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Study of β-Catenin Levels in sera and urine of Iraqi Women with Breast Carcinoma

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Abstract

Breast cancer is a complex and multifaceted disease, comprising diverse subtypes that can be characterized by distinct histopathological, molecular, and clinical features. The dysregulation of β-catenin, a key transcription factor in the Wnt/β-catenin signaling pathway, plays a critical role in the early stages of tumorigenesis, ultimately influencing clinical outcomes. This study aimed to determine the correlation between sera and urine β-catenin levels in Iraqi patients with breast cancer comparing with control and first relative groups and to assess whether urine can be utilized as a substitute to sera to measure this status. The levels of β-catenin in fasting sera and urine were measured in a cohort of 29 breast cancer patients, as well as in 29 of their first-degree relatives and 30 age-matched healthy individuals serving as controls. All patients and first relatives were attending to the Oncology Teaching Hospital in Medical City. Sera and urine β-catenin concentration were measured using enzyme-linked immunosorbent assay. Urine creatinine concentrations were measured by colorimetric assay using automatically Roche/Hitachi cobas c111 System. Sera levels of β-catenin were significantly lower in breast cancer patients than in first relative and controls ($p < 0.01$). While in urine, levels of β-catenin were significantly higher ($p < 0.001$) in breast cancer than in control and first relative groups. Low levels of β-Catenin in sera and high levels of this marker in urine of breast cancer patients comparing with control and first relative degree groups pointed out that β-catenin may serve as a diagnostic and prognostic biomarker, more studies needed to comfier these results.

Keywords: Keywords: β-catenin, Breast cancer, Wnt signaling, tumor marker, ELISA.

دراسة مستويات بيتا كاتينين في مصل و بول النساء العراقيات المصابات بسرطان الثدي

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الخلاصة

سرطان الثدي هو مرض معقد ومتعدد الأوجه، ويتكون من أنواع فرعية متنوعة يمكن تمييزها من خلال ميزات نسيجية وجزيئية وسريرية مختلفة. يلعب اختلال تنظيم بيتا كاتينين، وهو عامل نسخ رئيسي في مسار الإشارة Wnt/β–catenin ، دورًا حاسمًا في المراحل المبكرة من تكون الأورام، مما يؤثر في النهاية على النتائج السريرية. هدفت هذه الدراسة إلى تحديد العلاقة بين مستويات بيتا كاتينين في الأمصال والبول لدى المرضى العراقيين المصابين بسرطان الثدي مقارنة مع المجموعة الضابطة ومجموعة الاقارب من الدرجة الأولى وتقييم ما إذا كان يمكن استخدام البول كبديل للأمصال لقياس هذه الحالة. الطريقة: تم قياس مستويات بيتا كاتينين في المصل والبول بعد الصيام في مجموعة من 29 مريضة بسرطان الثدي، بالإضافة إلى 29 من أقاربهن من الدرجة الأولى و30 فردًا سليمًا في نفس الفئة العمرية كأفراد تحكم. كان جميع المرضى والأقارب من الدرجة الأولى يترددون على مستشفى الأورام التعليمي في مدينة الطب. تم قياس تركيز البيتا كاتينين في المصل والبول باستخدام تقنية الاليزا. وتم قياس تركيز الكرياتينين في البول بتقنية الاختبار اللوني باستخدام جهاز كوباس من شركة روش الالمانية. كانت مستويات بيتا كاتينين في المصل أقل بكثير لدى مرضى سرطان الثدي مقارنةً بأقاربهم من الدرجة الأولى والأفراد السليمين ($p < 0.01$) .بينما في البول، كانت مستويات بيتا كاتينين أعلى بشكل ملحوظ ($p < 0.001$) لدى مرضى سرطان الثدي مقارنةً بمجموعة التحكم وأقارب الدرجة الأولى. تشير المستويات المنخفضة من بيتا كاتينين في المصل والمستويات العالية من هذا الماركر في البول لدى مرضى سرطان الثدي مقارنةً بمجموعة التحكم وأقارب الدرجة الأولى إلى أن بيتا كاتينين قد يكون بمثابة علامة تشخيصية وتنبؤية، وتحتاج المزيد من الدراسات لتأكيد هذه النتائج.

1. Introduction

Breast cancer (BC) is a multifaceted condition with several subtypes based on its histology, molecular, and clinical symptoms [1, 2]. Based on the data from the Iraqi Cancer Registry (ICR) in 2022, BC is the most prevalent type of cancer among women, accounting for 33.81% of all cancer cases in Iraq [3]. The prognosis and treatment options vary, the majority of current treatments are based on clinic-pathologic features such tumor size, grade, and lymph node status in addition to estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) status [4]. Invasive ductal carcinomas (IDCs) are the most common type of breast cancer in women and making up around 80% of all cases of the disease [5]. IDCs are a heterogeneous category of tumors characterized according to cytoarchitectural parameters, because they have a wide range of morphological variance [6]. Multiple genetic variations have been identified in breast carcinomas that have invaded from the original site, with some holding value as prognostic or predictive biomarkers [7]. In humans, a multifunctional protein β-catenin is encoded by the CTNNB1 gene. Typically, it cooperates with E-cadherin on the cellular membrane and serves a crucial structural role in the adherens junctions. By joining the cadherin cytoplasmic domain with α-catenin, which anchors the cytoskeleton adhesion complex, β-catenin protein functions as a major regulator of the cadherin-mediated cell-cell adhesion mechanism [8]. Furthermore, β-catenin is involved in the Wingless/Wnt signaling cascade, a crucial transcription-activating process that promotes cell migration, polarity, and proliferation [9, 10]. According to a recent study, there is a correlation between high β-catenin levels and BC cell proliferation as well as miR106a overexpression [11]. The cell membrane, cytoplasm, and/or nucleus contain β-catenin. The protein binds to the intracellular portion of type I cadherin molecules. It links the actin cytoskeleton *via* α-catenin and is necessary for cadherin's structural organization and function on the cell membrane [12]. The destruction complex composed of casein kinase 1α (CK1α) and glycogen synthase kinase 3β (GSK3β) phosphorylates the cytoplasmic free β-catenin, this led to its ubiquitination and subsequent proteasomal degradation [13]. Nevertheless, in instances where certain elements of the destruction complex are impaired. When β-catenin moves to the nucleus, it helps regulation

gene transcription [14]. In the Wnt/β-catenin pathway, disrupted regulation of the transcription factor β-catenin causes the earliest stages of the carcinogenesis [9]. In human breast cancer, a heightened intracellular level of β-catenin has been involved with a higher tumor grade and unfavorable prospect [15]. Furthermore, non-metastasizing fibromatosis and metaplastic carcinomas have been linked to the greatest degree of β-catenin expression in up to 90% of cases [16]. The canonical Wnt signaling pathway is another name for the Wnt/β-catenin pathway, is an evolutionary conserved signaling axis involved in several physiological processes, including tissue homeostasis, apoptosis, invasion, migration, and proliferation [17]. Several established risk factors contribute to the development of breast cancer, including advancing age, genetic predisposition, reproductive history (such as prior pregnancies and fertility treatments), alcohol consumption, exposure to ionizing radiation, and a family history of breast cancer or precancerous lesions [18]. Deviant control of the transcription factor β-catenin within the Wnt/β-catenin pathway triggers the early phases of carcinogenesis, since β-catenin plays a vital role in this signaling system [19]. Human urine represents a vital bodily fluid that holds significant value in the routine medical testing. Numerous studies indicate that urine has the potential to harbor biomarkers crucial for the screening of breast cancer, encompassing metabolomic, proteomic, and exosomic analysis [20]. This study aimed to determine the correlation between sera and urine β-catenin levels in Iraqi patients with breast cancer comparing with control and first relative groups and to assess whether urine can be utilized as a substitute to sera to measure this status.

Martials and Methods

Patients and specimens

This study consisted of a total of 88 female participants, who were categorized into three distinct groups: Group 1 comprised 29 breast cancer patients, Group 2 consisted of 29 first-degree relatives of breast cancer patients, and Group 3 included 30 healthy controls. All patients and first relatives were attending to the Oncology Teaching Hospital in Medical City. They were conveyed by several hospitals in Baghdad and other governorates in Iraq over the time of November 2022 to April 2023. Ages of these individuals were (25-57) years. Prior to surgery and pharmaceutical treatment, all patients were diagnosed in accordance with the WHO categorization. All patients have invasive ductal carcinoma, with no suffer from any other malignant diseases, (45%) of them had family history with tumors, the other had no family history. All patients were estrogen receptor (ER) +ve, human epidermal growth factor receptor 2 (HER2) –ve, and grade II, stage I, II.

Fasting sera and urine samples were collected from all individuals. None of the individuals in the examined groups were found to have diabetes mellitus, liver disease, kidney disease, heart disease, or osteoporosis. All of them were nonsmokers, non-pregnant, and had not taking any inflammation treatment with non-steroidal anti-inflammatory drugs.

Samples

Fasting blood (5 mL) and early morning mid-stream random spot urine (10 mL) was collected after the study subjects gave their agreement and filling out the face-to-face questionnaire. At room temperature, blood was allowed to stand for 30 minutes to allow complete clotting. Urine samples were collected from study groups into disposable screw cup containers for estimation of study parameters. The collected specimens were centrifuged at 4000 rpm for ten minutes. The urine samples were divided into two portions, one of them used immediately for creatinine measurement. Creatinine concentration was measured in urine samples immediately. The sera and the second portion of urine was separated into small aliquots and stored at (-20) ºC until being used to measure β-catenin.

Methods

Calculation of Body Mass Index (BMI)

The BMI was determined using the following equation:

BMI= Weight (kg)/ Height (m^2).

Sera and urine markers analysis

By enzyme-linked immunosorbent assay (ELISA) assay, sera and urine β-catenin concentrations were determined using commercially available kit (Catalogue Number: SL1870Hu). At 450 nm, the optical density (OD) was determined on a 96-well microplate Human reader where the β-catenin concentrations are expressed in pg/mL. Urine creatinine levels were quantified using a colorimetric assay performed on a Roche/Hitachi cobas c111 System analyzer. Creatinine concentrations in urine samples were measured automatically via this biochemical analyzer and reported in units of milligrams per deciliter (mg/dL). β-Catenin concentration in urine was divided by the level of creatinine in urine for the purpose of providing a representative sample, enabling us to analyzed β-Catenin without the need for collecting urine for 24 hours.

Statistical analysis

The data were analysed using the SPSS software 25. Data was reported as mean ± SD, and using the ANOVA test, statistical comparisons were done with ($p < 0.05$) being considered statistically significant. Pearson correlation test was conducted to correlate among all parameters [21]. To assess the accuracy of β-Catenin marker, Receiver Operating Characteristic (ROC) and the area under the curve (AUC) were performed as a comprehensive way.

Results

In this study, 45% of the patients had a family history of tumors, while the remaining 55% did not report any familial tumor occurrences. There were no significant differences in the results between these patients. Some physical features of the study groups were given in (Table-1). The difference in age and BMI between these groups is not high ($p>0.05$). The post hoc comparison corroborates this, indicating that all three groups are statistically similar in terms of age and BMI.

Table 1: Mean ± SD of age and BMI across all studied parameters.

parameter	Breast cancer (BC) n=29	First relative (FR) n=29	Control (n=30)	P^a	P^b	P^c	P^d
Age (Year)	43.62±8.36	40.35±10.51	40.96±7.01	0.365	0.964	0.471	0.334
BMI (Kg/m^2)	28.68±3.66	26.99±3.12	27.07±2.67	0.147	0.996	0.138	0.090

P^a value between patients with breast cancer and first relative.
P^b value between first relative and control group.
P^c value between patients with breast cancer and control group.
P^d value among all studied group (ANOVA test).

Significant variations were observed in the mean serum β-catenin levels, expressed as mean ± standard deviation (SD), when comparing the different groups. The control group exhibited significantly higher values (51.69 ± 5.61) compared to the BC (34.62 ± 2.27) and FR (37.65 ± 3.89) groups, as highlighted by a ($p < 0.001$). The post hoc labels further specified these differences, with the control group being statistically different from both BC and FR groups (Table-2).

For urine, this parameter also presented significant variations among the groups ($p < 0.001$). The BC group exhibited the highest value, (63.07 ± 12.93), followed by the FR group (43.00

± 4.49) and the control group (33.52 ± 2.73). The post hoc labels reiterated these significant differences among all three groups (Table-2).

The mean ± SD of creatinine concentration in urine do not significantly differ across the BC (12.31 ± 5.29 mg/dL), control (13.50 ± 6.38 mg/dL), and FR (12.05 ± 5.47 mg/dL) groups. This is supported by a high (p=0.61) and corroborated by the post hoc labels, which indicated that all three groups were statistically similar in this parameter (Table 2).
In addition, Table 2 illustrates the ratio of β-Catenin in urine to the level of creatinine in urine, the results showed that there was a highly significant difference ($p < 0.001$) between BC and control group, and between BC and FR group, while non-significant differences between FR and control group.

Table 2: Mean ± SD of studied parameters in sera and urine which are presented in three groups: breast cancer patients, first-degree relative group, and healthy controls.

parameter	Breast cancer (BC) n=29	First relative (FR) n=29	Control (n=30)	P^a	P^b	P^c	P^d
β-Catenin sera (pg/mL)	34.62 ± 2.27	37.65± 3.89	51.69± 5.61	0.030*	0.000**	0.000**	0.000**
β-Catenin urine(pg/mL)	63.07± 12.93	43.00± 4.49	33.52± 2.73	0.000**	0.000**	0.000**	0.000**
Urine Creatinine (mg/dL)	12.31± 5.29	12.05± 5.47	13.50± 6.38	0.958	0.637	0.711	0.611
β-Catenin /Cr. Urine $*10^{-9}$	6.43±3.64	3.62±1.24	2.91±1.36	0.000**	0.547	0.000**	0.000**

P^a value between patients with breast cancer and first relative.
P^b value between first relative and control group.
P^c value between patients with breast cancer and control group.
P^d value among all studied group (ANOVA test).

Sera and urine β-catenin exhibited significant variations, thus potentially serving as valuable biomarkers. Specifically, sera β-catenin was markedly higher in the control group with respect to the BC and FR groups, while that in urine was substantially elevated in the BC group. These findings may possess significant ramifications for the diagnosis or prognosis of breast cancer and may prompt further investigations to unravel the underlying biological mechanisms.

Table 3: Correlations among levels of β-catenin and Age, BMI in sera of all studied groups.

parameter		β-Catenin (pg/mL) BC group	β-Catenin (pg/mL) FR group	β-Catenin (pg/mL) control group
Age (year)	r	-0.214	-0.131	-0.212
	p	0.264	0.553	0.270
BMI (Kg/m^2)	r	-0.072	-0.025	-0.211
	p	0.711	0.909	0.273

The Pearson correlation matrix presented in Table 3 offers valuable insights into the relationships between multiple variables in the context of sera BC. According to the results illustrated in this table, no strong correlation was observed between β-catenin and other parameters in all studied groups. To estimate whether urine can be used as an alternative fluid instead of sera, Pearson correlation was done between β-catenin in sera and urine. As seen in

(Table-4), there was no significant correlation between sera and urine β-catenin in all studied groups.

Table 4: Correlations among levels of β-catenin in sera and urine in all studied groups.

β-Catenin urine (pg/mL)	β-Catenin sera (pg/mL)				
			BC group	FR group	Control group
	BC group	r	0.311		
		p	0.101		
	FR group	r		0.398	
		p		0.060	
	control group	r			-0.068
		p			0.725

Receiver Operating Characteristic (ROC) analysis for β-Catenin in sera and urine in patients with breast cancer comparing with control subjects.

Figure 1 illustrates the efficacy of β-catenin as a diagnostic marker in distinguishing BC cases from control subjects. The results showed that sera and urine β-catenin, exhibited exceptional diagnostic accuracy, as indicated by a sensitivity and specificity of 100 and an AUC of 1.00, with a cutoff value <38.00 pg/mL for sera and > 38.00 pg/mL for urine. These findings suggested that this particular marker is extremely reliable for distinguishing breast cancer from controls.

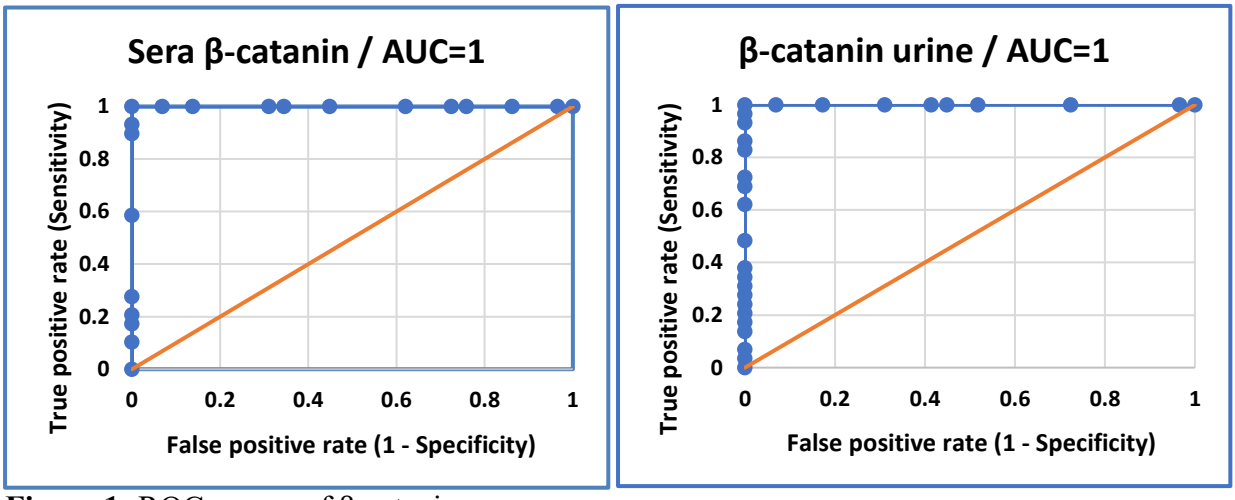

Figure 1: ROC curves of β-catenin concentrations in sera and urine of breast cancer patients

Discussion

Table 1 reveals that BMI spans a range from normal to overweight. Notably, a growing body of research has found a significant link between BMI and the risk of developing breast cancer. A comprehensive meta-analysis conducted by [22] has shed light on this association, providing valuable insights into the relationship between these two factors. There is a low positive correlation between breast cancer risk and BMI, with an increase in BMI of about 5 kg/m^2 requiring an increase in breast cancer risk of about 2% [23]. Nonetheless, among premenopausal women, a higher BMI reduces the risk of breast cancer. Obesity and overweight are thought to provide protection against premenopausal breast cancer, with the departure of women having a family history of breast cancer. Therefore, body fat may be a superior measure of breast cancer risk in postmenopausal women than BMI or body weight. Body fat propagation

may also influence breast cancer risk [24]. According to observational data, women with and without a family history of breast cancer may have a similar link between their BMI and risk of developing the disease [25].

In different types of breast cancer, there are many studies on β-catenin in tissue. A study carried out in China which analyzed the expression of β-catenin protein using immunohistochemistry in a group of 241 individuals diagnosed with breast cancer. The findings pointed to a notable presence of β-catenin in breast cancer, suggesting a significant role of β-catenin in promoting tumor growth [26]. In United State, a study by Shen *et al*., in 2016, on 72 patients with triple negative breast cancer. The researchers investigated the expression of β-catenin through immunohistochemistry and determined that β-catenin expression offers distinctive prognostic capability regardless of traditional pathological factors [27]. In Iran, a study on 88 invasive breast cancer cases, within this investigation, a correlation was noted between patient`s age over 50 years, HER2 negativity and abnormal β-catenin expression [28]. A separate investigation conducted in India involving a cohort of 25 patients diagnosed with breast cancer indicated that the presence of sera β-catenin potentially contributes to the development of breast cancer and holds promise as a prognostic indicator [29]. The results in our study showed that levels of β-catenin in sera decreased in BC patients compared with FR group and control groups (Table 2). Our findings contradict the results reported in the sole existing study on sera β-catenin within the literature [29]. This difference in results could be attributed to the differing in ethnicity and nature of the two countries in terms of environmental conditions, nutrition, and risk factors. In Iraq, there is only one research on β-catenin in tissue. The immunohistochemical results of this study revealed that over 70% of breast cancer samples were shown to have aberrant β-catenin expression [30].
In urine, there are no studies on urine β-catenin in Iraq and the world. The results in this study showed that β-catenin level was significantly higher than those in FR and control groups, that may be due to aberrant activation of β-catenin mediated transcriptional signaling and disruption of balance between cell proliferation and differentiation thus paving the way for tumorigenesis (Table 2). β-Catenin concentration in urine divided on the level of creatinine in urine for the purpose of providing a representative sample, enabling us to analyzed β-Catenin without the need for collecting urine for 24 hours. There are no significant differences in urine creatinine concentration among all groups.
No correlation was observed between β-catenin and age, BMI, that means β-catenin did not change with increasing age and BMI (Table 3). On the other hand, there was no correlation between sera and urine β-catenin. This meant that urine cannot be used as an alternative fluid than sera (Table 4).

The diagnostic utility of sera and urine β-catenin in BC disease was evaluated using ROC curves. The findings demonstrated the strong diagnostic efficiency of sera and urine β-catenin in the diagnosis of BC (Figure 1).

Conclusion

Low levels of β-catenin in sera and high levels in urine BC patients comparing with control and first relative degree groups and ROC results observed that β-catenin may serve as a diagnostic and prognostic biomarker for BC, more studies needed to comfier these results. The results of the Pearson correlation analysis indicate that urine cannot serve as a suitable alternative to serum.

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