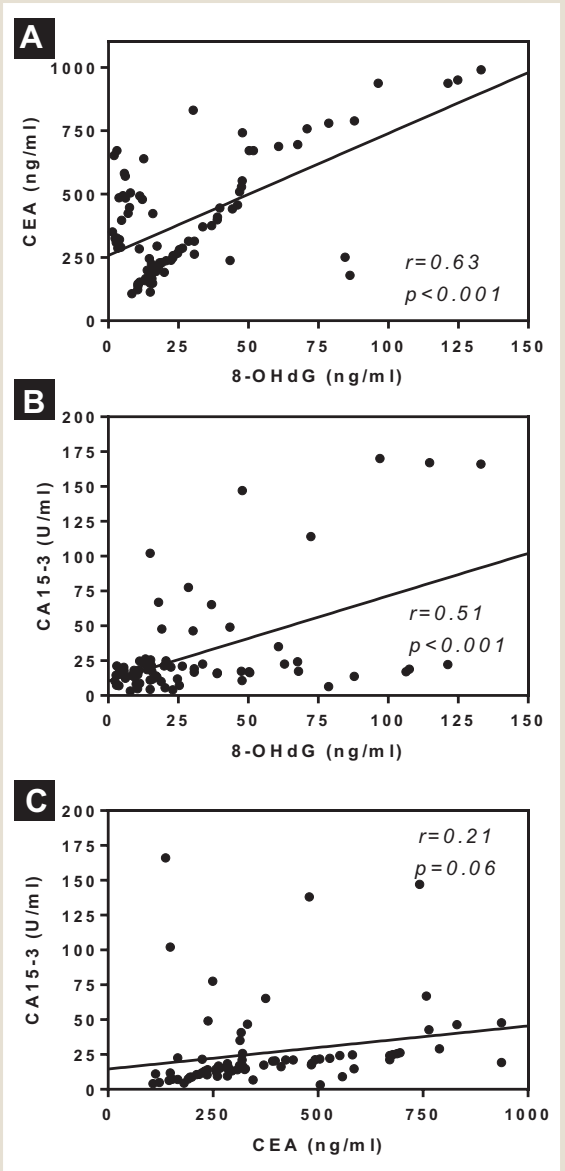
The serum level of 8-OHdG in BC was highly significantly increased in patients with BC than in patients with benign lesions, with mean values of 55.21 ng/dL and 30.21 ng/dL, respectively ($P < .001$) (Table 2 and Figure 1). In comparison with normal healthy controls (9.08 ng/dL), the serum levels of 8-OHdG in both the BC and benign groups were significantly higher ($P < .001$).

Figure 5 Serum Levels of 8-OHdG, CA15-3, and CEA in Patients With BC With and Without Family History of BC (A), With Different BC Types (Invasive/Infiltrating Ductal Carcinoma or Invasive Lobular Carcinoma) (B), and in Patients With and Without Metastasis (C). *P < .05, **P < .01, and ***P < .001



Abbreviations: BC = breast Cancer; CA15-3 = cancer antigen 15-3; CEA = carcinoembryonic antigen; 8-OHdG = 8-hydroxy-2'-deoxyguanosine.

Figure 6 Correlation Between the Serum Levels of 8-OHdG, CA15-3, and CEA



Abbreviations: CA15-3 = cancer antigen 15-3; CEA = carcinoembryonic antigen; 8-OHdG = 8-hydroxy-2'-deoxyguanosine.

Interestingly, the mean value of the levels of other 2 studied parameters, CEA and CA15-3, were sharply increased in the BC group compared with the control group (472.56 ng/dL [P < .001] and 57.28 ng/dL [P < .001], respectively) (Table 2 and Figure 1). By contrast, there was no significant difference between the levels of CEA (328.42 ng/dL) and CA15-3 (15.16 ng/dL) in the benign group compared with the control group. There was a significant difference between the BC and benign groups in the levels of both parameters, CEA and CA15-3 (P < .001), as shown in Figure 1.

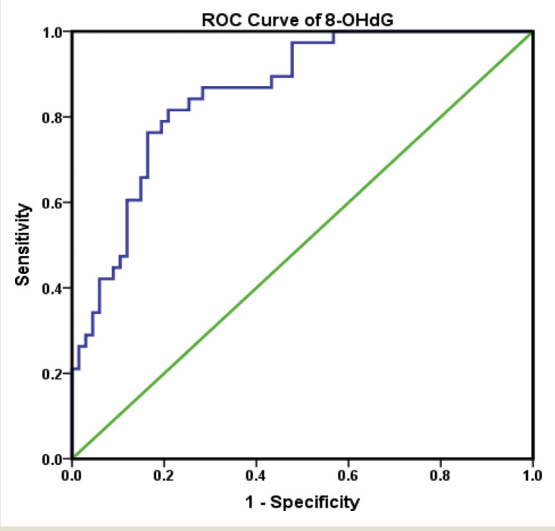
Serum Levels of 8-OHdG Increased in Early Invasive BC

The levels of 8-OHdG were significantly higher in stage I (81 ng/dL) compared with stage II (51 ng/dL; P < .05), stage III (38 ng/dL; P < .01), and stage IV (19 ng/dL; P < .001), whereas the levels of CA15-3 and CEA showed nonsignificant differences among the different invasive stages of BC (Figure 2).

Changes of the Levels of 8-OHdG in Patients With BC According to Clinical Presentations

The serum levels of 8-OHdG, CA15-3, and CEA in the patients with BC with different clinical presentations, mass, pain, and discharge, are presented in Figure 3. The levels of 8-OHdG

Figure 7 The ROC Curve of Serum Levels of 8-OHdG of Studied Subjects. The ROC Curve 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 the Blue Curve is Closer to the Left-hand Border and Then the top Border of the ROC Space, Then the Test is More Accurate. The Area Under the Curve is 0.86, Indicating Good Accuracy of the 8-OHdG Test



Abbreviations: 8-OHdG = 8-hydroxy-2'-deoxyguanosine; ROC = receiver operating Characteristic.

and CA15-3 were significantly lower in patients with BC with pain ($P < .01$) and discharge ($P < .001$). However, in patients with BC with mass, significant increased levels of 8-OHdG and CA15-3 ($P < .001$) were detected. There was a nonsignificant difference in the levels of CEA among the different clinical observations for patients with BC.

Association Between Predictive Immunohistochemistry (IHC) and 8-OHdG

Serum levels of 8-OHdG, CA15-3, and CEA in the patients with BC with different histopathology observations, including ER^+, PR^+, and $HER2^+$ are illustrated in Figure 4.

Relation of 8-OHdG and Family History of BC and Metastasis

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

Table 4 Analysis of Binary Logistic Regression Analysis of Serum 8-OHdG and the Risk of Breast Cancer

Odds Ratio	Significance	95% Confidence Interval	
		Lower	Upper
74.1	0.001	8.97	613.56

Abbreviation: 8-OHdG = 8-hydroxy-2'-deoxyguanosine.

Correlations of 8-OHdG with CA15-3 and CEA

The studied marker 8-OHdG showed significant positive correlations with CEA (r = 0.63; $P < .001$), and CA15-3 (r = 0.51; $P < .001$). A nonsignificant positive correlation between CEA and CA15-3 was observed (r = 0.21), as shown in Figure 6.

Diagnostic Performance of Serum 8-OHdG for BC

The analysis of the ROC curve of 8-OHdG serum levels of studied subjects was applied to elucidate the discrimination of the 8-OHdG test between the samples with and without BC. Figure 7 shows the area under the ROC curve. A significant area under the curve (AUC) was observed from the data analysis of the ROC curve (0.86; $P < .001$). The sensitivity (82%) and specificity (80%) were selected at a cutoff value of 8-OHdG equal to 21.4 ng/mL (Table 3).

Serum 8-OHdG and the Risk of BC

The estimated OR of BC 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 with the lowest quartile group (low-risk). OR was 74.1 ($P < .001$).

Discussion

Oxidative stress has been considered as a cause and/or a reason for BC. 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-OHdG, which was recently used as a reliable and sensitive marker of oxidative stress and carcinogenesis, and was found in high levels in the biological fluids of several patients with cancer.[19]

In this study, the levels of 8-OHdG were high in the BC group compared with the benign lesion and healthy control groups, which is compatible with previous published studies.[15-17,20] Before tumor removal, 8-OHdG level was higher for breast cancer patient than after tumor removal.[21] Additionally, the levels of 8-OHdG were

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%

Abbreviations: AUC = area under the curve; 8-OHdG = 8-hydroxy-2'-deoxyguanosine; ROC = receiver operating characteristic; SE = standard error.

found to be elevated in patients with BC compared with healthy controls.[15] Kuo et al found that the urine levels of 8-OHdG were significantly higher in patients with BC compared with a control group, which supports 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) significantly elevated the levels of 8-OHdG in patients with BC with DM compared with those without DM.[16] Therefore, it would be important to take into account the status of chronic diseases such as DM, hypertension, and osteoporosis when measuring oxidative stress biomarkers in patients with cancer.

Our study observed a significant gradual decrease in the 8-OHdG levels in invasive BC stages from stage I to stage IV, whereas no significant differences were observed in CA15-3 and CEA levels. These results agree 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 have reported that benign lesion and early stage BC could be differentiated by the detection of 8-OHdG.[22] Furthermore, the levels of 8-OHdG significantly decreased in invasive breast carcinomas, compared with noninvasive lesions in patients with 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 types, such as lung cancer, it was reported that the levels of 8-OHdG decreased in advanced cancer stages compared with early stages. Yano et al studied the urinary levels of 8-OHdG in patients with lung cancer; they noticed that the average of 8-OHdG in the late stages of the disease was significantly lower than 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 patients with BC with clinical observation of breast mass presence, whereas these results were opposite in patients with BC with pain or discharge. At the molecular level, there are several studies that reported the expression of 8-OHdG is significantly different in cancerous and noncancerous breast tissues.[6,20] These data, along with our findings, are supportive of the hypothesis that oxidative DNA damage is an important risk factor for BC. However, others observed no significant differences in 8-OHdG levels in cancerous versus noncancerous tissue.[25,26] One of the explanations for the contradicted data is the methodological problem that arises during isolation and extraction of the DNA from the samples, which includes oxidation and degradation of DNA content.

The levels of 8-OHdG, CA15-3, and CEA were significantly higher in patients with BC who were $HER2^+$. There was no difference in the levels of these parameters in patients with BC who were ER^+ and PR^+. Previously, Sova et al found that there was no significant association between 8-OHdG levels and patients with BC who were ER^-, PR^-, and $HER2^-$.[27] For example, our result might be helpful in confirmation of an $HER2^+$ test, thus can determine which patients may get benefit from HER2-targeted therapy such as trastuzumab (Herceptin), lapatinib (Tykerb), pertuzumab (Perjeta), and T-DM1 (Kadcyla). These targeted treatments can improve the survival rate in patients with $HER2^+$ invasive BC. The average level 8-OHdG is slightly higher in patients with BC who were ER^-. This could be used to aid the targeting therapy when using estrogen-targeting drugs such as tamoxifen and/or aromatase inhibitors in patients with BC.[28] However, more work should be conducted and a large sample size included to investigate the potential discrimination role of 8-OHdG in ER status. Therefore, more genetic studies should be conducted to reveal the correlation between the biomarkers of BC, especially serum 8-OHdG, and the 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 a similar pattern of both established biomarkers. This similarity confirms that 8-OHdG is important oxidative biomarker that could be approved as a diagnostic tool for BC. However, a large-scale study that includes more patients in different stages of BC would be important before starting any clinical trial to evaluate the use of 8-OHdG in the diagnosis of early stage BC.

Ductal carcinoma in situ (DCIS) is a noninvasive type of BC.[29] In our study, we have not assessed 8-OHdG in patients with DCIS owing to the lack of samples for study. Therefore, future work should include a 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 to protect high-risk groups of women from BC. Consuming natural products that are high in antioxidants 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 a history of BC.[30] Furthermore, lycopene (carotenoid) in tomatoes was shown to be an antioxidant against the effects of free radicals and hence diminishes oxidative stress.[31,32] Green tea polyphenol consumption diminished 8-OHdG urinary levels in individuals who were at high risk of liver cancer.[33] Garlic also diminished 8-OHdG levels in the brain and plasma of rats that were 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 for this high-risk group.

Conclusions

We can conclude that the increased levels of serum 8-OHdG in patients with BC compared with patients with benign lesions and healthy controls may have a significant effect on BC development and might help as a potential biomarker for assessing individuals with high risk of BC. 8-OHdG could be used as a confirmatory and/or surrogate marker for BC. This could decrease false-positives or false-negatives during BC 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 BC and assist in distinguishing malignant from benign lesions. However, a large sample size from different stages and types of BC should be included in any future study to confirm

the present results before translating the findings into routine clinical application.

Clinical Practice Points

- One of the most prominent product of oxidative DNA damage is 8-OHdG, which was recently used as a reliable and sensitive marker of oxidative stress and carcinogenesis, and was found in high levels in the biological fluids of several patients with cancer. The levels of 8-OHdG were high in the BC group compared with the benign lesion and healthy control groups.
- Our study observed a significant gradual decrease in the 8-OHdG levels in invasive BC stages from stage I to stage IV, whereas no significant differences were observed in CA15-3 and CEA levels. The levels of 8-OHdG, CA15-3, and CEA were significantly higher in patients with BC who were $HER2^+$. No difference in the levels of these parameters in patients with BC who were ER^+ and PR^+.
- 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 BC and assist in distinguishing malignant from benign lesions. Our result might be helpful in confirmation of an $HER2^+$ test, thus can determine which patients may get benefit from HER2-targeted therapy such as trastuzumab (Herceptin), lapatinib (Tykerb), pertuzumab (Perjeta), and T-DM1 (Kadcyla). These targeted treatments can improve the survival rate in patients with $HER2^+$ invasive BC. The average level of 8-OHdG is slightly higher in patients with BC who were HER^-. This could be used to aid the targeting therapy when using estrogen-targeting drugs such as tamoxifen and/or aromatase inhibitors in patients with BC.

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Disclosure

The authors have stated that they have no conflicts of interest.

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