ISSN: 0067-2904

Evaluation of cancer biomarkers as target for breast cancer progression

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Received: 20/5/2024 Accepted: 1/10/2024 Published: 30/10/2025

Abstract

This study aimed to elucidate the roles of heparin, adenosine, N-cadherin, and β-catenin in cancer, with the ultimate goal of identifying a novel therapeutic target that can be combined with chemotherapy to enhance the host immune response against cancer. A pilot study was conducted in the main medical hospital in Baghdad (medical city-oncology teaching hospital). A total of 60 blood samples were collected from women with breast cancer aged 25-65 years, along with 30 blood samples from apparently healthy women aged matched with patients. The samples were divided into three groups: 30 newly diagnosed patients untreated breast cancer, 30 patients undergoing chemotherapy (Adriamycin + cyclophoshomide), and 30 health control individuals. Three millilitres peripheral blood samples were drawn from each participant. Serum was isolated, and an ELISA assay was carried out to determine serum level of studied parameters. The results indicated a significant increasing in the serum level of heparin, adenosine, N-cadherin and β-catenin in all patients (early diagnosed and treated) versus control. Additionally, there were significant differences in serum level of patient's according to the age, stage, grade of disease and patient's hormonal status of some studied parameters. In conclusion, all studied parameters may consider a good therapeutic target to stop the breast cancer progression.

Keywords: Cancer, Breast cancer, Metastasis, Heparin, Adenosine, N-cadherin, β-catenin.

تقييم المؤشرات الحيوية للسرطان كهدف لتطور سرطان الثدي

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الخلاصة

هدفت هذه الدراسة إلى توضيح أدوار الهيبارين، الأدينوزين، N–كادهيرين، وبيتا–كاتينين في السرطان، بهدف نهائي يتمثل في تحديد هدف علاجي جديد يمكن دمجه مع العلاج الكيميائي لتعزيز الاستجابة المناعية للجسم ضد السرطان. تم إجراء دراسة تجريبية في المستشفى الطبي الرئيسي في بغداد (مدينة الطب–مستشفى الأورام التعليمي). تم جمع ما مجموعه 60 عينة دم من نساء مصابات بسرطان الثدي تتراوح أعمارهن بين 25–65 عامًا، بالإضافة إلى 30 عينة دم من نساء يتمتعن بصحة جيدة ويطابقن المرضى في العمر. تم تقسيم العينات إلى ثلاث مجموعات: 30 مريضة تم تشخيصهن حديثًا بسرطان الثدي ولم يتلقين علاجًا، 30 مريضة يخضعن للعلاج الكيميائي (أدريامايسين + سيكلوفوسفاميد)، و30 من الأفراد الأصحاء كعينة ضابطة. تم سحب ثلاثة ملليترات من الدم المحيطي من كل مشارك. تم فصل المصل، وأجري اختبار ELISA لتحديد مستوى

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المصل للمعايير المدروسة. أشارت النتائج إلى زيادة كبيرة في مستوى الهيبارين، الأدينوزين، N–كادهيرين وبيتا–كاتينين في جميع المرضى (المشخصين حديثًا والمعالجين) مقارنة بالعينات الضابطة. بالإضافة إلى ذلك، كانت هناك فروقات كبيرة في مستوى المصل بين المرضى وفقًا للعمر، المرحلة، درجة المرض، والحالة الهرمونية لبعض المعايير المدروسة. في الختام، يمكن اعتبار جميع المعايير المدروسة هدفًا علاجيًا جيدًا لوقف تقدم سرطان الثدي.

1. Introduction

Cancer is a complex disease characterized by the uncontrolled division of cells and their potential metastasis to other organs [1, 2]. Breast cancer, the most prevalent malignancy affecting female breast tissue, leads to abnormally and uncontrollably [3, 4]. In 2022, 1,335 women died from cancer, with a mortality rate of 12.87 per 100,000 females (F), according to the Iraqi ministry of health (2022) [5]. The highest mortality rate was for females diagnosed with breast cancer, accounting for 22.58% (6.22 per 100,000 females), while bronchial and lung cancer was 15.99% (4.48\100,000 F) [6, 7]. Metastasis is the process by which cancer cells travel from the original tumor to different organs or tissues in the body [8]. Bones, liver, lungs, and brain are the most common sites of metastasis in breast cancer. However, it is likely to spread to other organs as well [9, 10]. Heparin has been found to modulate immune responses and restore anti-tumor immune activity. By enhancing recognition and activation of the immune system, heparin may help treat and slow the ability of cancer cells to spread. Additionally, some researchers have proposed that heparin possesses anti-cancer properties. It has been shown to inhibit various steps of the metastatic cascade in cancer, which includes the adhesion of cancer cells to the endothelium (the inner lining of blood vessels) [11, 12]. This adhesive interaction is mediated by adhesion molecules, and the specific mechanisms through which heparin affects adhesion molecules in breast cancer may vary [13]. Heparin has demonstrated promise as a potential anti-cancer agent in a number of research. These include preventing tumor cell adhesion, invasion, angiogenesis (the creation of new blood vessels to sustain tumor growth), interact with growth factors, cytokines, and adhesion molecules and metastasis [3, 14]. Furthermore, adenosine promotes breast cancer tumor development and metastasis, as well as cell signaling and immune response modulation [15]. These effects may ultimately promote the growth of breast cancer cells [16]. Adenosine is being studied in breast cancer due to its potential role in regulating adhesion molecule expression. Heparin and adenosine influence adhesion molecules associated with breast cancer differently depending on the environment and tumor characteristics [17, 18]. N-cadherin, also known as Neural Cadherin, is typically absent in normal breast epithelial cells but can be increased in invasive breast cancer [19, 20]. N-cadherin induce cell motility and migration through its interaction with epidermal growth factor receptor-1 (EGF-R), and it's also can stimulate MAPK/ERK (mitogen-activated protein kinases/extracellular signal-regulated kinases) signaling pathways that play a role in carcinogenesis [21, 22]. The upregulation of N-cadherin expression is linked to the development of more aggressive forms of breast cancer and a worse prognosis for patients [23]. Additionally, N-cadherin facilitates the separation of cancer cells from the main tumor, hence promoting metastasis [24]. β-catenin plays a crucial role in gene expression, signal transmission, and adheres to cells [25]. Due to its involvement in the Wnt signaling pathway-a key player in cell proliferation, differentiation, and cancer progression—researchers have concentrated on its potential role in breast cancer. Research has connected the abnormal activation of the β-catenin pathway to the acceleration of breast cancer spread. Mutations in the CTNNB1 gene stabilize beta catenin, leading to its accumulation in cancer cells [26, 27]. β-catenin promotes the progression of tumor via suppressing the T cell response, which initiates a cascade that enhances cancer cell invasion, migration, angiogenesis (the development of new blood vessels), and metastasis (the colonization of distant organs) [28], [29]. The tumor milieu

is characterized by high levels of heparin, adenosine, N-cadherin and β-catenin [24]. Heparin and its derivatives reduce the emergence of metastatic lesions by generate heparinase and thrombin or inhibiting adherence of cancer cells to vascular endothelium. Adenosine play a critical role in tumor immunity, angiogenesis and metastasis process. N-cadherin induce cell migration through its interaction with (EGF-R), and also stimulate MAPK/ERK signaling pathways that play a role in carcinogenesis. β-catenin promotes the progression of tumor via suppressing the T cell response [23]. The current study aims to highlight the role of these molecular factors as potential new therapeutic targets to combine with chemotherapy to improve the host immune response against cancer instead of acting directly on tumor cells.

2. Materials and Methods

Study design:

A pilot study was carried out on samples of Iraqi women with breast cancer who visited the Iraqi Medical City (oncology teaching hospital) in Baghdad between August 2023 and December 2023. A total of 90 blood specimens were collected. Partitioned the samples into three groups early patients (newly diagnosis without any treatment), treated groups (patient's with breast cancer under Adriamycin treatment) control (healthy individual without any disease) with an age range of 25–65 years for all groups.

Inclusion and exclusion criteria

The inclusion criteria for this study focused on women diagnosed with breast cancer. Specialists in breast cancer were responsible for selecting and diagnosing the patients. Mammography and histological findings verified the diagnosis. The following conditions were not considered for inclusion in the study: other types of cancer, autoimmune illnesses, infectious diseases, pregnancy, breastfeeding, and other serious acute or chronic medical conditions. For this research, the entire sample consisted of women.

Samples collection:

Each patient had a 3 ml Venus blood specimen drawn for analysis. The blood was placed in a gel tube. The tube was centrifuged at 3000 rpm for a duration of 10 minutes. Serum samples were placed in Eppendorf tubes and frozen at -20 °C until use.

Principle of Double Antibody Sandwich ELISA Kits:

The levels of heparin, adenosine, N-cadherin, and beta-catenin protein in serum samples were measured using a commercial double-antibody sandwich ELISA kit (USNF, USA) categories number are (L230718487, L30346222, L230718489 and L230718491) respectively. In this method, target antigens are sequentially bound by particular antibodies in this method. Target antigen from the sample binds to a capture antibody immobilized on the ELISA plate. A secondary detection antibody linked to an enzyme (e.g., horseradish peroxidase) binds to the captured antigen-antibody complex. The enzyme subsequently combines with a chromogenic substrate to produce a colorimetric product whose optical density (OD) is proportional to the sample's target antigen concentration. Heparin, adenosine, N-cadherin, and beta-catenin levels were quantified by comparing sample OD values to a standard curve produced using known antigen concentrations from the ELISA kit. To ensure assay accuracy and consistency, the manufacturer's guidelines for sample preparation were obeyed.

Statistical analysis:

The data were analyzed using the SPSS program, specifically the Independent T test and One Way ANOVA test. The *P value* was measured using the Least Significant Differences (LSD) method, as well as the Pearson chi square and ROC test. The data were reported as the

mean ± standard error (S.E.), and a *p-value* less than 0.05 was considered statistically significant.

3. Results

Study parameters serum level

Table 1 presents the serum levels of study parameters for both the patients and the control group, with result expressed by (mean±SE), for heparin and β-catenin there was a high significant increasing between early diagnosed patients (63.38±7.38 and 24.77±2.56) respectively compared to control the result was (26.88±3.53 and 6.90±1.32) respectively at *p value* (<0.001). For *Adenosine and N-cadherin* significant increases in serum levels for early diagnosed and treated patients (217.02±21.30, 203.72±15.14) and (2.68±0.54, 1.98±0.11) respectively, compared to controls (72.01±4.22 and 0.59±0.12) respectively, at *P value* <0.001. The results further indicated a significant difference between early diagnosed and treated in heparin and β-catenin at *p value* <0.001**

Table 1: Study parameters serum level between patient and control

Groups	Parameter concentration (Mean±S.E.) (pg/mL)			
	Heparin	**Adenosine**	**N-cadherin**	**β-catenin**
Control	26.88±3.53	72.01±4.22	0.59±0.12	6.90±1.32
Early patients	63.38±7.38 [a]	217.02±21.30	2.68±0.54	24.77±2.56 [a]
Treated patients	36.41±3.94	203.72±15.14	1.98±0.11	8.59±1.12
P value	**<0.001****	**<0.001****	**<0.001****	**<0.001****
Between early and treated	-	-	-	-
P value	**<0.001****	0.54 NS	0.14 NS	**<0.001****

[a] vs. control **= high significant, NS= no significant

The association between studied parameters according to the age.

The groups of age were categorized into two main age groups, ≤50 and >50 years, in patients and control, which distributed in Figure 1, and the mean of each parameter was compared to age groups of control and also within two age groups of studied patients.

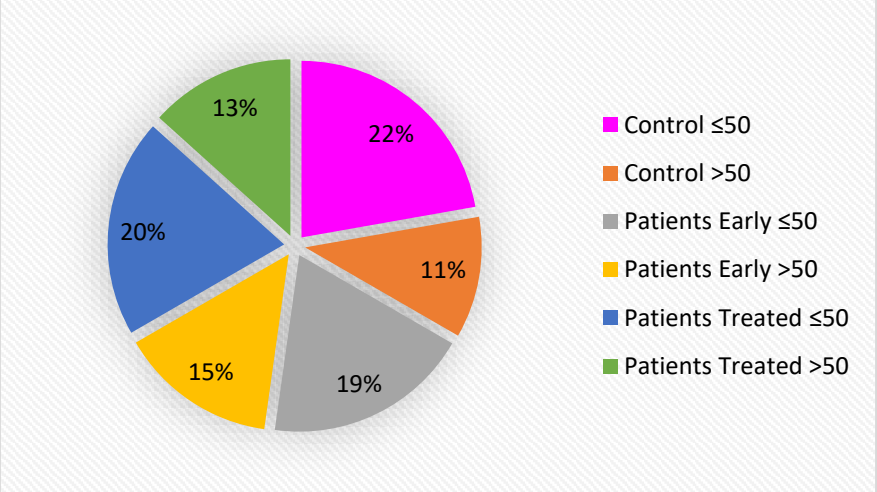

Figure 1: percentage age distribution in all studied groups

Study parameters serum level distribution according to age in patients (early and treated) and control.

The data presented in Table 2 illustrates the levels of heparin, Adenosine, N-cadherin and β-catenin across different age groups of the examined subjects. The results indicate a

significant rising of heparin, Adenosine, N-cadherin and β-catenin serum levels in both the ≤50 and >50 age groups of early diagnosed and treated patients, compared to the corresponding age groups in the control group at *p value* (<0.001). Additionally, no significant differences were found between ≤50 and >50 age groups within both studied patient's groups (early diagnosed and treated) for all study parameters except in β-catenin there is a notable disparity in the level of the condition between individuals under the age of 50 and those over the age of 50 who were diagnosed early.

Table 2: Study parameters serum level in patients and control according to age in all studied groups.

Parameter	Parameter concentration (Mean±S.E.) according to age					
	Control		Early patients		Treated patients	
	≤50	>50	≤50	>50	≤50	>50
Heparin	23.74±4.03	33.16±6.71	69.82±10.89 [ab]	54.95±9.33 [a]	40.12±5.42	30.84±5.45
P value	**<0.001****		0.16 NS		0.39 NS	
Adenosine	68.63±5.70	78.77±5.26	213.26±31.48 [ab]	221.94±28.26 [ab]	196.93±17.74 [ab]	213.90±27.69 [ab]
P value	**<0.001****		0.78 NS		0.59	
N-cadherin	0.72±0.16	0.33±0.08	3.00±0.95 [ab]	2.25±0.17 [ab]	1.95±0.14 [ab]	2.02±0.20 [ab]
P value	**<0.001****		0.26 NS		0.92 NS	
β-catenin	7.62±1.72	5.47±1.97	28.71±4.07 [ab]	19.62±1.96 [ab]	7.17±1.40	10.71±1.73
P value	**<0.001****		**0.01***		0.32 NS	

[a] vs. control <50, *= significant [b] vs. control >50, **= high significant, NS= no significant

The association of studied parameters in breast cancer patients according to the grade of disease

This study examined the correlation between the amounts of analyzed parameters and the stage of breast cancer in the patients. Three grades (I, II, III) were identified in early diagnosed patients and two grades only (II, III) in treated patients which distributed with different percentage, as shown in Figure 2.

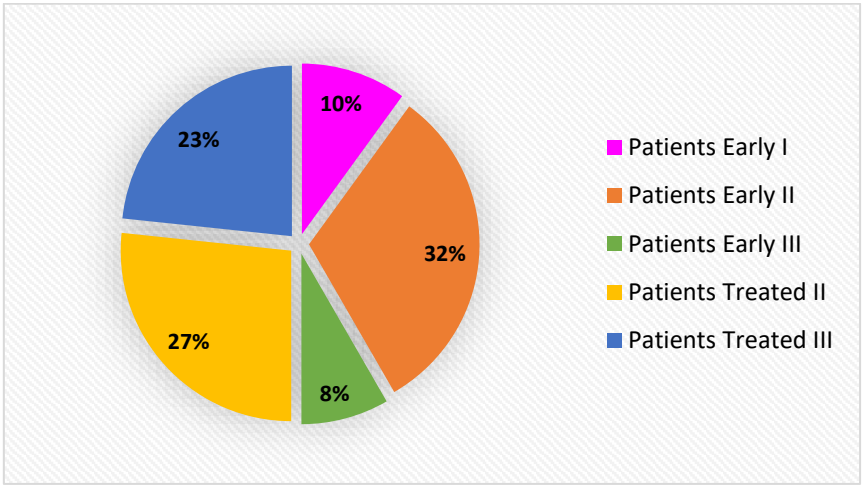

Figure 2: the percentage of disease grades among patients groups.

Study parameters serum level distribution according to diseases grade in patients (early and treated):

The data presented in Table 3 illustrates the levels of heparin, Adenosine, N-cadherin and β-catenin across different diseases grade of the examined subjects. There was no significant

difference in the serum levels of heparin, Adenosine, N-cadherin and β-catenin among individuals who were early diagnosed patients at *p value* (0.65, 0.23, 0.11 and 0.35) respectively. Similarly, there were no significant difference in the serum levels of heparin, Adenosine, N-cadherin and β-catenin among individuals who were treated patients at *p value* (0.48, 0.47, 0.61 and 0.59) respectively. On the other hand, a significant decreasing in heparin level was recorded between early diagnosed and treated patients in grade II but not in grade III at *p value* (0.005). Additionally, there was a significant difference was showed between early diagnosed and treated in grade II and III ($p<0.001$, $p<0.01$) respectively, as β-catenin was decreased in treated patients. Adenosine and N-cadherin showed no significant difference was notice in grade II and grade III between early diagnosed and treated patients.

Table 3: Study parameters serum level in patients and control according to grade in all studied groups.

Patients groups	Grade groups	Parameter concentration (Mean±S.E.) (pg/mL)			
		Heparin	Adenosine	N-cadherin	β-catenin
Early diagnosed patients	I	61.12±15.02	278.03±47.19	1.51±0.21	30.88±9.02
	II	67.96±10.17	189.75±22.31	2.41±0.32	24.54±2.79
	III	48.68±13.71	247.41±76.33	5.11±2.99	18.31±3.07
P value		0.65 NS	0.23 NS	0.11 NS	0.35 NS
Treated patients	II	33.77±4.77	193.17±20.26	2.04±0.18	8.01±1.46
	III	39.43±6.55	215.77±23.12	1.92±0.14	9.25±1.77
P value		0.48 NS	0.47 NS	0.61 NS	0.59 NS
Between early & treated	II	-	-	-	-
P value		**0.005****	0.91 NS	0.35 NS	**<0.001****
Between early & treated	III	-	-	-	-
P value		0.5 NS	0.6 NS	0.08 NS	**0.01***
NS= no significant, **= high significant					

The correlation between the examined variables in breast cancer patients based on the disease stage

This study investigated the relationship between the levels of the examined parameters and the stage of breast cancer in patients, seeking to identify potential correlations. It was found two stages (I, II) in early diagnosed patients and three stages (I, II, III) in treated patients which distributed with different percentage as shown in Figure 3.

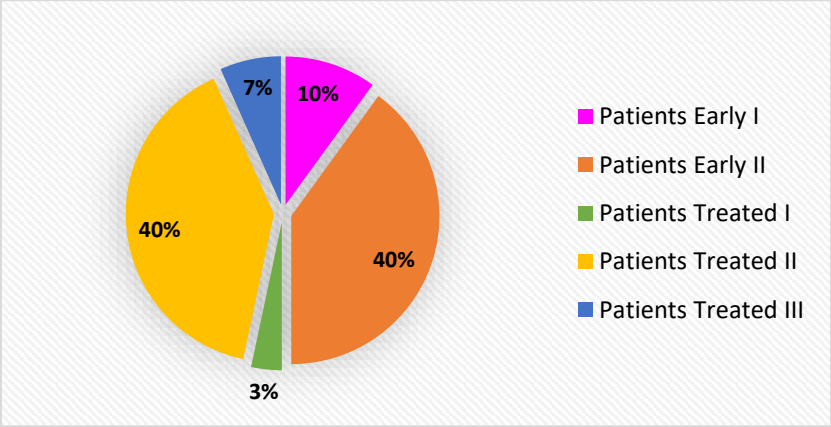

Figure 3: the percentage of stages of disease among patients groups.

Study parameters serum level distribution according to diseases stage in patients (early and treated):

The data presented in Table -4, illustrates the levels of heparin, Adenosine, N-cadherin and β-catenin in the different diseases stages of the examined subjects. It has been found that there

was no significant difference in its level between stage I and II in early diagnosed patients at *p value* (0.99, 0.53, 0.55 and 0.35) respectively, as well as among stage I, II and III in treated patients at *p value* (0.6, 0.66, 0.22 and 0.12) respectively. Also, no significant effect was found between early diagnosed and treated patients in stage I in heparin, Adenosine and N-cadherin at *p value* (0.29, 0.69 and 0.16), but there was a significant changes in beta-catenin levels was detected between two studied patients in stage I at *p value*(<0.001). In stage II it was found significant difference in heparin and β-catenin *p value* (0.006 and <0.001) respectively.

Table4: Study parameters serum level in patients and control according to grade in all studied groups.

Patients groups	Stage groups	Parameter concentration (Mean±S.E.) (pg/mL)			
		Heparin	Adenosine	N-cadherin	β-catenin
Early diagnosed patients	I	63.55±9.97	189.77±47.62	2.01±0.20	19.93±1.25
	II	63.34±8.97	223.83±24.12	2.84±0.67	25.98±3.15
P value		0.99 NS	0.53 NS	0.55 NS	0.35 NS
Treated patients	**I**	41.25±12.75	151.95±38.15	2.72±0.53	0.19±0.05
	II	34.42±4.28	206.20±17.61	1.91±0.13	9.41±1.15 [a]
	III	45.95±14.61	214.75±40.22	2.04±0.21	7.83±4.11
P value		0.6 NS	0.66 NS	0.22 NS	0.12 NS
Between early & treated	**I**	-	-	-	
P value		0.29 NS	0.69 NS	0.16 NS	**<0.001****
Between early & treated	**II**	-	-	-	
P value		**0.006****	0.56 NS	0.18 NS	**<0.001****
NS= no significant, **= high significant, [a] vs. stage I in treated group					

The association of studied parameters in breast cancer patients according to the hormonal status

This study explored the correlation between the levels of specific indicators and the hormonal status of breast cancer patients. The data obtained revealed the presence of five hormonal states (ER+PR+HER2-, ER+PR-HER2-, ER-PR-HER2+, ER+PR+HER2+, ER-PR-HER2-) in patients detected early, and three statuses (ER+PR+HER2-, ER+PR-HER2-, ER-PR-HER2+) in treated patients. These statuses were distributed in varying percentages, as depicted in Figure 4.

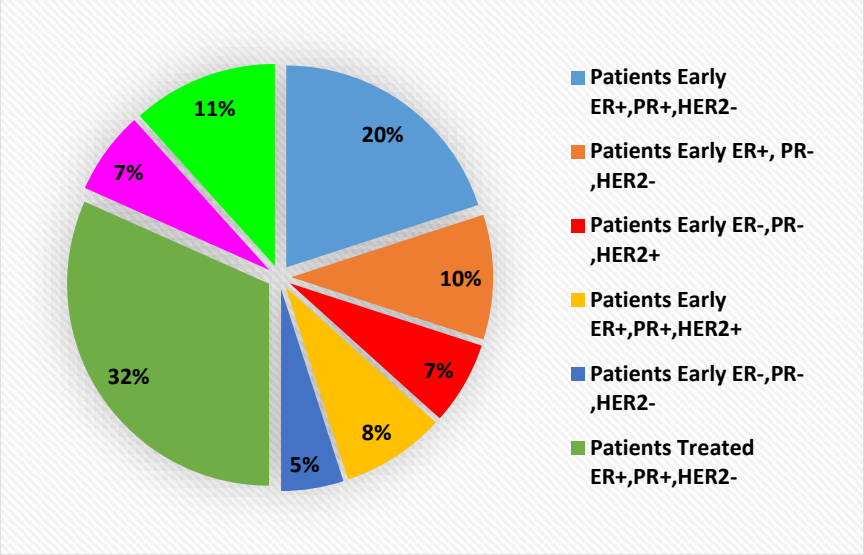

Figure 4: the percentage of hormonal statuses of disease among patients groups.

Study parameters serum level distribution according to hormonal status in patients (early and treated):

The data presented in Table 5 illustrates the levels of heparin, Adenosine, N- cadherin and β-catenin across different hormonal status of the examined subjects**.** The serum levels of heparin in both studied patients in all different hormonal statuses. The statistical analysis of the data indicated that there were no significant changes in heparin level among all hormonal statuses in early diagnosed patients and also in treated patients.

The serum levels of adenosine in both studied patients in all hormonal statuses. The statistical analysis of the data showed that there were no significant changes in adenosine level among all hormonal statuses in early diagnosed patients and also in treated patients.

N-cadherin serum levels according to hormonal status, it has been showed that there was a significant difference in its levels among all hormonal statuses in early diagnosed patients (p 0.05) and the highest level was in triple negative status. While there was no significant differences in treated patients.

According to the current study, hormonal status has no impact on β-catenin serum level in early diagnosed patients (p=0.08). Notably, a statistically significant difference was observed in the treated patients, with a p-value of 0.05, indicating a significant effect of the treatment.

Table 5: Study parameters serum level in patients and control according hormonal status all studied groups.

Patients groups	Hormonal status groups	Concentration (Mean±S.E.)			
		Heparin	Adenosine	N-cadherin	β-catenin
Early diagnosed patients	ER+,PR+,HER2-	64.43±15.13	227.20±30.99	2.41±0.52 [b]	33.33±5.39
	ER+, PR-,HER2-	61.77±11.20	206.36±23.35	1.95±0.25 [b]	20.29±1.02 [d]
	ER-,PR-,HER2+	35.62±3.56	156.40±50.67	1.95±0.35 [b]	15.35±2.93 [d]
	ER+,PR+,HER2+	65.58±14.30	223.00±88.61	1.99±0.38 [b]	21.10±1.62
	ER-,PR-,HER2-	105.73±15.65 [a]	290.67±107.46	7.33±4.84 [b]	20.10±3.80
P value		0.23 NS	0.63 NS	0.05*	0.08 NS
Treated patients	ER+,PR+,HER2-	37.87±5.51	192.58±18.17	2.13±0.14	9.82±1.44 [e]
	ER+,PR-,HER2-	26.05±3.36	273.87±56.50	1.44±0.22 [c]	11.07±1.62 [e]
	ER-,PR-,HER2+	38.36±7.74	193.87±24.72	1.87±0.26	3.82±1.80
P value		0.6 NS	0.2 NS	0.11 NS	0.05*

[a] vs. ER-,PR-,HER2+ in early diagnosed patients group, [b] vs. ER-,PR-,HER2-in early group, [c] vs. ER+,PR+,HER2- in treated group, [d] vs. ER+,PR+,HER2- in early group, [e] vs. ER-,PR-,HER2+ in treated group NS= no significant *= significant n cad

The Correlation between the serum levels of the studied parameters

The Table 6 presents the correlation between parameters serum level and the result as follows, there is a weak positive correlation between adenosine and (heparin, beta-catinen) the result (0.365, 0.270) respectively. Additionally, there is a medium positive correlation between adenosine and N-cadherin, the result (0.502). A weak positive correlation was found between heparin and (N-cadherin, beta-catinen) the result (0.249, 0.215) respectively. There is a weak positive correlation between N-cadherin and beta-catinen, with the result of 0.268.

Table 6: the correlation between markers serum level

Parameter		Heparin	Adenosine	N-cadherin	β-catenin
Heparin	Pearson Correlation	1			
	Sig. (2-tailed)				
Adenosine	Pearson Correlation	**0.365****	1		
	Sig. (2-tailed)	**<0.001**			
N-cadherin	Pearson Correlation	**0.249***	**0.502****	1	
	Sig. (2-tailed)	**0.018**	**<0.001**		
β-catenin	Pearson Correlation	**0.215***	**0.270***	**0.268***	1
	Sig. (2-tailed)	**0.042**	**0.01**	**0.011**	
	Sig. (2-tailed)	0.891	0.002	0.273	0.269

Receiver Operating Characteristics test

ROC (Receiver Operating Characteristics) test used to identify the good predicted marker using ROC curve analysis. ROC and AUC (Area under the Curve) are metrics used to evaluate how effectively a test can differentiate between patients and healthy individuals. According to the Table 7 and Figure 5, Heparin have a high sensitivity and high specificity the result was (80%,73%) *P value* (<0.001), AUC (0.83), cutoff (30.65). adenosine have a high sensitivity and high specificity the result was (85%,100%) *P value* (<0.001), AUC (0.95), cutoff (107.7). N-cadherin have a high sensitivity and high specificity the result was (99%,83%) *P value* (<0.001), AUC (0.93), cutoff (0.99). β-catinen have a high sensitivity and high specificity the result was (90%,83%) *P value* (<0.001), AUC (0.92), cutoff (15.9), as shown in the Table 7.

Table 7: ROC curve results for all studied parameters in patients with breast cancer comparing with controls

Parameters	AUC	cutoff	Sensitivity	Specificity	P Value
heparin	0.83	30.65	80%	73%	**<0.001****
adenosine	0.95	107.7	85%	100%	**<0.001****
N-cadherin	0.93	0.99	99%	83%	**<0.001****
β-catenin	0.92	15.9	90%	83%	**<0.001****

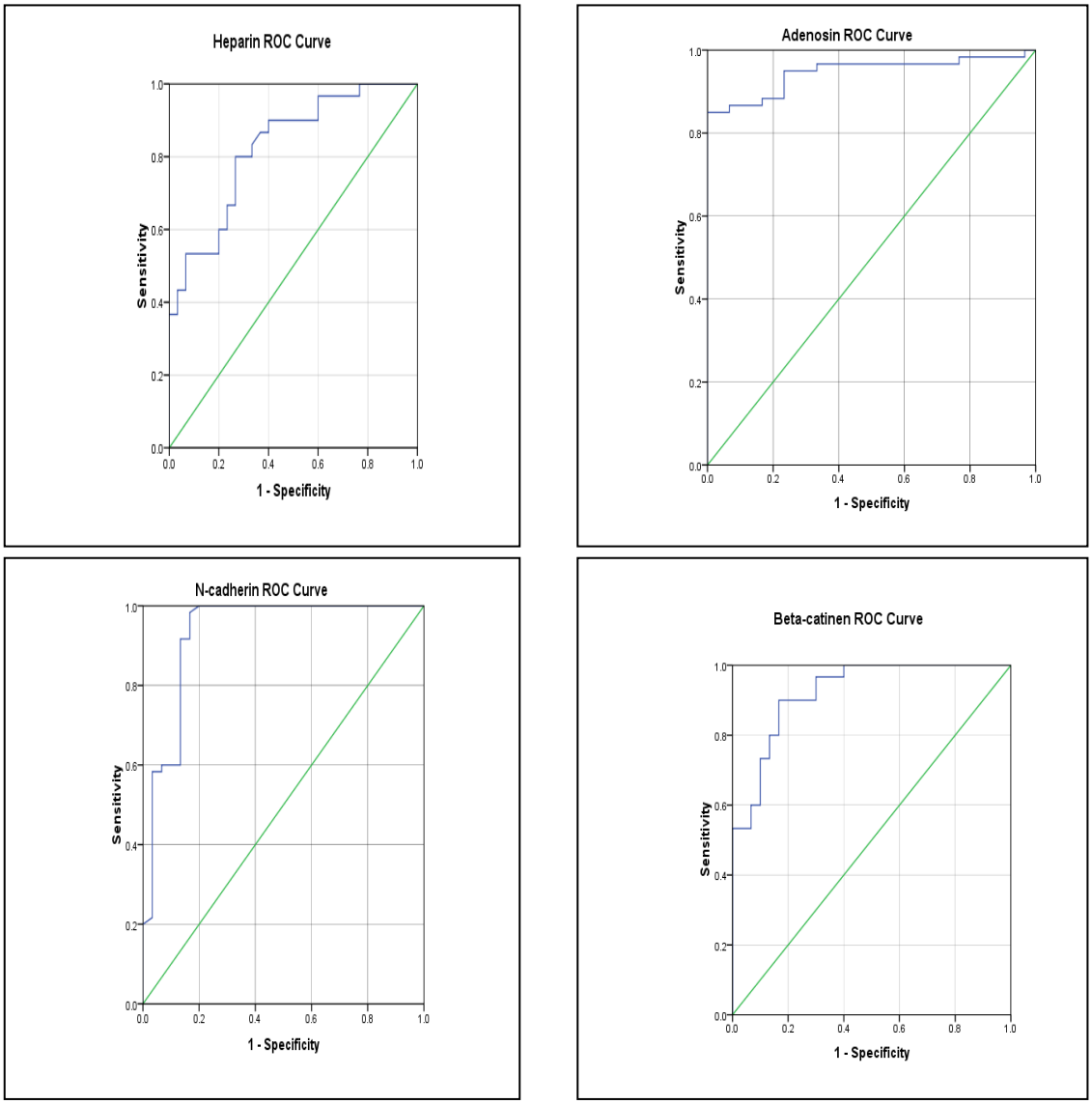

Figure 5: Model discrimination of the ROC curve and AUC of the predicting model.

3. Discussion:

Despite the remarkable progress in technology that has contributed to longer lifespans for humans, researchers are still facing challenges in finding effective treatments to address the growing prevalence of cancer. The current study investigates the potential role of (heparin, adenosine, N-cadherin and beta-catinen) in breast cancer development and if can consider these factors as a potential therapeutic target to combat the cancer and particularly breast cancer.

Recent findings show a significant elevation in serum heparin levels in patients compared to the control group. This increase may be attributed to its role as a crucial ingredient in facilitating the metastasis process, which is a critical characteristic contributing to the advancement of cancer. Heparin delivered the growth factor to its receptor and protect it from degradation. Heparin released from hydrogel help in mimicking extracellular matrix. Where vascular endothelial growth factor A (VEGFA) heparin complex bind with vascular endothelial growth factor receptor (VEGFR) and inhibite the signal cascade for angiogenesis. It's well known that VEGFR is regulated by heparin a natural glycosaminoglycan [30] so this may explain the elevated serum level in patients. There are no previous studies that have addressed

the same object of our study. All previous studies addressed heparin as a therapy in patients suffer from cancer such as [24]. Also recorded a significant different according to age in patient's groups under and above 50 years as compared with control, this mean that there was a strong impact of heparin in related with age. An elevated risk of venous arterial thromboembolic complications is a hallmark of cancer, an acquired thrombophilic disorder. The complicated underlying pathophysiology of cancer includes cancer cells' prothrombotic features, which can be exacerbated by anticancer treatment techniques like chemotherapy, hormone drugs, and surgery. The activation and inhibition of procoagulants and anticoagulants are naturally balanced in a normal coagulation-fibrinolysis system [31]. Cancer cells can affect this equilibrium by producing procoagulant and fibrinolytic chemicals, releasing proinflammatory and proangiogenic cytokines, and directly interacting with vessels and blood through adhesion molecules [32]. The result also recorded a significant increasing in heparin serum level in early patients with grade II, stage II as compared with treated patients' same grade and stage, it can be conclude that the chemotherapy treatment has a good impact because the heparin concentration return close to the normal level, but this depended on the number of chemotherapy dose, In related to hormonal status there is no impact on its level in the two patients groups.

Adenosine serum level was also significantly higher in the patients compared to the control group in the most recent results. Its function as an immune suppressive factor may account for this increase. [33]. ATP plays a significant role in controlling inflammatory signals and can function as a source of harm. Associated molecular pattern (AMP). ATP, functioning as a DAMP, can stimulate the activation of NLRP3 in the inflammasome within cells through its interaction with purinergic P2X7 receptors. NLRP3 has been identified as a crucial factor in the tumor microenvironment (TME) that facilitates tumor-promoting inflammation, immune evasion, proliferative signaling, invasion, angiogenesis, and suppression of apoptosis. The findings of our investigation are consistent with the research conducted by [34], which reported elevated blood levels of CD73. CD73 serves as both a signaling molecule and an enzyme involved in the synthesis of adenosine in colorectal cancer. There is no correlation between the level of age, grade, stage, hormonal status, and the two patient groups.

Regarding N-cadherin, it is a member of the cadherin family of proteins. Research has shown that N-cadherin has a role in promoting cancer aggressiveness. The latest findings indicate a substantial elevation in the serum level of N-cadherin in patients compared to the control group. Homotypic and heterotypic cell-cell adhesion are mediated in part by this calcium-dependent signal-chain transmembrane glycoprotein, which its level is likely to increase. According to [35] N-cadherin is overexpressed in many human malignancies, including breast, prostate, lung, and liver cancers. The current findings are in line with a study by [36] that also discovered a high level of N-cadherin expression in an invasive breast cancer cell line. Consistent with our findings, a previous study by [35] demonstrated that N-cadherin plays a positive regulatory role in promoting the invasive, migratory, and EMT properties of prostate cancer cells, and further revealed that overexpression of N-cadherin activates downstream signaling pathways. In study conducted by [37] which reported a significantly over expression of N-cadherin in metastasis then in related primary tumor (p 0.039) [38]. Also, a study done by [39]on patients with breast cancer using IHC assay to determined fibronectin expression which is an adhesion molecule, the result showed a progressive increase in fibronetin which increased as the disease progressed. In related to grade and stage there is no impact of its level in the two patients groups. Our result also showed that early diagnosed patients with hormonal status (triple negative) has the highest N-cadherin serum level versus other hormonal status, this mean that this status contribute with breast cancer progression. while there was no significant among hormonal status in treated patients. β-catenin also a significant serum level elevated in patients versus control. A study done by [9] which using IHC assay to elevate the expression of beta-

catenin in breast cancer and they recorded that 70% of samples had beta-catenin expressed normally in the membrane, while it was abnormally expression in 30% of samples. So, the current result is disagreed with this study, but the different may contribute to the type of samples it's a tumor biopsiy while our samples were serum. The reason for increasing of bata-catenin serum level in patients may contribute to its role in formation a new blood vessel (angiogenesis). Evidence suggested that both canonical and non-canonical wnt signaling pathway are involve with metastasis in a variety of organs in normal and pathological condition [40]. Mutation in wnt signaling pathway have resulted in up regulation of VEGF expression and induce new vasculeration of malignant cell and has an increased expression in tumor cell [41]. A study done by [42] on patients with non-hodgkins lymphoma using IHC assay, the result found the positive staining in cytoplasmic as perinuclear of β-catenin in tumor cell was detected in 29\37 (78%) case of NHLs and 8\37 (21%) was negative for β-catenin. Also, another study done by [43] on patients with bladder cancer using IHC assay the result found expression of β-catenin in 93% of male and 100% of female($p<0.01$), and the most β-catenin expression was found in plasma membrane and cytoplasm. β –catenin also recorded a significant different in its serum level in early patients age groups (>50 and <50) years. The >50 years recorded that the highest level versus to <50 and this level a high significant different in two patients' groups versus control. In related to disease grade and stage, there was a significant increasing in early patients with (grade and stage) II versus treated patients with same grade and stage. Additionally, early patients with grade III showed a significantly higher β-catenin serum level compared to treated patients with the same grade. There was a notable rise in the β-catenin serum level in treated individuals with hormonal status (ER+, PR+, Her-; ER+, PR-, Her-) compared to (ER-, PR-, Her+) when it came to hormonal status. A high level of β-catenin activity was found to be substantially associated with a poor prognosis for breast cancer patients, according to a study conducted by [44]. The researchers also discovered an overexpression of β-catenin [45], established a link between abnormal β-catenin expression, patient age beyond 50 years, and Her2 negative. This finding aligns with the current results [46], which indicated that β-catenin is highly expressed in breast cancer and strongly linked to tumor promotion. They used IHC assay to measure β-catenin expression in nuclei plasma samples from 41 patients, which was correlated with tumor histological grade ($p<0.05$). Grade I had little β-catenin expression, while grade III had significant expression.

Conclusion

All studied parameters showed a significant increasing in both early and treated patients compared to control. Additionally, the statistical analysis revealed a significant impact of some demographic characteristics of patients of the serum level of the studied parameters, so according to this finding we conclude that (heparin, adenosine, N-cadherin, and β-catenin) may be effective therapeutic targets to halt disease progression.

5. Acknowledgements

The authors would like to University of Baghdad and the Iraqi Ministry of Health to provide the necessary facilities to conduct the research.

6. Ethical approval

The study obtained ethical permission from the Iraqi Ministry of Health, specifically the Department of Medical Teaching at City Oncology Teaching Hospital (permission No. 7724, dated 25/7/2023). Subject to the agreement of the patient. The oncologist directed suitable patients for referral.

7. Conflict of interest

There are no conflicts of interest.

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