Short Communication

Detection of Receptor for Advanced Glycation End Products (RAGE) and High Mobility Group Protein Box 1 HMGB1 Levels in Patients' Serum with Breast Cancer

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Abstract

Background: Breast cancer (BC) is an unchecked proliferation of epithelial cells that begin in the breast lobules or ducts. BC develops and spreads as a result of the high mobility group protein box 1 (HMGB1). The survival, development, and metastasis of tumor cells have all been analyzed for the patients from Oncology Center in Merjan Medical City, Babylon Governorate. HMGB1 and receptor for advanced glycation end products (RAGE) levels in patients and controls were assessed using the enzyme-linked immunosorbent assay technique. **Objectives:** The current study's goal is to analyze the blood levels of HMGB1 and RAGE in both BC patients and healthy volunteers and evaluate how their expression changes as the disease progresses. **Materials and Methods:** Samples collected from BC levels exhibited a 76% sensitivity and a 70% specificity, respectively. Serum RAGE levels were 74% sensitive and 70% specific for the diagnosis of BC, respectively, and their substantial *P* value = 0.023 correlated with tumor size. **Results:** Patients had significantly higher HMGB1 and RAGE levels than did the healthy control group. In order to identify BC, serum HMGB1 is linked to HMGB1 binding to the RAGE receptor. **Conclusion:** The presence of HMGB1 in the serum may serve as a helpful biomarker for the detection of BC. BC RAGE is useful for monitoring the growth of tumor size.

Keywords: Breast cancer, high mobility group box 1 (HMGB1), receptor for advanced glycation end products (RAGE)

Introduction

The most frequent kind of cancer among women globally is breast cancer (BC).[1] BC develops from an unregulated growth of epithelial cells in the breast lobules or ducts. This condition includes BC that has metastasized to distant organs, migrated to distant lymph nodes, or invaded the surrounding breast stroma (primary invasive BC).[2] Additionally included are early, non-invasive breast malignancies such ductal carcinoma *in situ* and lobular carcinoma in situ.[3]

BC accounts for 23% of the 1.1 million new cases of female cancer diagnosed each year, making it the most common disease among women in 2020, BC was the most common type of cancer in Iraq.[4]

According to the most recent Iraqi Cancer Registry, BC is the most prevalent kind of female malignancy, accounting for around one-third of the reported female malignancies.[5,6] This demonstrates that, among the whole Iraqi population, BC outpaces even bronchogenic cancer as the most common cancer kind. Recent rises in BC incidence in Iraq indicate a severe health problem.[6]

Protein Superfamily of the high mobility group with secretory and intracellular activity includes high mobility group protein box 1 (HMGB1), which has both secretory and intracellular activity.[7] A 215 amino acid long protein known as HMGB1 is present in the nucleus and has two DNA-binding domains (HMG A box and HMG B box). HMGB1 primarily interacts with DNA through its A and B box domains.[8,9]

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HMGB1 possesses both pro- and anti-tumorigenic biological actions that play a complicated role in carcinogenesis. HMGB1 up- or down-regulation varies in intensity depending on the kind of cancer.[9] The findings suggested that HMGB1 could be able to promote the growth of BC cells in a laboratory setting. Anti-HMGB1 increases apoptosis and HMGB1 overexpression in BC cell lines by binding to its receptor for advanced glycation end products (RAGE), activating NF-B, up-regulating leukocyte adhesion molecules, and producing proinflammatory cytokines and angiogenic factors in both cancer drugs and radiation.[10] HMGB1 may prove to be useful biomarker to detect the progression of BC and could be targeted by gene therapy technique for cure.[11]

1. Advanced glycosylation end products (AGEs) and numerous S100 family members are among the active ligands that are combined with the immunoglobulin superfamily's multi-ligand receptor, advanced glycation end product receptor (RAGE), on the cell surface.[12] Damage-Associated Molecular Pattern molecules (DAMPs) generated following tissue injury include HMGB1, many S100 proteins, and others. RAGE is thought to be a receptor for these molecules.[13] AGEs, which are formed as post-translational modifications of proteins and lipids, are the results of nonenzymatic glycation condensation and oxidation between carbonyl groups of reducing sugars and free amine groups of nucleic acids, proteins, or lipids.[14,15] It has been anticipated that RAGE expression will negatively affect tissue rearrangement since it is induced by increased oxidative stress and heightened inflammatory responses after tissue injury.[16]

RAGE serves as a master regulator of tumor genesis, invasion, and metastasis by interacting with AGEs, HMGB1, or the S100 group of proteins, which are primarily produced during pathological conditions of glycation and inflammation.[2] The receptor-ligand pair is the main target for cancer prevention and efficient therapy, regardless of the tumor's origin, molecular subtype, or clinical stage. When found, these drugs might be used in concert with traditional chemotherapy to effectively suppress the growth and spread of cancer without having any detrimental effects on normal cells. It gives drugs that target RAGE and its ligands particular cytotoxicity.[17]

The goal of the current study is to examine the serum levels of HMGB1 and RAGE in BC patients and healthy volunteers as potential biomarkers and to determine how their expression changes as the illness progresses.

Materials and Methods

A case–control study design was used for the current research. Two groups of participants in this study were created; the first group was made up of 50 female participants who had received a clinical diagnosis of BC at the Babylon Oncology Center in Mergan Medical City. Their ages ranged from 30 to 69, with an average age of 45.11 ± 6.66 years. The second group, which included 50 female participants, was a healthy control group. Their ages varied from 30 to 69, with an average age of 42.2 ± 7.02 years.

The enzyme-linked immunosorbent assay approach was used to biochemically quantify the amounts of serum HMGB1 and RAGE.

Ethical issues

All participants in this study were informed before to collecting samples, and verbal agreement was obtained from each of them. According to the document number 14, the local ethics committee evaluated and approved the research protocol, subject information, and consent form in August 29, 2022 that issued from College of Medicine, University of Babylon.

Results

Demographic/body mass index characteristics of patients and control

Demographic characteristics of the 100 studied subjects, 50 BC, and 50 control subjects, as shown in Table 1. The mean differences of age and body mass index (BMI) between the BC group and the control group is statistically non-significant $P > 0.05$. This matching is crucial to get rid of any variations in the findings that might be caused by these parameters' discrepancies.

The presence of family history is an important contributory factor in BC. This study showed 21 (42%) of BC patients have positive family history, and 29 (58%) of BC patients have negative family history, and these results were significantly associated with the risk of BC when compared to the healthy control ($P < 0.0001$), as displayed in Table 2.

The results shown in Table 3 indicate the frequency distribution of patients by tumor size and reveal that 28 (58.3%) of patients with BC had tumors with a T2 size. The table reveals that only 24 (48%) patients had HER 2 positivity, while 37 (74%) had estrogen receptor positivity and 32 (64%) had progesterone receptor positivity.

HMGB1 in BC is being evaluated in patient serum showed that the mean and standard deviation (SD) for the patients HMGB1 serum level were significantly ($P \leq 0.05$) increased

Table 1: Means and standard deviation of age and BMI in patients and control

Parameter	Patient (50) Mean ± SD	Control (50) Mean ± SD	*P*-value
Age	45.11 ± 6.66	42.2 ± 7.02	0.26
BMI	28.4 ± 4.23	27.23 ± 2.33	0.3

SD = standard deviation

*$P < 0.05$ is consider significant,

Table 2: Distribution of patients and controls according to family history

Family history	Patient (50)		Control (50)		*P*-value
	N	%	*N*	%	
Positive	21	42	0	0	0.0001
Negative	29	58	50	100	

N: number of cases

Table 3: Patient distribution for breast cancer based on correlate with the specific characteristic parameter (tumor size, hormone receptor expression)

Associated characteristic	Patients	
	N	%
Tumor size		
T1	8	16.7
T2	28	58.3
T3	6	12.5
T4	6	12.5
Estrogen receptor		
Positive	37	74
Negative	13	26
Progesterone receptor		
Positive	32	64
Negative	18	36
HER2		
Positive	24	48
Negative	26	52

Serum levels of HMGB1 and RAGE in the studied groups
HER2, human epidermal growth factor receptor 2

Table 4: Comparison of HMGB1 and RAGE level in patients and control groups

Parameter	Patient (50) Mean ± SD	Control (50) Mean ± SD	*P*-value
HMGB1 (ng/mL)	5.52 ± 0.74	4.93 ± 0.99	0.01
RAGE (ng/mL)	0.56 ± 0.5	0.47 ± 0.14	0.003

*$P < 0.05$ is consider significant

(5.52 ± 0.74) ng/mL compared with healthy control group (4.93 ± 0.99) ng/mL, respectively, as indicated in [Table 4]. There were highly significant ($P \leq 0.05$) increase in RAGE serum levels of patients (0.56 ± 0.5) when compared with healthy control group (0.47 ± 0.14) ng/mL, respectively, as shown in [Table 4].

Receiver operating characteristic curve for high mobility group protein box 1

Receiver operator characteristic (ROC) curve analysis was performed to assess the HMGB1 cutoff value as well as to forecast the BC as diagnostic tests or adjuvant diagnostic tests. The results are shown in Figure 1. The HMGB1 cutoff value was >5.069, with an area under the curve of 0.79 (0.676–0.91), sensitivity 76%, specificity

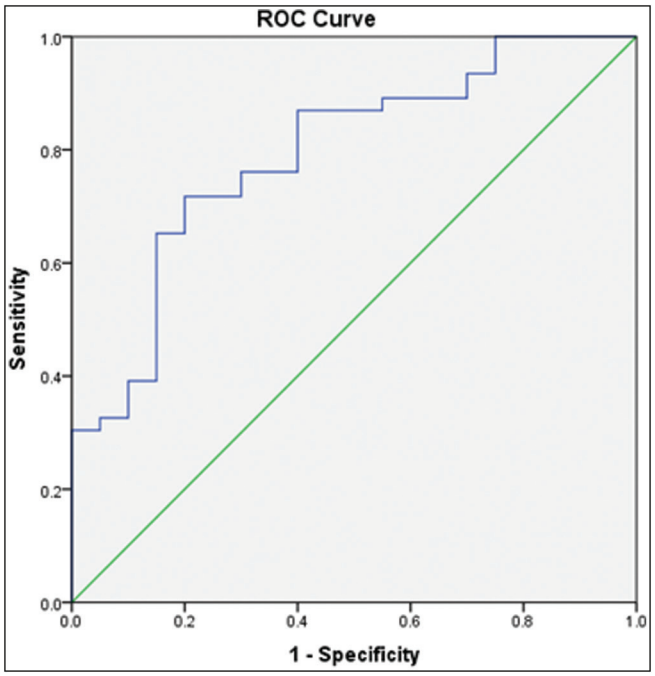

Figure 1: ROC curve for HMGB1

Table 5: Median levels of serum HMGB1 in patients with breast cancer and control subjects

HMGB1 (pg/mL)	Patients *N* = 50	Control *N* = 50	*P*-value
Median	5.474	5.055	0.017

70%, positive predictive value (PPV) 89.19%, and negative predictive value (NPV) 55.17.

For HMGB1, our results stated that fair diagnostic value in determining a BC diagnosis.

According to Table 5 findings, patients with BC had higher median blood HMGB1 levels than the values for the control group (5.474 pg/mL vs. 5.055 pg/mL), and this difference was statistically significant (P 0.017).

When compared to the T1, T2, and T4 volume, the mean serum HMGB1 level rose at T1 was 5.54 ± 0.78, 5.48 ± 0.65, and 5.14 ± 0.98, respectively ($P = 0.48$). Grades one and three had levels that were lower than grade two (5.66 ± 0.803) and higher than grade three (5.13 ± 0.473, respectively) ($P = 0.08$). The relationship between mean serum HMGB1 levels and cancer stage is non-significant; however, the value was greater in stage 1 (5.68 ± 0.7) than in stage 2, stage 3, and stage 4.

Receiver operating characteristic curve for receptor for advanced glycation end products

In order to anticipate whether BC diagnostic tests or adjuvant diagnostic tests will be used, the ROC curve analysis was carried out. The results are shown in Figure 2. The RAGE cutoff value was more than 0.548,

Table 6: Distribution of serum RAGE level according to tumor characteristics

	HMGB1 level				
Tumor size	**T1**	**T2**	**T3**	**T4**	***P*-value**
Mean ± SD	5.77 ± 0.44	5.54 ± 0.78	5.48 ± 0.65	5.14 ± 0.98	0.48 NS
N	8	28	6	6	
Tumor grade	**G1**	**G2**	**G3**	***P*-value**	
Mean ± SD	5.7 ± 0.36	5.66 ± 0.80	5.13 ± 0.47	0.08 NS	
N	3	34	13		
Stage of cancer	**Stage 1**	**Stage 2**	**Stage 3**	**Stage 4**	***P*-value**
Mean ± SD	5.68 ± 0.7	5.35 ± 0.91	5.2 ± 0.53	5.62 ± 0.57	0.38 NS
N	25	14	6	5	

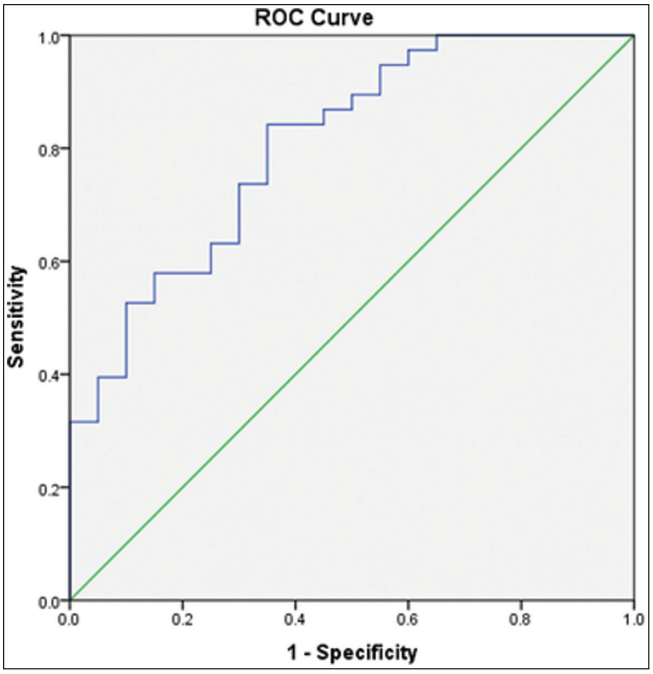

Figure 2: ROC curve for RAGE

Table 7: Median levels of serum RAGE in patients with breast cancer and control subjects

RAGE (pg/mL)	Patients *N* = 50	Patients *N* = 50	*P*-value
Median	0.557	0.533	0.003 HS

with sensitivity 74%, specificity 70%, PPV 82.35%, NPV 41.67%, and an area under the curve of 0.81 (0.691–0.923).

Our findings indicated that RAGE had a strong diagnostic value in the identification of BC.

In accordance with Table 7 findings, patients with BC had median serum RAGE levels that were higher than those of the control group, at 0.557 pg/mL versus 0.533 pg/mL, a difference that was highly significant (*P* 0.003).

Taking into account the features of the tumor, Table 8 shows mean serum RAGE levels. The results of this investigation showed a strong correlation between the tumor size and the mean blood levels of RAGE. The mean of serum RAGE rose as tumor size grew, reaching high levels in T4 tumors (1.34 * 1.92) as opposed to T1, T2, and T3 tumors in the patient group, with respective values of (0.52 ± 0.1, 0.56 ± 0.05, and 0.51 ± 0.12) (*P* 0.023).

Grade three had values that were (0.92 ± 1.31) higher than grades 1 and 2 (0.54 ± 0.078 and 0.55 ± 0.082, respectively) (*P* = 0.24). The value was greater in stage 2 (0.85 ± 1.27) compared to stages 1, 3, and 4, which were each associated with non-significantly lower mean serum RAGE levels (0.57 ± 0.046, 0.55 ± 0.056, and 0.54 ± 0.056) (*P* > 0.62).

There was a non-significant correlation between HMGB1 and RAGE (*P* > 0.4, *r* = 0.13) as in Figure 3.

Discussion

The findings demonstrated elevated HMGB1 levels in BC patients, with a significant difference in levels between the patient and control groups (*P* < 0.01). There was no relationship between the changes in the tumor's size and stage and the blood levels of HMGB1. The results support Sun *et al.*[16]

This could be as a result of cancer cells only secreting relatively little HMGB1. In addition, our study is limited by the very few samples we used. The diagnostic utility of HMGB1 is supported by our early findings, which call for a bigger research.

Age differences were not significantly different between the sick group and the control group (*P* > 0.05). These findings were consistent with several authors worldwide.[18,19]

Considered important mediators of cancer formation and progression are RAGE and its ligand HMGB1 through activation of oncogenic signaling cascades linked to tumor cell proliferation and metastasis.[20]

RAGE levels between the sick group and the control group were significantly different, according to the data (*P* 0.00). RAGE serum level and tumor growth were shown to be significantly correlated (*P* = 0.023). Higher levels of serum RAGE in T4 size (1.34–1.92) in comparison to T1, T2, and T3 size in the patient group, with a positive link. This outcome is consistent with Nankali *et al.*[21]

Table 8: Distribution of serum RAGE level according to tumor characteristics

	RAGE level				
Tumor size	**T1**	**T2**	**T3**	**T4**	***P*-value**
Mean ± SD	0.52 ± 0.1	0.56 ± 0.05	0.51 ± 0.12	1.34* ± 1.92	0.023 S
N	8	28	6	6	
Tumor grade	**G1**	**G2**	**G3**	***P*-value**	
Mean ± SD	0.54 ± 0.07	0.55 ± 0.08	0.92 ± 1.31	0.24 NS	
N	3	34	13		
Stage of cancer	**Stage 1**	**Stage 2**	**Stage 3**	**Stage 4**	***P*-value**
Mean ± SD	0.57 ± 0.04	0.85 ± 1.27	0.55 ± 0.056	0.54 ± 0.056	0.62 NS
N	25	14	6	5	

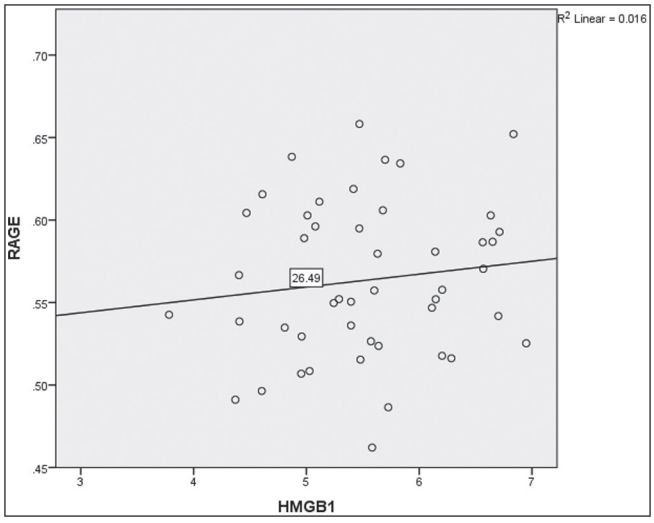

Figure 3: Correlation between HMGB1 and RAGE

Conclusions

Possible helpful biomarker for the detection of BC is serum HMGB1. RAGE is useful for monitoring the growth of tumor size.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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