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| RESEARCH ARTICLE

Detection of Catalase and 4-Hydroxynonenal as Detective Biomarkers in Iraqi Breast Cancer

Anfal Akeel Taha[1] ✉ Hind Salman Jasim[2] and Wafaa Fadhil Hamad[3]

[1]Department of Medical Laboratory Techniques, College of Health and Medical Techniques, Middle Technical University (MTU), Baghdad, Iraq

[2]Baquba Technical Institute- Middle Technical University (MTU), Baghdad, Iraq

[3]College of Health and Medical Techniques, Middle Technical University (MTU)

Corresponding Author: Anfal Akeel Taha, **E-mail**: edc0087@mtu.edu.iq

| ABSTRACT

Breast cancer is the most common malignant tumor among women and a leading cause of cancer-related death. Early diagnosis is critical for improving treatment outcomes. The objective of this study is to determine and examine catalase (CAT) and 4-hydroxynonenal (4-HNE) markers as non-invasive biomarkers for early detection in Iraqi breast cancer. A case-control study of 150 participants was further divided into 75 women with a diagnosis of breast cancer and 75 healthy controls. In the present study, blood samples were collected for the determination of serum CAT and 4-HNE levels using an enzyme-linked immunosorbent assay technique. The biochemical markers urea and creatinine were determined by automated Cobas c311. Hemoglobin concentration (HB)-and total white blood cell (WBC) count was done by an automated hematology analyzer. Statistical analyses were performed using the SPSS version 24, where receiver operating characteristics (ROC) analysis was used to assess diagnostic performance. CAT activity was significantly reduced in the patient group (6.07±1.19 pg/ml) compared to controls (12.11±2.74 pg/ml), while 4-HNE was significantly elevated in the patient group (11.43±2.41 pg/ml) compared to control (3.83±1.02 pg/ml). Among hematology analyzer, there was a significant difference in HB and WBC between patients and controls. Roc analyses demonstrated a sensitivity of 100% for CAT and 98% for 4-HNE. Reduced serum levels of CAT and elevated 4-HNE levels in Iraqi breast cancer may serve as new diagnostic biomarkers for breast cancer, highlighting their role in disease progression.

| KEYWORDS

Breast cancer; Antioxidants; Catalase (CAT); Reactive oxygen species (ROS); 4-Hydroxynonenal (4-HNE).

| ARTICLE INFORMATION

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1. Introduction

The most common cancer among women and the primary cause of death for this population is breast cancer (Huang *et al*., 2021).The growth of a malignant tumor results from the aberrant proliferation of some breast cells, which divide at a pace faster than that of normal cells. These malignant cells have the ability to spread within the lymph nodes and breast tissue and potentially disseminate to other parts of the body (Fatima *et al*., 2020). Advances in early diagnosis, creative treatment approaches, and a better understanding of the disease have significantly enhanced survival rates and gradually reduced breast cancer mortality (Loibl *et al*., 2024). Early detection inhibits malignant cells from spreading to other regions of the body, thus improving the results of treatment (Allugunti, 2022). Oxidative stress is defined as an imbalance between the production of reactive oxygen species (ROS) and antioxidants and is considered the main risk to cause breast cancer (Jelic *et al*., 2021). This imbalance results in angiogenesis, DNA damage, inflammation, and cell proliferation—all of which are contributors to cancer onset and progression(Herdiana *et al*., 2023). ROS are oxygen-containing molecules with reactive properties, including radicals like O2 − (superoxide) HO• (hydroxyl) as well as non-radicals like H2O2 (hydrogen peroxide) (Kirtonia *et al*., 2020). Radicals are chemical compounds with unpaired

electrons, making them highly reactive (Bhattacharjee *et al*., 2018), while non-radicals do not have unpaired electrons and are not considered radicals. However, these non- radical species can readily initiate free radical reactions in living organisms (Martemucci *et al*., 2022). Increased ROS may initiate lipid peroxidation, which affects polyunsaturated fatty acids (PUFAs) in cell membranes and makes 4-hydroxynonenal (4-HNE) (Yahia *et al*., 2023). 4-HNE is an important biomarker in investigating levels of oxidative stress in the cell and tissues, reflecting how much lipid peroxidation and damage due to oxidation have taken place (Tufail *et al*., 2024). Negatively impacts breast cancer through multiple mechanisms. First, 4-HNE produces covalent adducts with amino acids such as cysteine, histidine, and lysine, among others, leading to protein dysfunction and inhibition of important enzymes, further enhancing oxidative stress (Milkovic *et al*., 2023). Second, it causes DNA damage by forming adducts with DNA bases through the production of genomic instability and promoting carcinogenesis (Sharma *et al*., 2022). ROS, which causes oxidative damage to cells, is prevented by the significant antioxidant enzyme catalase(Galasso *et al*., 2021). Catalase neutralizes a potentially hazardous byproduct of cell metabolism and helps to prevent oxidative damage to cells by improving the conversion of hydrogen peroxide into water and oxygen (Anwar *et al*., 2024). The aim of the study is to determine and examine CAT and 4-HNE biomarkers as non-invasive markers for early detection of breast cancer.

2. Materials and Methods

2.1 Study Design

This observational case-control study included 75 Iraqi women aged 23–72 years who were newly diagnosed with breast cancer without a history of receiving any treatment like chemotherapy or radiotherapy. Samples were collected from Al-Amal National Hospital for Oncology Treatment and Al-Alwaiya Maternity Teaching Hospital in the Women's Health Department Unit of Early Detection of Breast Cancer in Baghdad, Iraq, from February 2024 to June 2024. Blood samples were obtained from these women after performing several diagnostic procedures, comprising clinical examination, ultrasound, mammogram, and multiple laboratory tests. A biopsy was conducted to confirm the diagnosis of breast cancer. In addition, a control group of 75 healthy women aged 22–70 was included in the study. They did not have any tumor, fibrosis, mass, or inflammation in the breast without any surgical intervention or nipple secretions, and they were confirmed by conducting a clinical examination, ultrasound, and mammography to ensure that there was no tumor in the breast or other problems. The clinicopathological characteristics included tumor grade and hormonal status as well as HER2 status. Verbal consent was obtained from all participants to ensure that they were fully informed of the study objectives and benefits. Exclusion criteria were pregnancy, metastatic breast cancer, individuals who had received radiation or chemotherapy, immunotherapy, hormonal therapy, or had undergone lumpectomy or mastectomy. In addition, women with breast cancer and chronic medical conditions such as diabetes, hypertension, cardiomyopathy, kidney disease, pancreatic disease, and lung disease, as well as breast fibroadenomas, were also excluded.

2.2 Sampling

Five milliliters of venous blood were collected and distributed into two parts. Two milliliters of blood were placed in an EDTA tube, while three milliliters of blood were placed in a dry, clean gel tube. The EDTA tubes were used to perform a complete blood count to assess hemoglobin and white blood cells by an automated hematology analyzer. The blood in the gel tube was allowed to clot for 10–15 minutes at room temperature, then centrifuged at 2000–3000 RPM for 10 minutes to separate the serum. The serum was divided among three sterile, carefully sealed Eppendorf tubes and kept in a deep freezer at -80°C until it was analyzed. A Roche Cobas C311 Chemistry Analyzer was used to measure creatinine and urea. ELISA (Cloud-Clone Corp., USA) was used to measure the levels of CAT in the serum, and Elabscience (USA) was used to measure the levels of 4-HNE.

2.3 Statistical analysis

SPSS program version 24 was used to perform the statistical analysis. The digital frequencies and percentages were calculated. An independent sample t-test was then applied to evaluate the significance of CAT, 4-HNE, and hematology tests with biochemical tests (hemoglobin, white blood cells, urea, and creatinine). A receiver operating characteristic (ROC) analysis was also done to find out how sensitive and specific serum CAT and 4-HNE were at telling the difference between people with breast cancer and healthy controls. Furthermore, Pearson's correlation analyses were used to determine the correlation between CAT and 4-HNE parameters. Respectively, a P value<.05 was considered to be statistically significant.

3. Results

3.1 Histologic grade of breast cancer

The distribution of breast cancer cases across three grades is displayed in Table 1. Grade 2 accounted for 72.0% of the total cases and included 54 individuals. This was the highest grade. Grade 1 had the least number of cases, with only 3 patients making up (4.0%). However, Grade 3 comprised of 18 patients, or 24.0%. This suggests that Grade 2 breast cancer was the diagnosis made for the majority of individuals.

Table 1 shows the histologic grade of breast cancer.

Grade	No.	%
Grade 1	3	4.0
Grade 2	54	72.0
Grade 3	18	24.0
Total	75	100.0

3.2 Distribution of estrogen receptor (ER), progesterone receptor (PR), and HER2 among breast cancer patients.

The distribution of estrogen receptors, progesterone receptors, and human epidermal growth factor receptor 2 among breast cancer patients is displayed in Table 2. The percent of ER positive was 70.0%, and negative was 29.3%. Regarding PR, the positive rate was 56.0%, while the negative rate was 44.0%. Additionally, the positive rate was 41.3%, and 58% was negative rate. Because these receptors have a major impact on treatment choices and patient outcomes, our findings demonstrate the necessity of assessing them in breast cancer.

Table 2: distribution of ER, PR, and HER2 among breast cancer patients.

	No.	%
ER positive	53	70.7
ER negative	22	29.3
Total	75	100.0
PR positive	42	56.0
PR negative	33	44.0
Total	75	100.0
HER2 positive	31	41.3
HER2 negative	44	58.7
Total	75	100.0

3.3 Comparison of hematological and biochemical tests between patients and control.

Table 3 shows a significant rise in WBC in the patient group (8.17±3.83) compared to the control group (6.40±1.00), with a p-value of less than 0.01. There is also a significant decline in HB in the patient group (12.61±1.37) compared to the control group (13.20±0.71), with a p-value fewer than 0.01. Conversely, no significant differences were shown in creatinine levels between the patient group (0.64±0.18) and control group (0.64±0.09), with a p-value greater than 0.05. Additionally, no significant differences in urea levels are found between the patient group (30.39 ± 4.75) and the control group (29.21 ± 3.29), with a p-value greater than 0.05.

Table 3: Comparison of hematological and biochemical tests between patients and controls.

	Group Study	Mean± Std.	t-test	P-Value
WBC(4.00-11.00)U/L	Control	6.40±1.00	3.455	P=.001
	Patient	8.17±3.83		P<0.01(HS)
HB(12.0-18.0)g/dl	Control	13.20±0.71	2.980	P=.003
	Patient	12.61±1.37		P<0.01(HS)
Urea(16-47)mg/dl	Control	29.21 ± 3.29	1.089	P=.278
	Patient	30.39 ± 4.75		P>0.05(NS)
Creatinine(<0.95)mg/dl	Control	0.64±0.09	0.136	P=.892
	Patient	0.64±0.18		P>0.05(NS)

3.4 Comparison of CAT and 4-HNE markers between patient and control groups.

As shown in Table 4, CAT levels were significantly decreased in the patient group (6.07±1.19 pg/ml) compared to the control group (12.11±2.74 pg/ml), with a p-value of 0.000. Similarly, 4-HNE levels were markedly elevated in the patient group (11.43±2.41 pg/ml) compared to the control group (3.83±1.02 pg/ml), with a p-value of 0.000.

Table 4: Comparison of CAT and 4-HNE markers between patient and control groups.

	Group Study	Mean± Std.	t-test	P-Value
CAT (pg/ml)	Control	12.11±2.74	15.882	P=.000 P<0.01(HS)
	Patient	6.07±1.19		
4-HNE (pg/ml)	Control	3.83±1.02	22.491	P=.000 P<0.01(HS)
	Patient	11.43±2.41		

3.5 Pearson Correlations between CAT and 4-HNE markers in patient group.

The results in Table 5 show the Pearson correlations among the markers CAT, 4-HNE, LPA, and ATX. CAT shows no significant correlation with 4-HNE.

Table 5: Pearson correlations between CAT and 4-HNE in the patient group.

		CAT	**4-HNE**
CAT	**Correlation**	**1**	**-0.07**
	Sig. (P-Value)		**0.5NS**
4-HNE	**Correlation**	**-0.07**	**1**
	Sig. (P-Value)	**0.5 NS**	
NS: No significant value			

3.6 ROC analysis for studied parameters among breast cancer and healthy control groups.

Based on Table 6 and figures (1 and 2), the ROC analysis revealed that CAT and 4-HNE each had an area under the curve (AUC) of approximately 0.99. CAT exhibited a high sensitivity of 100% with a specificity of 98%. And 4-HNE exhibited 98% sensitivity and 100% specificity. Each had significant p-values less than 0.001, indicating strong statistical robustness.

Table 6: ROC analysis for studied parameters among breast cancer and healthy control groups.

Markers	AUC	P-Value	Cut-off Point	Sensitivity	Specificity
CAT (pg/ml)	.99	.000	8.81	100%	98%
4-HNE (pg/ml)	.99	.000	6.828	98%	100%

AUC: Area Under Curve ROC: Receiver Operating Characteristic, H Sig. at P value < 0.000

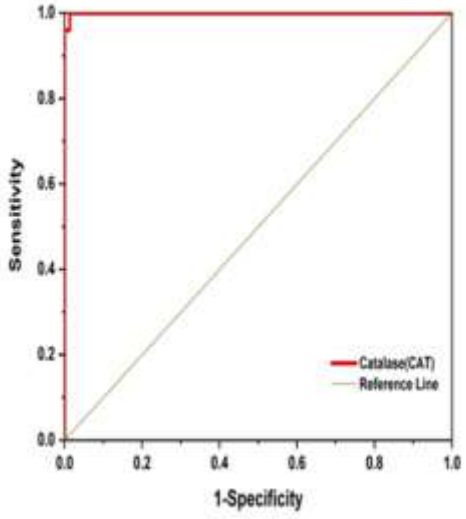

Fig. 1 ROC curve for Catalase activity

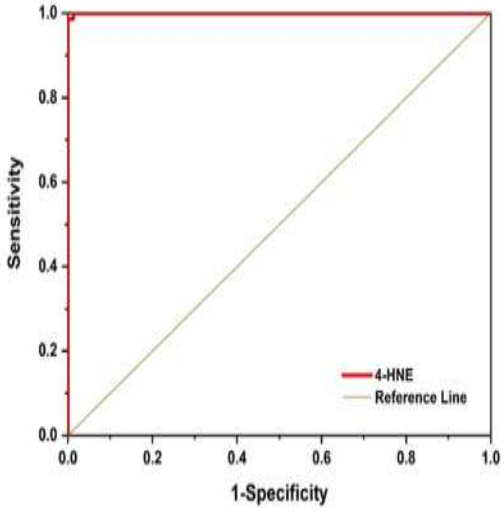

Fig. 2 ROC curve for 4-HNE activity

4. Discussion:

Histological grade is one of the most frequently utilized prognostic indicators for patients diagnosed with breast cancer (Skoog *et al*., 2017). In this study, the majority of patients were diagnosed with grade 2 breast cancer, with grade 2 accounting for 72.0% of the total cases. This finding is in line with Hameed et al. (2022), where the majority of patients who were diagnosed with grade 2 constituted (53.3%) of their cases. Grade 2 breast cancer is characterized by being of moderate differentiation and a histologic score of six to seven. Compared to healthy, normal cells, grade 2 cancer cells appear different and tend to proliferate and divide at a somewhat faster rate (Elfgen *et al*., 2022). ER, PR and HER2 receptors are key biomarkers used to classify breast cancer subtypes. The expression levels of these markers are critical in determining treatment strategies and predicting prognosis (John *et al*., 2024). In this study, patients with positive ER were 70.7%, while those with negative ER were 29.3%. This finding agrees with Hameed *et al*. (2022), who reported a positive rate of 70.0% and a negative rate of 30.0%. ER-positive tumors are more common than ER-negative tumors and tend to have a better prognosis, as they respond well to endocrine therapy compared to ER-negative tumors, thus avoiding the harmful effects of chemotherapy. In addition, since ER-positive tumors are associated with slower growth and lower histology grade, this contributes to increased overall survival (Nisa *et al*., 2022). Regarding progesterone receptors (PR), the study showed a positive rate of 56.0% and a negative rate of 44.0%. This aligns with findings from Hameed *et al*. (2022), who documented a positive rate of 53.33% and a negative rate of 46.67%. Higher PR positivity is correlated with enhanced outcomes in breast cancer. PR negativity and low status are associated with significantly worse disease-free survival compared to those with high PR status (Clelland *et al*., 2023). For HER2 receptors, the results indicated that 41.0% of patients had a positive rate and 58.7% had a negative rate. This study agrees with Hameed *et al*. (2022), who found a positive rate of 33.3% and a negative rate of 66.67%. The higher prevalence of HER2-negative status indicates an association with enhanced overall survival in breast cancer patients, as HER2-low status is often related to less aggressive tumor behavior (Molinelli *et al*., 2023). In this study, the WBC count in the patient group was significantly elevated compared to that in the control group, with a p-value of less than 0.01, although the mean WBC persisted within the normal range. Our results are consistent with Divsalar *et al*. (2021) findings that the patient group had higher WBC counts compared to the control group despite the mean WBC being normal. Similarly, Akinbami *et al*. (2013)found higher WBC counts in breast cancer patients than in controls. This increase is likely due to the association of neoplasms with neutrophilia and the demargination of 50% of neutrophils, which are normally adhered to vessel walls and not usually represented in blood count (Akinbami *et al*., 2013). HB concentration was found to be lower in the patient group compared to the control group, with a highly significant difference, though the mean HB remained within the normal range. This observation is in agreement with Shilpa *et al*. (2020), who also found a reduction in HB concentration in patients compared to the controls, with a highly significant difference. Chronic diseases, as well as cancers, can lead to reduced erythropoietin production, resulting in declining red blood cell production and anemia. In addition, low socioeconomic status plays a role in nutritional anemia (Shipla *et al*., 2020). Oxidative stress plays a critical role in the progression, proliferation, and invasion of breast cancer. Decreased CAT contributes to oxidative stress, which is implicated in aging and the pathogenesis of various diseases, such as cancer (Salman et al., 2020). In the current study, CAT activity was significantly reduced in the patient group compared to the control group, which agrees with Batool *et al*. (2023) and Sahu *et al*. (2015), both of whom reported reduced CAT levels in breast cancer patients. Similarly, Maurya *et al*. (2022) show a significant decrease in CAT activity in patients compared to controls. The reduced CAT levels highlight a positive

relationship between oxidants and a negative relationship between antioxidants and increased breast cancer incidence (Malik *et al*., 2021). The production of ROS is a normal physiological process, but an imbalance can lead to oxidative stress, which is related to multiple pathologies, including cancer. This imbalance accelerates lipid peroxidation of PUFAs in cell membranes and produces 4-HNE (Yahia *et al.,* 2023). Increased 4-HNE levels play a role in cell signal transduction, promoting cell growth and differentiation, and are shown at higher levels in invasive human breast cancer (Jakovcevic *et al*., 2020). In the present study, 4-HNE levels were significantly higher in the patient group compared to the control group, aligning with the research of Yahia *et al*. (2023), who additionally found a significant rise of 4-HNE in patients. In this study, CAT demonstrated special diagnostic performance in this investigation, with an area under curve (AUC) of 0.99, sensitivity of 100%, and specificity of 98%, demonstrating its high efficiency in distinguishing between breast cancer patients and healthy controls. A lower AUC of 0.831 for CAT activity, with a sensitivity of 80% and a specificity of 70%, was observed in another work by Salman *et al*. (2020). Despite these differences, both studies agree on the potential of catalase as a diagnostic marker, although our study shows a higher sensitivity. The variations in sensitivity and AUC may be attributed to variances in study design, sample sizes, or measurement techniques. There is no study that involved ROC curve analyses for 4-HNE in breast cancer.

5. Conclusion

This study aimed to identify key biomarkers in breast cancer patients and assess their diagnostic significance for distinguishing patients from controls. The study showed that serum levels of CAT were significantly reduced and significantly raised serum 4-HNE levels in Iraqi breast cancer patients. These oxidative stress markers revealed promise as diagnostic tools, demonstrating high sensitivity and specificity. However, there are several limitations that should be acknowledged, like the sample size, which was relatively small and may limit the reproducibility of our findings to a larger population. Further studies should expand sample sizes to validate CAT and 4-HNE diagnostic potential in breast cancer patients, especially for early diagnosis and treatment strategies. The study relied only on blood samples, while CAT and 4-HNE levels may be different in cancerous tissues. Therefore, analyzing tissue samples may give more accurate information about the role of these markers in the tumor itself.

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Orcid

Anfal Akeel Taha (https://orcid.org/0009-0004-4652-3226),
Hind Salman Jasim (https://orcid.org/0000-0002-7380-3061)
Wafaa Fadhil Hamad https://orchid.org/0000-0003-1078-5158)

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