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RESEARCH ARTICLE

Evaluation of Heat Shock Protein-90 as a Potential Risk Marker in Sera Women with Breast Cancer

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Abstract

Background: Breast cancer (BC) the second most common kind of newly diagnosed malignancy worldwide, is the most common illness among women. Heat shock protein 90 (HSP-90) plays a crucial in the growth and spread of cancer cells by maintaining the stability of overexpressed signaling proteins. **Aim of study:** Evaluate serum levels of HSP-90 as potential risk markers in breast cancer patients as compared to healthy individuals. **Subject and methods:** The research included 180 participants. Ninety females with BC (23 stage I, 36 stage II, 24 stage III, and 7 stage IV) contrasted with 90 healthy women as a control group matched with patients in age. Serum HSP-90 was measured using the ELISA technique kits. Additionally, lipid profiles and liver enzyme were assessed. **Results:** Serum HSP-90 levels were significantly elevated across all stages in women with BC compared to healthy women ($P < 0.001$), as well as among different stages of the disease. There was an increase in serum total cholesterol (TC), triglycerides (TG), low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) in patients. In contrast, there was a decrease in serum high density lipoprotein cholesterol (HDL-C) levels compared to healthy controls. Additionally, HSP-90 showed a significant positive correlation with age, ALP, TG, TC, HDL-C, LDL-C, ALT, AST, and VLDL-C with the exception of body mass index (BMI). HSP-90 demonstrated good diagnostic efficiency in breast cancer patients, with a cut-off value for HSP-90 at 300.5 pg/mL. This marker exhibited a sensitivity of 88.9% and a specificity of 87.8% with an area under the curve (AUC) of 0.907 (95% CI: 0.86-0.954; $P < 0.001$). **Conclusion:** HSP-90 may be a biomarker for breast cancer monitoring and early detection.

Keywords: Breast cancer- Heat shock protein 90- Sera Women

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Introduction

Breast cancer (BC) is still a serious threat in the context of global health issues because of its complex etiology and wide range of clinical presentations, which make prevention and treatment extremely difficult [1]. Understanding the complex nature of breast cancer is essential to creating efficient strategies for treatment, as the disease's incidence is still rising globally [2]. The most common cancer in Iraqi females is breast cancer, which accounted for 34.27% of instances that were recently reported in 2016 [3]. When it comes to health, the WHO found that patients may have a reduced death rate if they get the treatment they need quickly after a diagnosis. The low survival rates of BC patients in Iraq may be attributed mostly to inadequate treatment decisions and inadequate early diagnosis methods [4]. Being a highly conserved member of the heat shock protein family, HSP-90 is a potential target For cancer treatment since it controls several proteins and signaling pathways that are implicated in cancer. In breast cancer, there are a number of abnormal signaling pathways; among them, Hsp-90 is

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abundant in breast cells [5]. HSP-90, a heat shock protein of 90 kDa, helps stabilize the shape and mature the action of several proteins that promote cancer. Previous research has found over 300 HSP-90 clients, which points to Hsp90's essential function in cancer cell fate determination [6]. In addition to being essential components of cells, lipids also play a role in metabolism and signal transmission inside cells. Studies have demonstrated that lipids have an ever-increasing role in cancer, including its genesis, development, migration, and cell death. As lipidomics technology develops and advances, this link is predicted to increase [7]. With a disproportionately high incidence among women and a catastrophic impact on people's lives worldwide, breast cancer is a serious public health problem in today's society. Because of this issue, researchers are investigating the link between lipids and breast cancer [8-10].

Materials and Methods

The present study is a case-control study samples were collected from the National Cancer and Hematology Institute in Najaf Al-Ashraf, Iraq during the period from December 2023 to April 2024. For the current study, 180 participants between the ages of 36 - 65 years. The study included two groups: Ninety women with BC newly diagnosed with breast cancer by a specialized physician (the women were screened for breast cancer using ultrasound, mammography, and magnetic resonance imaging as well as, the histopathological results were used to categorize the breast cancer patients) and ninety apparently healthy women of the same age, who met the exclusion criteria.

Exclusion criteria

The study excluded all women who were pregnant, suffering from autoimmune disorders, microbial infections, chronic diseases (such as hypertension, cardiovascular disease, kidney disease, and thyroid disorders), taking oral contraceptives or any hormonal medications, and undergoing surgery.

Samples Collection

Following interviews regarding the participants' age range, a questionnaire developed by the researcher was used to collect information on their place of residence, employment, marital status, and family history of BC. Subsequently, blood samples for 5 mL were taken from each participant's veins. Gel tubes were utilized to transport blood samples following a breast cancer diagnosis and prior to the initiation of any treatment. the blood is centrifuged at 3000xg for 10 min to separate the sera into four Eppendorf tubes, then the samples are stored at -80°C until analysis.

Immunological and Routine Assays

Serum Hsp90 was measured using enzyme-linked immunosorbent assay (ELISA) kit from Melsin, China. This company has been utilized in previous research study [11]. Serum activities of ALT, AST, and ALP were measured spectrophotometrically, while TC, TG, and HDL-C were assessed using the enzymatic colorimetric method (Biolabo, France). LDL-C values were calculated by the Friedewald formula [12].

Statistical Analysis

The Kolmogorov-smirnov test was used to examine the distribution types of the results group. To compare the parameters between two groups, we used the Student's t-test. The analysis of variance one way (ANOVA) test was employed to assess differences in scale variable between diagnostic groups. The results were expressed as (mean±standard deviation) for normally distributed value. For correlation study, the Pearson's correlation coefficients (r) examine association between scale variable to find out the correlation between parametric parameter and other variable. Statistical significance was determined for all hypothesis tests with p-values less than 0.05 (two-tailed). Receiver operating characteristics (ROC) curves were measured to examine the diagnostic ability of the measured biomarkers to diagnose the disease. The cut-off values of the concentrations produce the best sensitivity and specificity from the area under the curve (AUC). IBM's Statistical Package for Social Sciences, version-27 (SPSS, Chicago, Illinois, USA), was used to compile and analyze the data.

Ethical Approval

All participants provided written informed permission in compliance with this present edition of the Helsinki Declaration, subsequent to clearance from the ethics commission (IRB) of the College of Science, University of Kufa, Iraq (order 41507 in 12/October/2023).

Results

The study included 90 breast cancer patients and 90 apparently healthy controls. The patients were divided into four groups according to the stages (stage I (23), stage II (36), stage III (24), and stage IV (7)). Compared with the control groups, the mean of HSP-90 level was significantly higher in the BC group (390.911 ± 94.528 vs. 279.333 ± 36.169 pg/mL, $p < 0.0001$; Table 1). Laboratory data such as liver enzymes and lipid profiles were significantly increased in the BC patients except for HDL-C, which was reduced when compared with healthy individuals. There

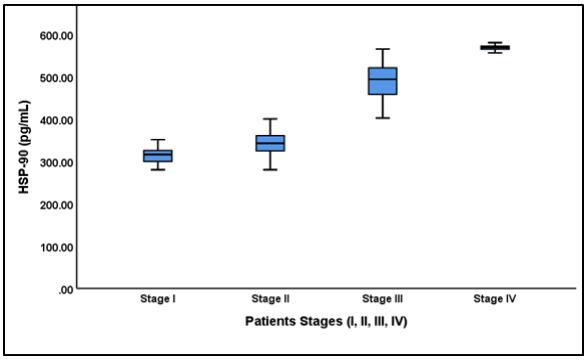

Figure 1. Comparison of Serum HSP-90 Levels in Patients' stages.

Table 1. Clinical and Demographic Information of Breast Cancer Patients Healthy Controls

parameters	Patients group (mean ± SD)	Controls group (mean ± SD)	P- value
Number	90	90	-------
Age (years)	48.344 ± 6.657	49.988 ± 8.074	0.1378 (NS)
BMI (kg/m^2)	28.758 ± 4.209	28.956 ± 6.559	0.8096 (NS)
ALT (IU/L)	22.185 ± 7.882	7.566 ± 3.187	P< 0.0001
AST (IU/L)	20.557 ± 4.773	7.683 ± 3.078	P< 0.0001
ALP (IU/L)	241.233 ± 62.521	86.733 ± 31.102	P< 0.0001
TG (mg/dL)	209.387 ± 25.511	196.721 ± 33.714	P< 0.005
TC (mg/dL)	235.642 ± 28.936	214.047 ± 34.246	P< 0.0001
HDL-C (mg/dL)	34.559 ± 5.067	48.661 ± 5.537	P< 0.0001
LDL-C (mg/dL)	159.205 ± 26.159	126.041 ± 30.651	P< 0.0001
VLDL-C (mg/dL)	41.877 ± 5.102	39.344 ± 6.742	P< 0.0001
HSP-90 (pg/mL)	390.911 ± 94.528	279.333 ± 36.169	P< 0.0001

Data represented as mean ± SD, SD: Standard Deviation, NS: non-significant, BMI: Body Mass Index, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, ALP: Alkaline phosphatase, TG: Triglyceride, TC: Total Cholesterol, HDL-C: High Density Lipoprotein-Cholesterol, LDL-C: Low density Lipoprotein-Cholesterol, VLDL-C: Very Low Density Lipoprotein-Cholesterol, HSP-90: Heat shock protein 90.

Table 2. Comparison of Variables between the Stages of Breast Cancer Patients

Variables	Patients Stages (No.= 90)			
	Stage I	Stage II	Stage III	Stage IV
Number (n%)	23 (25.555%)	36 (40%)	24 (26.666%)	7 (7.777%)
Age (years)	43.739 ± 5.941	49.388 ± 6.442	50.041 ± 5.834	52.285 ± 6.156
	p-value: a) 0.001 b) 0.001 c) 0.002 d) 0.001 e) 0 .687 h) 0.397			
BMI (kg/m^2)	27.815 ± 3.574	29.122 ±4.561	29.292 ± 4.232	28.154 ±4.489
	p-value : a) 0.251 b) 0.235 c) 0.853 d) 0.879 e) 0 .582 h) 0.533			
ALT (IU/L)	14.095 ± 4.031	20.586 ± 4.558	28.379 ± 5.175	35.757 ± 1.912
	p- value: a) 0.0001 b) 0.0001 c) 0.0001 d) 0.0001 e) 0.0001 h) 0.0001			
AST (IU/L)	17.982 ± 4.478	20.077± 4.923	22.937 ± 3.682	23.328 ± 3.552
	p-value: a) 0.079 b) 0.0001 c) 0.006 d) 0.016 e) 0.078 h) 0.837			
ALP (IU/L)	185.913 ± 46.193	235.778 ± 54.168	296.458 ± 42.601	261.714±38.573
	p-value: a) 0.0001 b) 0.0001 c) 0.0001 d) 0.0001 e) 0.197 h) 0.097			
TG (mg/dL)	211.196 ±29.001	201.235 ±22.372	217.036 ± 26.149	219.140±16.535
	p-value: a) 0.138 b) 0.424 c) 0.462 d) 0.01 e) 0.085 h) 0.845			
TC (mg/dL)	231.408 ± 32.218	230.633 ± 28.745	243.430 ± 27.695	248.615±13.983
	p-value: a) 0.92 b) 0.154 c) 0.168 d) 0.094 e) 0.132 h) 0.675			
HDL-C (mg/dL)	34.224 ± 4.874	32.335 ± 5.151	37.261 ± 4.011	37.832 ± 2.338
	p-value: a) 0.131 b) 0.028 c) 0.075 d) 0.0001 e) 0.005 h) 0.775			
LDL-C (mg/dL)	154.944 ± 28.872	158.051 ± 26.590	162.761 ± 25.969	166.954±13.637
	p-value: a) 0.66 b) 0.312 c) 0.294 d) 0.499 e) 0.416 h) 0.712			
VLDL-C (mg/dL)	42.239 ± 5.800	40.247 ± 4.474	43.407 ± 5.229	43.828 ± 3.307
	p-value: a) 0.138 b) 0.424 c) 0.462 d) 0.01 e) 0.085 h) 0.845			
HSP-90 (pg/mL)	310.478 ± 36.913	342.055 ± 33.448	489.541 ± 40.302	568.285±7.718
	p-value: a) 0.001 b) 0.0001 c) 0.0001 d) 0.0001 e) 0.0001 h) 0.0001			

Data represented as mean ± SD, SD: Standard Deviation, BMI: Body Mass Index, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, ALP: Alkaline phosphatase, TG: Triglyceride, TC: Total Cholesterol, HDL-C: High Density Lipoprotein-Cholesterol, LDL-C: Low density Lipoprotein-Cholesterol, VLDL-C: Very Low Density Lipoprotein-Cholesterol, HSP-90: Heat shock protein 90. Significant differences between stages; a) Stage I vs. Stage II, b) Stage I vs. Stage III, c) Stage I vs. Stage IV, d) Stage II vs. Stage III, e) Stage II vs. Stage IV, h) Stage III vs. Stage IV.

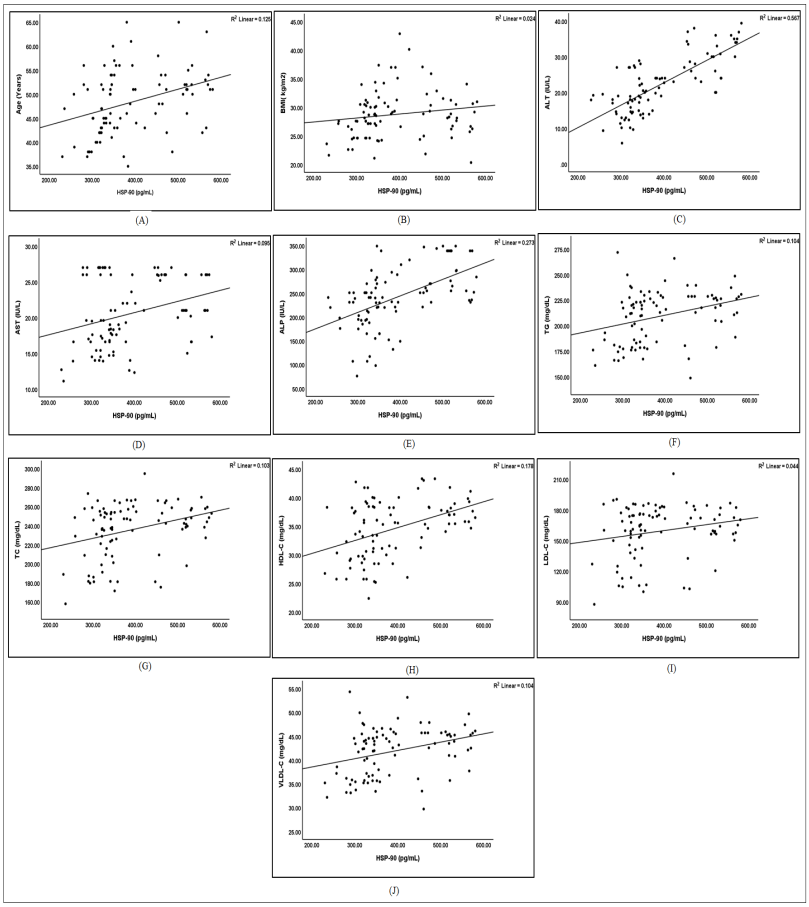

Figure 2. Correlation between HSP-90 Levels and A, Age; B, BMI; C, ALT; D, AST; E,ALP; F, TG; G, TC; H, HDL-C; I, LDL-C; J, VLDL-C.

Table 3. Correlation between HSP-90 and Studied Parameters in Breast Cancer Patients

Parameters	r	p-value	Parameters	r	p-value
Age (years)	0.354**	0.001	TG (mg/dL)	0.322**	0.002
BMI (kg/m^2)	0.156	0.142	TC (mg/dL)	0.321**	0.002
ALT (IU/L)	0.753**	0.0001	HDL-C (mg/dL)	0.422**	0.0001
AST (IU/L)	0.308**	0.003	LDL-C (mg/dL)	0.21*	0.047
ALP (IU/L)	0.523**	0.0001	VLDL-C (mg/dL)	0.322**	0.002

**. A significance level of 0.01 (2-tailed) indicates a correlation. *. Assuming a 2-tailed significance threshold of 0.05, the correlation is evident. r, The Pearson correlation coefficient.

was no significant difference between the two groups (patients and healthy groups) with respect to age and body mass index (p = 0.1378, p = 0.8096, respectively).

Table 2 and Figure 1 revealed that the level of HSP-90 was significantly higher for all stages. No significant difference was observed in TC, TG, LDL-C, or VLDL-C levels in the four stages. However, HDL-C showed significant variation between II vs. III and II vs. IV stages. There was no BMI difference between the four stages.

The linear regression analysis was used to verify the relationship of biochemical parameters with the serum level of HSP-90. As shown in Table 3 and Figure 2 were significant positive correlation of all parameters with HSP-90. Despite the significant decrease in HDL-C levels in patients with PCOS, a positive correlation with HSP-90 was observed. This correlation arose because many samples exhibited an increase in HSP-90 that corresponded an increase in HDL-C.

However, there was no correlation between HSP-90 and BMI.

The results of the receiver operating curve (ROC) and area under the curve (AUC) analysis for HSP-90 are presented in Table 4 and Figure 3. The AUC was 0.907 (CI of 0.86-0.954), with sensitivity of 87.8% and a specificity of 88.9%. The cut-off value at 300.5 (pg/mL) demonstrated the marker ability to diagnose breast cancer.

Discussion

Normal cells typically express HSP-90 at modest levels, but when under stress, its expression rises to defend biological proteins from damage or target aggregation. However, HSP-90 is often elevated in several cancer types, including breast cancer. It stabilizes and activates its

Table 4. Analysis of the Area under the Curve for Receiver Operating Characteristics of the Diagnosis for Breast Cancer Patients vs. Healthy Control

Variables	HSP-90 (pg/mL)
Area Under Curve (AUC)	0.907
Cut-off value	300.5
P-value	p < 0.0001
Specificity	88.90%
Sensitivity	87.80%
Confidence interval 95%	0.86-0.954

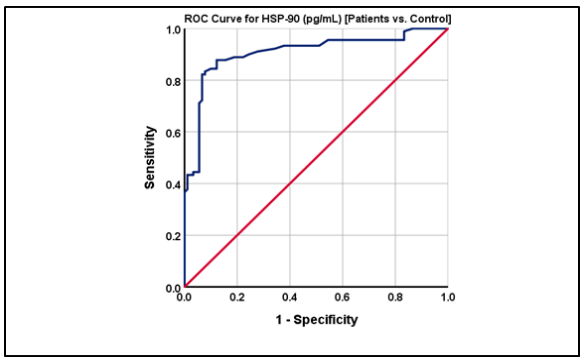

Figure 3. Receiver Operating Characteristic Curve for Breast Cancer Patients vs. Healthy Control.

targets, many of which are oncogenes, such as transcription factors, kinases, and downstream gene regulators, to enhance tumor cell adhesion, motility, and metastasis [13]. Heat shock protein 90 is a preserved molecular chaperone that aids in folding cytosolic proteins, maintain structural integrity, and control signal transduction and cell cycle [14]. This could contribute to clarifying because HSPs are essential for controlling processes in cells, such as apoptosis, metastasis, and proliferation. Furthermore, inside the tumor microenvironment, several of the crucial HSPs also control the delicate balance between the immune responses that are destructive and protective [15]. HSP-90 satellite proteins influence various stages of cancer progression, including growth, immortality, apoptosis, angiogenesis, invasion, and metastasis. The presence of a high-affinity transcript is essential for cancer cells with altered satellite proteins, as the proportion of HSP-90 in high-affinity states may impact cancer progression and therapeutic efficacy [16]. High levels of HSP-90 expression in breast cancer are linked to the disease's recurrence and lymph node metastases. Research has demonstrated that elevated expression of HSP- 90 is a strong predictor of BC survival and a disease prognostic factor in itself of itself [17]. HSP-90 is crucial for the stability of a number of proteins involved During the course of, survival, and tumor development in breast cancer. It also plays a significant role in the stabilization of various proteins associated with cancer growth and survival [18]. Because HSP-90 is found on the surface of tumor cells and is released by them via exosomes, it is a potential and readily available biomarker. In BC, elevated levels of HSP-90 are linked to a poor prognosis [19]. The ongoing production of inflammatory signals and the elevation of intracellular HSPs as a result of enhanced cellular turnover are the causes of the increased release of HSPs into the extracellular environment [20]. Breast cancer patients in the advanced stages have high levels of HSP-90 specific autoantibodies; the HSP-90 chaperone contributes to the development of a variety of human malignancies, including breast cancers [21]. Given evidence that HSP-90 This molecular chaperone has emerged as a promising therapeutic target due to its central involvement in breast cancer biology and its capacity to inhibit the function of several receptors, kinases, and transcription factors implicated in human cancer [22]. Hsps expression has been related to tumor cell differentiation and proliferation, as well as to poor prognosis and resistance to apoptosis. HSPs are elevated in BC tissue, as evidenced by several findings, and the degree of the increase corresponds to the degree of malignancy [23].

In conclusions, the promising results regarding HSP-90 may reveal its potential as a reliable biomarker in the diagnosis of breast cancer. Elevation of HSPs in breast cancer tissue underscores a significant relationship between these proteins and malignancy. Higher levels of HSP-90 are not merely byproducts of cellular stress but are closely associated with the degree of malignancy present in tumors. HSP-90 levels could serve as a valuable biomarker for assessing tumor aggressiveness and potentially guiding treatment strategies.

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Conflict of Interest

Author declares no conflict of interest.

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