RESEARCH ARTICLE

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Expression Analysis of Oxidative Stress Markers 8-hydroxydeoxyguanosine and Protein Carbonyl in Breast Cancer and Their associations with Certain Immunological and Tumor Markers

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Abstract

Objective: Breast cancer (BC) is a heterogeneous disease, recognized as a major public health problem worldwide, with several risk factors including oxidative stress, an imbalance between the production of reactive oxygen species (ROS) and antioxidant capacity. The aim of this study is to evaluate oxidative stress markers 8-Hydroxydeoxyguanosine (*8-OHdG*) and protein carbonyl and their associations with pathological and immunohistochemical parameters as well as the immune system in particular Interleukin-6 (*IL-6*) in BC patients from western Algeria. **Methods:** We analyzed serum concentrations of *8-OHdG* and *IL-6* by Eliza technique and protein carbonyl by derivatization of protein carbonyl groups with 2,4-dinitrophenylhydrazine (DNPH), then associated them with immunohistochemical and pathological parameters, as well as in the different molecular and histological groups of BC. **Results:** Our results show that there was no significant difference in serum concentrations of *8-OHdG* and protein carbonyl, between controls and patients, and a negative correlation between these markers and lymph node count as well as with proliferation protein Ki-67. We also found a significant association between the DNA oxidation marker *8-OHdG* and Human epidermal growth factor receptor 2 (HER2) expression. No association was found between oxidative stress markers and *IL-6*. Furthermore, no significant differences in serum levels of *8-OHdG* and protein carbonyl were found in the different histological types and molecular classes of BC. **Conclusion:** Our results show that our patients do not have oxidative stress and that physiological ROS concentrations represent a good prognostic indicator, particularly in cancer cell proliferation through their negative correlation with Ki-67, and also metastatic lymph node count. Moreover, HER2 probably has the capacity to modify the state of oxidative stress, notably DNA oxidation. Finally, different molecular and histological types of BC have the same oxidative stress level and antioxidant capacity.

Keywords: Human epidermal growth factor receptor 2- interleukin-6- Ki-67- Oxidative stress- reactive oxygen species

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Introduction

Breast cancer (BC) is a global healthcare problem that has a considerable impact on women's well-being [1]. According to recent global cancer statistics, BC has become the most frequently diagnosed cancer worldwide, accounting for 11.7% of new cancer cases in 2020 [2]. In addition, research into the Global Burden of Disease shows that BC accounts for 30% of all female cancers, and that its incidence rate has continued to rise over the past decade [3].

BC develops and occurs as a result of a number of internal and external factors: poor lifestyle choices, environmental factors and socio-psychological factors are all linked to its onset. It has been shown that 5-10% of BC can be attributed to genetic mutations and family history, and that 20-30% of BC can be attributed to modifiable factors [4]. Another risk factor oxidative stress has long

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been considered one of the mutation factors leading to carcinogenesis through mutation of the oncogene or tumor suppressor gene [5].

Reactive oxygen species (ROS), though products of normal cellular metabolism, are regarded as having a substantial influence on cancer development, partly because of their ability to react with DNA, when the quantity of ROS surpasses the capacity of ROS scavenging systems, oxidative stress occurs, and this unbalanced redox status leads to increased DNA damage [6].

Among the various mechanisms involved in oxidative stress, protein carbonylation is an important modification associated with the pathogenesis of many diseases, including cancer [7], as well as DNA damage, the most studied of which is 8-Hydroxydeoxyguanosine (*8-OHdG*), determined by ROS levels and the function of *8-OHdG* repair enzymes, principally 8-oxoguanine DNA glycosylase 1 (OGG1) [8]. ROS levels may be higher in tumors than in surrounding healthy tissue, but the specific protein carbonylation induced by ROS and its unique role in cancer progression or suppression are poorly understood [9]. In addition, low levels of *8-OHdG*, both in serum and in BC cells, strongly indicate more aggressive disease, particularly in ductal carcinomas. Previous studies have reported conflicting data on the function of *8-OHdG* as a prognostic factor in different types of cancer. The mechanism behind these findings is an interesting topic for future studies [6].

Inflammatory processes can induce DNA mutations in cells through oxidative/nitrosative stress. This condition occurs when the production of free radicals and active intermediates in a system exceeds the body's ability to neutralize and eliminate them. Inflammatory and cancer cells themselves produce free radicals and soluble mediators such as arachidonic acid metabolites, cytokines and chemokines [10].

The complexity and heterogeneity of BC, and the limitations of new markers such as oxidative stress markers for early detection and prevention of this disease. In addition, it remains unclear whether there is an association between oxidative DNA damage, oxidative stress and the molecular features of BC, as well as with inflammatory parameters in the progression or suppression of BC. Knowledge of these associations and molecular mechanisms can contribute to prevention and the development of new therapeutic targets in BC.

The aim of this study is to evaluate markers of oxidative stress the *8-OHdG*, as well as protein peroxidation, and their associations with pathological parameters (tumor size, SBR grad and number of lymph nodes), immunohistological (IHC) parameters (ER, PR, Ki-67 and *HER2*), and inflammatory including Interleukin 6 (*IL-6*), and find the influence of histological types and molecular subtypes on serum levels of these molecules in patients with BC from western Algeria.

Materials and Methods

Subjects

In order to achieve our previously announced objectives we carried out a case-control study of 70 BC patients and 23 presumed healthy controls were recruited from October 2020 to February 2022 at the University Hospital (EHU) of Oran (medical oncology department and gynecology-obstetrics department), all of whom consented to participate by answering questionnaires and agreeing to all biological examinations performed on their blood.

This study did not include patients who had received chemotherapy or any other type of anticancer treatment, or patients with incomplete medical records. Information collected by the baseline questionnaire included sociodemographic and anthropometric characteristics (weight and height), fertility history, use of hormones for infertility and menopause, date of cancer diagnosis, anatomopathological characteristics and family and/or personal history of BC.

Methods

Blood was collected by venipuncture in the fold of the arm on a heparinized tube, and the plasma was separated by centrifugation at 3000 rpm for 10 minutes, and then stored at -80°C.

Assays for *8-OHdG* and *IL-6* were performed by ELISA technique, and protein carbonyl determined by the derivatization of protein carbonyl groups with 2,4-dinitrophenyl-hydrazine (DNPH), at the Biology of Development and Differentiation Laboratory, Ahmed Ben Bella University, Oran 1, and biochemistry laboratory at EHU of Oran. Histopathological and IHC studies of patients' *HER2*, PR, ER and Ki-67 markers were carried out at the anatomopathology laboratory of EHU Oran.

The *8-OHdG* assay was performed using a competitive ELISA technique for quantitative measurement of *8-OHdG* (kit ELISA OxiSelect ™ Oxidative DNA Damage catalog number STA-320). Unknown *8-OHdG* samples or *8-OHdG* standards are first added to a pre-absorbed *8-OHdG* / BSA-conjugated microplate. After a brief incubation, an anti-*8-OHdG* monoclonal antibody is added, followed by an HRP-conjugated secondary antibody. The *8-OHdG* content in unknown samples is determined by comparison with the predetermined *8-OHdG* standard curve. Optical density is measured immediately at 450 nm (this test is competitive, so there is an inverse correlation between sample concentration and measured optical density). A convenient and sensitive sequential sandwich colorimetric Elisa test was established for quantitating *IL-6* in serum.

The suggested dilution for the serum of the normal plasma sample is 2-fold. A volume of 100 ul of each standard and sample are added to the appropriate wells. Incubation is performed for 2.5 hours at room temperature. The wells are washed four times with 300 ul of wash solution followed by adding 100 ul of detection antibody in each well. A second incubation is performed for 1 hour at room temperature with gentle shaking. In the same way as before, the wells are washed, and then 100 µl of streptavidin solution is added to each well and incubated for 45 minutes at room temperature with gentle shaking. After a final wash, a solution of 100 µl of TMB substrate reagent is added to each well. The final incubation is performed for 30 minutes at room temperature in the dark

with gentle shaking. The reaction is stopped by adding 50 µl of stop solution. The optical density is measured at a wavelength of 450 nm. The minimum detectable dose of Human *IL-6* was determined to be 3 pg/ml (IL6 concentration lower than 3pg/ml is considered negative and equal or higher than 3 pg/ml is considered positive) (Product number: Rab 0306).

The Protein Carbonyl Content Assay Kit (Catalog Number MAK094) provides a simple and direct procedure for measuring carbonyl content in a variety of biological samples. Carbonyl content is determined by the derivatization of protein carbonyl groups with 2,4-dinitrophenylhydrazine (DNPH) leading to the formation of stable dinitrophenyl (DNP) hydrazoneadducts, which can be detected spectrophotometrically at 375 nm, proportional to the carbonyls present. Transfer 5 uL of Sample to another set on wells and perform a protein assay to determine the amount of protein per sample. Generate a protein standard curve according to assay protocols. It is recommended to use bovine standard albumin for the Protein Standard curve. Determine the protein content from the Protein assay Standard curve.

Determine the carbonyl content as follows: C (nmole/well) = A 375/ 6.364 x 100

Where: 6.364 = Millimolar extinction coefficient. 100 = Total volume (V) in well (uL). CP (nmole carbonyl/mg protein) = C/P x 1000 x *DF*

Where: C = Amount of carbonyl in sample well (nmole/well). P = Amount of protein from standard well x 20 = ug/well. Conversion factor of 20 is from 5 uL Sample used for Protein assay to 100 uL Sample used for Carbonyl assay. DF = Dilution factor for Sample. DF = 1 for undiluted samples. 1000 = Conversion factor (ug to mg).

Statistical analysis

Statistical Package for Social Sciences (SPSS) version 21.0 was used to perform statistical analysis. A descriptive analysis of the basic parameters was performed. Student t test was applied to compare means between patients and controls, and found a possible association between oxidative stress parameters (*8-OHdG*, Protein Carbonyl) and qualitative IHC parameters (*HER2*, ER, PR) and *IL-6*. The Person Correlation Test was used to analyze the correlation between levels of oxidative stress parameters and other quantitative pathological parameters. One-way ANOVA was used to compare serum oxidative stress parameters concentrations in different histological and molecular classes.

Results

The average age of our patients was 53 years, the average height is 1.61 cm, the average weight is 73.45 kg, and the average age at menopause is 47.87 years.

The majority of our patients are diagnosed with BC in 2020 (51%), more than half of our patients are T2 size (57%), 57% of patients do not take contraception versus 43% who do, almost all patients have no personal history of BC (Table 1).

Table 1. Patient Distribution by Year of Diagnosis, Tumor Size, Contraceptive Intake and Personal History of BC

Paramaters	Number (pourcentage)
Diagnostic year of BC	
2018	1 (1)
2020	36 (51)
2021	33 (48)
Tumor size	
T1	13 (19)
T2	40 (57)
T3	11 (15)
T4	6 (9)
Contraceptive intake	
Yes	30 (43)
No	40 (57)
Personal history of BC	
Yes	69 (98.5)
No	1 (1.5)

Calculation of the body mass index (BMI) shows that 53 patients (76%) are over 25 and 17 patients (24%) are under 25.

Comparative analysis of *8-OHdG* between patients and controls shows that there is no statistically significant difference with p= 0.52. Also, no significant difference in Protein Carbonyl between patients and controls with p = 0.86. The results of the comparative analysis are presented in Table 2.

Analysis of the correlations of 8-Ohdg with pathological, immunohistochemical parameters shows that there is a negative correlation between *8-OHdG* and lymph node count with P= 0.048 and pearson correlation (PC) = -0.27. Also, a negative correlation with cell proliferation protein Ki-67 with p= 0.04 and PC= -0.35. No significant correlations were found between 8-Ohdg and other parameters (SBR grade, tumor size with P= 0.47, 0.56 respectively). A negative correlation was found between Protein Carbonyl and the lymph node count with P = 0.03 and PC= -0.28, also a correlation with Ki-67 with p= 0.043 and PC = -0.36. No correlation between Protein Carbonyl and SBR tumor grade and tumor size with P= 0.45, 0.42 respectively. The results of the analysis are reported in Table 3.

No significant association was observed between *8-OHdG* and Il-6 with P = 0.46. No association was

Table 2. Comparison Analysis of *8-OHdG* and Protein Carbonyl Serum Levels between Patients and Controls

Parameters	Mean ± SD	p-value
8-OHdG (ng/ml)		
Patients	2.78 ± 1.61	
Controls	2.92 ± 0.33	0.52
Protein Carbonyl (nmole carbonyl/mg protein)		
Patients	2.6 ± 2.76	0.86
Controls	2.69 ± 1.78	

Table 3. Correlation Analysis of Patient's Serum 8-OHdG, Protein Carbonyl with Lymph Node Count, *Ki-67*, SBR Grade and Tumor Size

Parameters	lymph node count	Ki-67	SBR grade	tumor size
8-OHdG				
p-value	0.048	0.04	0.47	0.56
Pearson correlation	-0.27	-0.35	-0.09	-0.07
Protein Carbonyl				
p-value	0.03	0.043	0.45	0.42
Pearson correlation	-0.28	-0.36	-0.07	0.1

Table 4. Analysis of the association between *8-Ohdg*, Protein Carbonyl and Immunohistochemical and Immune Parameters

Parameters	8-OHdG	Protein Carbonyl
HER2+		
Positive Mean ± SD	3.26 ± 2.46	3.07 ± 3.12
Negative Mean ± SD	2.17 ± 1.29	2.52 ± 2.75
p-value	0.038	0.53
ER		
Positive Mean ± SD	2.49 ± 1.94	2.62 ± 3.02
Negative Mean ± SD	2.44 ± 1.54	2.74 ± 2.41
p-value	0.9	0.88
PR		
Positive Mean ± SD	2.47 ± 2.03	2.42 ± 2.95
Negative Mean ± SD	1.52 ± 2.58	2.74 ± 2.4
p-value	0.82	0.42
IL-6		
Positive Mean ± SD	2.1 ± 1.36	2.65 ± 2.64
Negative Mean ± SD	2.44 ± 1.96	2.57 ± 2.88
p-value	0.46	0.9

also found between *8-OHdG* and hormone receptors (HR), including ER with P = 0.9 and PR with P = 0.82. A significant association was found between *8-OHdG* and *HER2* with P= 0.038. No significant association was found between Protein Carbonyl and IHC parameters including *HER2* with p= 0.53, ER with p= 0.88, PR with p= 0.9. Also, no significant association was found between Protein Carbonyl and *IL-6* with p=0.9. The results are shown in Table 4.

No significant difference was found in the analysis of serum levels of *8-OHdG* in the different molecular subtypes of BC, with p= 0.49. The same applies to Protein Carbonyl no significant difference was observed with p =0.92 (Table 5).

Analysis of serum *8-OHdG* concentrations in the different histological types showed that there was no significant difference between the different histological groups, with p= 0.76. No significant difference was also found in Protein Carbonyl concentrations, with p= 0.92. The results of the analysis are shown in Table 6.

Discussion

Breast cancer is the most common malignancy in the world, and the leading cause of cancer-related death. Several factors, both modifiable and non-modifiable, are associated with an increased risk of developing BC. Modifiable risk factors can be changed or avoided, and include obesity, a sedentary lifestyle and exposure to exogenous hormones [11]. Obesity can have a marked

Table 5. Comparison Analysis of 8-Ohdg and Protein Carbonyl Serum Levels in Different Subtypes Molecular of Breast Cancer

Subtypes molecular	Luminal B	Luminal A	Triple negative	Luminal	HER2+	p-value
8-OHdG						
Mean ± SD	3.6 ± 2.36	2.63 ± 0.85	3.08 ± 1.37	1.85 ± 1.94	2.14	0.49
Protein Carbonyl						
Mean ± SD	2.02 ± 2.47	3.5 ± 3.77	2.5 ± 2.27	2.44 ± 1.96	6.63	0.36

Table 6. Comparison Analysis of 8-OHdG and Protein Carbonyl Serum Levels in Different Histological Types of Breast Cancer

Histological types of BC	Invasive carcinoma of no special type	invasive pleomorphic ductal carcinoma	Invasive lobular carcinoma	Metaplastic breast carcinoma	Pure mucinous breast carcinoma	Invasive micropapillary breast carcinoma	Ductal carcinoma in situ	p-value
8-OHdG								
Mean ± SD	2.87 ± 1.75	2.71 ± 0.3	1.13 ± 0.55	2.82 ± 0.26	3.07	1.49 ± 1.04	2.41 ±	0.76
Protein Carbonyl								
Mean ± SD	2.61 ± 2.8	2.28 ± 2.65	3.53 ± 4.6	1.04 ± 0.07	1.26 ± 0.31	2.77 ± 0.95	0.53	0.92

impact on women, as it is associated with an increased risk of many chronic diseases, as well as an increased risk of malignant tumors such as BC [12]. BMI calculations show that the majority of our patients (53) are overweight (BMI over 25). The exact mechanisms underlying the positive association between high BMI levels and BC risk remain poorly understood. It has been suggested that greater tissue density be a consequence of increased epithelial cell concentrations, elevated levels of growth factors such as insulin-like growth factor I (IGF-1), stromal fibrosis and/or epithelial hyperplasia, all of which represent potential risk factors for BC [13]. More work is required on the role and molecular mechanism of obesity and adipose tissue and the effects of molecules secreted by adipocytes in the initiation, promotion and progression of cancer cells in order to develop new therapeutic targets and adapt a lifestyle to reduce the risk of BC.

ROS generated by oxidative stress have long been implicated as one of the triggering factors for DNA mutations leading to oncogenesis, particularly in BC. Serum levels of *8-OHdG* are one of the most extensively studied biomarkers of oxidative stress in nuclear and mithocondrial DNA damage [5]. In a large prospective study, we demonstrated recently that negative *8-OHdG* immunostaining is an independent prognostic factor for BC-specific [6]. A study conducted by nagashima et al. found that *8-OHdG* levels in BC DNA were not significantly different from those in the corresponding non-cancerous breast tissue [14]. Another study by Charles et al. also found that *8-OHdG* concentrations were non-significant between cancerous and non-cancerous tissue [15]. In the current study, we found that there was no significant difference in *8-OHdG* between patients and controls. Our results are in line with previous literature data.

Another marker of oxidative stress protein carbonyl, represents the most the most frequently used biomarker of protein oxidation, and their accumulation has been observed with aging and in several human diseases, including cancer [16]. In the present study, we did not find a significant difference in protein carbonyl between controls and patients. A study showed that there was no significant difference in protein carbonyl levels between cases and controls in BC.[17]. Another study performed by Söylemez et al. [18] showed that there was no statistically significant difference in serum carbonyl protein levels between the control group and BC patients after chemotherapy. Our results are consistent with previous data in the literature.

The prognostic value of histological factors such as axillary lymph node status, tumor size, tumor grade and hormone receptor status, other indicators for refining the risk of relapse, predicting individual patient prognosis and adjusting treatment have yet to be identified. Other necessary markers for refining the risk of relapse, predicting individual patient prognosis and tailoring treatment have yet to be identified [19]. One of the important findings of our study is that there is a negative correlation between serum concentrations of *8-OHdG*, protein carbonyl and the lymph node count and Ki-67. A study by soprano et al. on human BC cell lines (MCF-7; MDA-MB231) found that induction of ROS production exerted both an anticancer and an antiproliferative effect [20], bearing in mind that the Ki-67 protein has been widely used as a marker of human tumor cell proliferation for decades [21]. Another study showed that *8-OHdG* on BC cells is an independent prognostic factor of poor prognosis, and that low serum and tissue levels of *8-OHdG* are characteristic of more aggressive BC [6]. A study in non-small-cell lung cancer found that *8-OHdG* expression correlated with negative lymph node status [22]. Ramırez-Exposito at al. Found that protein oxidation, analyzed as carbonyl group content and lipid peroxidation, showed significantly lower levels in the sentinel lymph node of women with macrometastatic BC compared with negative sentinel lymph nodes [23]. Several mechanisms may explain the inverse association between *8-OHdG* levels and tumor aggressiveness. Low levels of *8-OHdG* in serum, plasma or urine may indicate impaired repair of oxidatively damaged DNA or enhanced antioxidant defense, rather than low ROS production. The main *8-OHdG* repair enzyme is human 8-oxoguanine DNA glycosylase 1 (hOGG1), whose proper functioning is crucial for the prevention of G-to-T transversion mutations [24]. Reduced levels of hOGG1 significantly increase the relative risk of carcinoma initiation [25]. With impaired hOGG1 function, cells are unable to cleave damaged guanosine from DNA, resulting in lower levels of *8-OHdG* in extracellular fluids. However, DNA repair defects do not explain the low expression of *8-OHdG* in the tumor tissues of the most aggressive BC [6]. Excessive ROS production can also lead to cell death or senescence, and cancer cells typically acquire and depend on high antioxidant capacity to compensate for the damaging effects of high ROS production. This is why therapeutic strategies designed to disrupt the antioxidant defense system in cancer are being actively pursued. Excessive ROS production causes various types of DNA damage, including base damage, single-strand breaks (SSBs) and double-strand breaks (DSBs) [26, 27]. Literature data and previous studies supports our findings on the negative correlation of oxidative stress markers (8-Ohdg and protein carbonyl) with the cell proliferation protein ki-67 and lymph node count. More detailed studies are needed to understand the influence of ROS on BC cell proliferation and lymph node metastasis.

In the current study, we also investigated the association between oxidative stress parameters and SBR grade as well as tumor size. Karihtala et al. and Beketic-Oreskovic et al. and Sova et al. reported that there was no significant difference between 8-Ohdg expression and tumor grade or size [6, 8, 19]. Another study by Napiórkowska-Mastalerz et al. found no significant difference between protein oxidation and tumour grade in [28]. Our results also show that there is no association between markers of oxidative stress (8-Ohdg and protein carbonyl) and tumour size or grade. Previous studies support our findings [19].

Around 20% of BC patients overexpress human epidermal growth factor receptor 2 (*HER2*), which is associated with increased tumor malignancy. The impact of *HER2* overexpression on oxidant/antioxidant

parameters in humans remains unknown [29]. Another important result of our study is the significant association between 8-Ohdg and *HER2*. Mohamed Nour Eldin et al. found that *8-OHdG* levels were significantly higher in BC patients with *HER2*+ [30]. A study on SkBr3 (*HER2*+) cell lines showed that *HER2*+ cells presented the greatest increase in intracellular ROS [31]. Another study shows that *HER2* positive cells present an increased state of lipid peroxidation associated with reduced antioxidant capacity, confirming that *HER2* amplification is capable of modifying oxidative stress in BC cells [29].

The coexpressions between *8-OHdG* and *HER2* are probably due to the nature of *8-OHdG* as a marker of oxidative stress-derived DNA mutations in general, and suggest that ROS have a function in the formation of *HER2* mutations [32]. Our results agree with previous findings in the literature. The mechanism behind these results is an interesting subject for future studies.

Oxidative stress is an attractive mechanistic hypothesis to explain the biological heterogeneity of ER-positive breast cancers, including PR status. ROS are essential mediators of growth factor receptor signaling and estrogen-induced cell proliferation [28]. Karihtala et al. [8] found no association between hormone receptors (HR) and oxidative stress markers including 8-Ohdg and the lipid peroxidation marker 4-hydroxy-2-nonenal (HNE). The results of sova et al. also show that there is no significant association between 8-Ohdg and HR [6]. The results of previous studies concur with ours, namely that there is no association between hormone receptors and oxidative stress markers, which could explain that ROS do not influence ER and PR expression.

Chronic inflammation is a key factor in promoting cellular changes that lead to increased production of ROS and cell proliferation [33]. Inflammatory processes can induce DNA mutations in cells through oxidative/nitrosative stress. This condition occurs when the generation of free radicals and active intermediates in a system exceeds the system's ability to neutralize and eliminate them. Inflammatory and cancer cells themselves produce free radicals and soluble mediators such as arachidonic acid metabolites, cytokines and chemokines, which act by generating reactive species. These, in turn, strongly recruit inflammatory cells in a vicious circle [34]. A study on stomach cancer carried out by Mustafa found no correlation between the oxidative stress marker 8-Ohdg and cytokine *IL-6* [35]. Alsancak et al. in a study in the development of diabetes found no association between oxidative stress and *IL-6* [34]. Our results also show that there is no association between oxidative stress parameters and *IL-6*. These results confirm previous findings, in which the authors concluded that *IL-6* does not influence ROS production. But further studies with a larger sample size are needed to understand the molecular mechanism and confirm previous findings.

Molecular subtypes of BC can be differentiated according to gene expression, therapy and prognosis. Luminal A tumors are known to be the subtype with the best prognosis of all BC subtypes. Luminal A tumours generally only require hormone therapy, while the majority of luminal B tumours and triple-negative tumours require chemotherapy combined with Herceptin for *HER2*-positive tumours. In contrast, *HER2*-enriched tumours and triple negative are characterised by a more aggressive phenotype and poor survival [36]. In a recently published study by our team, we found that there was no significant difference between marker of oxidative stress and lipid peroxidation 4-HNE in the different molecular groups of BC [37]. Zhang et al. [38] showed that there was no statistical difference between BC subtypes and systematic oxidative stress (based on 5 biomarkers of systematic oxidative stress). Another study by Bel'skaya et al. [39] demonstrated that there was no statistical difference in antioxidant activity in the different subtypes of BC. Based on the previous literature, our results suggest that antioxidant capacity is equivalent in the different subtypes of BC. However, further studies are needed to understand the role of oxidative stress and antioxidant capacity in each molecular subtype of BC.

BC can be and are classified according to several of their aspects, histological presentation being the basis of the World Health Organisation's (WHO) classifications of breast tumours for a long time in successive editions of the 'blue book' [40]. In the current study we compared the serum concentrations of protein carbonyl and 8-Ohdg in the different histological classes, and concluded that there was no significant difference between the different groups. Our results are in agreement with the study by Karihtala et al. [8] who also concluded that there was no significant difference in two parameters of oxidative stress including *8-OHdG* and 4-HNE in the different histological types of BC. Another study by Sova et al. did not find a significant difference in *8-OHdG* in the different histological groups of BC [6]. These results may be explained by the fact that the different histological types have an equivalent antioxidant capacity and also an equivalent ROS production profile. In addition, our study was based on a small sample size and a larger sample size will be needed in future studies to confirm these results, and further studies are also needed to investigate the association of ROS with cell signaling pathways in each histological type and to explain and understand the molecular mechanisms involved.

The limitations of this study are the small number of patients at our hospital due to certain inclusion criteria, and the fact that we were unable to analyze our patients' total antioxidant capacity and other inflammatory markers due to budgetary constraints.

In conclusions, our results show that there is no significant difference in serum concentrations of oxidative stress markers, notably *8-OHdG* and protein carbonyl, between controls and patients which means that our patients do not have oxidative stress with physiological concentrations of ROS, and a negative correlation between these markers and lymph nodes count and also with proliferation protein Ki-67. These results indicate that physiological concentrations of ROS may have à good prognostic effect on BC, notably through its negative effect on lymph node metastases and cancer cell proliferation.

We also found that there was a significant association between the DNA oxidation marker *8-OHdG* and *HER2* expression, these data can be explained by the ability of

HER2 to modify the state of oxidative stress, notably DNA oxidation, No association was found between oxidative stress markers and *IL-6*, probably indicating that *IL-6* does not influence ROS production.

In future research, we plan to study the molecular mechanism of oxidative stress association and its markers with the different signaling pathways involved in initiation and progression of BC to develop new strategies and therapeutic targets for this disease.

Author Contribution Statement

SY, ZT, AM : Literature search and preparation of manuscript, performance of techniques ; NB : Recruitment of patients, collection of data from records, MM : help with analysis and interpretation of *IL-6* results and information on the protein carbon protocol ; MR : Biostatistical analysis of results and help with writing the article ; HO : Aid for 8-OhDG analysis ; SA : Aid for protein carbonyl analysis; SS : Purchase of kits, help with various techniques ; TS : Director of our laboratory, help with various techniques, Help with writing and guiding our work, planning our work.

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Ethical Declaration

Our study was approved by ethical committee of Batna 2 University. Batna, Algeria. Ref /R/U.B.2.

Statement on data availability

The data supporting the results of this study are available from the corresponding author, [SY], upon reasonable request.

Conflict of Interests

All the authors declare no conflict of interests.

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