Journal of Pioneering Medical Sciences
Received: October 05, 2025 | Accepted: February 07, 2026 | Published: March 05, 2026
Volume 15, Issue 02, Pages 143-150

DOI https://doi.org/10.47310/jpms2026150219

Investigation of CD8, CD20 and their Correlation with Heat Shock Protein 90 in Breast Cancer Patients

Tuqa Alaa Nuaman[1*] and Hayfaa Mahmood Fahad[2]
[1,2]Department of Biology, College of Medicine, Aliraqia University, Iraq

*Corresponding author: Tuqa Alaa Nuaman (e-mail: tuqa.a.naaman@aliraqia.edu.iq).

Abstract **Objectives: Background:** One of the main causes of cancer-related fatalities globally, breast cancer is the most prevalent malignancy in women. Many individuals are still detected at advanced stages and its occurrence keeps rising due to lifestyle, hormonal and hereditary factors. The disease is biologically diverse, with molecular subtypes (luminal, HER2-positive, triple-negative) that influence prognosis and therapy. Despite progress in screening and targeted treatments, recurrence and therapy resistance remain major challenges, highlighting the need for new prognostic markers and improved therapeutic strategies. **Objective:** Analytic immunological markers for assessment of prognostic values of CD8, CD20 and their correlation with heat shock protein 90 in breast cancer. **Methods:** Al-Iraqi University's College of Medical Sciences gave its approval for the project. The trial, which lasted three months (November to February 2025), involved 140 females from the medical city (Oncology Educational Hospital) who donated three ml of their blood divided into two sets of participants. One group consisted of 70 patients with breast cancer, while the other group consisted of 70 healthy people. Serum levels of CD8, CD20 and HSP-90 were estimated using ELISA assays. Participants in the study provided demographic information, including age, sex, family history and medical history. **Results:** The study samples ranged in age from 19 to 85 years, with a mean of 49.86±12.713. The majority of the sample (46.4%) was in the 46–60 age range. The mean levels of CD8 and CD20 were significantly higher (18.93541±4.743529 Vs 4.82896±1.858044), with significant mean differences of -14.106457 (t = -23.167, df138, p = 0.000) for CD8 and (1.71293±0.212769 Vs 0.91524±0.206988), with significant mean differences of - 0.797686 (t = -22.483, df138, p = 0.000) for CD20.The mean value of HSP-90 was significantly higher (47.85257±3 8.893877 Vs 14.57260±5.176012), with a significant mean difference of -33.279971 (t = -27.058, df: 138, p = 0.000) respectively. **Conclusion:** CD8, CD20 and HSP-90 can be used to evaluate the immune response of breast cancer disease patients and serve as a marker for early diagnosis.

Key Words Breast Cancer, CD8, CD20, HSP-90

INTRODUCTION

Breast cancer is characterized by the uncontrolled proliferation of abnormal breast cells, resulting in the formation of tumors. Without intervention, these tumors may metastasize to other parts of the body and lead to mortality.

Breast cancer is a multistep process influenced by various elements that include complex genetic and epigenetic interactions, hormonal factors and environmental determinants that lead to the development of malignant mammary epithelial cells. The World Health Organization (WHO) estimates that the disease accounts for approximately 2.3 million new cases and over 685,000 deaths globally yearly [1].

Epidemiological estimates indicate a significant increase in disease burden during the next few decades. By 2050, breast cancer incidence is projected to rise by 38% and the new cases would be around 3.2 million per year. Concurrently, mortality is predicted to increase by 68%, or about 1.1 million deaths a year. This anticipated increase has been ascribed to causes such as world population growth, demographic aging and better diagnostic techniques resulting in both earlier and more frequent discovery [2].

The infiltration of $CD8^+$ cytotoxic T lymphocytes and $CD20^+$ B lymphocytes in breast tumors was demonstrated to associate with a better survival outcome. Specifically, patients with triple-negative breast cancer in which these immune cells were observed at postoperative higher densities had better disease-free and overall survival, indicating their significance as valuable prognostic biomarkers [3].

Cytotoxic immune cells like CD8+ T lymphocytes and Natural Killer (NK) cells can recognize and destroy tumor cells. The ratio effector of these cells is a major determinant of the immune response against breast tumors. An increased CD8+ ratio is usually accepted as a favorable prognostic factor, representing a more active anti-tumor immune environment [4].

$CD20^+$ B lymphocytes were also found in the tumor stroma and tertiary lymphoid structures of breast cancer samples. The exact biological function of them has not been thoroughly elucidated, while there is a growing body of evidence to suggest that they may participate in antitumor immunity by generating antibodies, enhancing AP and producing cytokines. The tumor-infiltration of $CD20^+$ B cells was correlated with good clinical outcome in some subsets of breast cancer patients; nevertheless, there is a need to clarify the prognostic or functional relevance for the behavior of this cellular subset [5].

Consequently, heat shock protein 90 has also been shown to be highly expressed in many cancers, including breast cancer can also regulate the immune response in the tumor microenvironment, possibly affecting immune cell infiltration and function [6].

Heat Shock Protein 90 (HSP90) is a molecular chaperone that facilitates the proper folding and stabilization of many client proteins, including several that contribute to oncogenic signaling. High expression of HSP90 has been found in breast cancer and it may serve as a diagnostic indicator due to its correlation with aggressive disease and resistance to therapy [7].

Although its intracellular function is well characterized, HSP90 alpha also affects tumor–immune crosstalk. Tumor cells may secrete HSP90 to the extracellular space and in this location, it contributes to shaping the immune microenvironment by stimulating dendritic cell activation and promoting maturation of antigen-presenting cells; by also modifying cytokine production. Meanwhile, HSP90 might also promote immune evasion through stabilizing molecules in checkpoints or inducing the function of Tregs [8].

Objectives of the Study

This study was required in Iraq since the number of cases of Breast Cancer (BC) has dramatically increased over the past few decades. The current study's goal is to use the ELISA technique to measure the serum levels of the following immunological markers that are present in breast cancer patients.

- Evaluation of immunosuppressive markers CD8+ and CD20+
- Calculating the blood serum's proportion of heat shock protein 90 (HSP-90)

METHODS

Sample Collection

This research was designed as a case-control study to investigate the association between immune biomarker levels and breast cancer. The study was conducted at the Teaching Oncology Hospital in Baghdad Medical City. The current study was carried out for 16 weeks from the first of November 2024 to 31 February 2025.

The target population was breast cancer patients. Blood samples were donated by 140 women. The total number of samples, which were divided into control and case groups, included 70 women who had been recently diagnosed with breast cancer at various stages and 70 other women who were completely healthy.

Three milliliters of venous blood sample were promptly transferred to a plain gel tube and allowed to coagulate at room temperature (20–25°C) for 15 minutes. Following a 10-minute centrifugation at 3000 rpm to separate the serum, the serum was transferred to five Eppendorf tubes and stored in a cooling box at -80°C until analysis.

Data Collection

Demographic data were obtained from the patients, such as age, sex, social status, Family history and medical history, through face-to-face interviews, depending on the feasibility and scope of the study. With the consent of the participants.

Statistical Analysis

The analysis utilized SPSS version 26 and STATISTICA version 9 for data entry and verification. Descriptive statistics for qualitative data included frequency distribution tables, numbers and percentages, while quantitative data were analyzed using mean, standard deviation and range. For assessing differences between study subgroups, unpaired Student's T-tests, Chi-square tests and Likelihood ratio tests were applied. A logistic regression model and ROC curve were used to determine the optimal cut-off value for immunological characteristics as a predictive noninvasive breast cancer risk marker, with statistical significance established at a p-value of less than 0.05.

Inclusion Criteria

Women of all ages who have been newly diagnosed with breast cancer at any stage of the disease.

Exclusion Criteria

For the group of cases, women with breast cancer who had received any type of treatment, whether chemotherapy, hormone therapy, or radiation, were excluded.

Evaluation of CD8 and CD20 Levels in the Serum of Study Groups

In this study, ELISA kits use the Sandwich-ELISA principle for each marker; two serological tests were performed (CD8, CD20) to estimate their levels.

The Immunological Kits were Human CD8 ELISA kit/Elabscince/U.S.A, Human CD20 ELISA kit/Fine Test/Germany, with the results being recorded using a Hs-human reader/Germany (Automated ELISA reader) and Combiwash hs/Germany (Automated ELISA washer).

The Standard Curve of each CD8 and CD20 in the Figure 1 and 2.

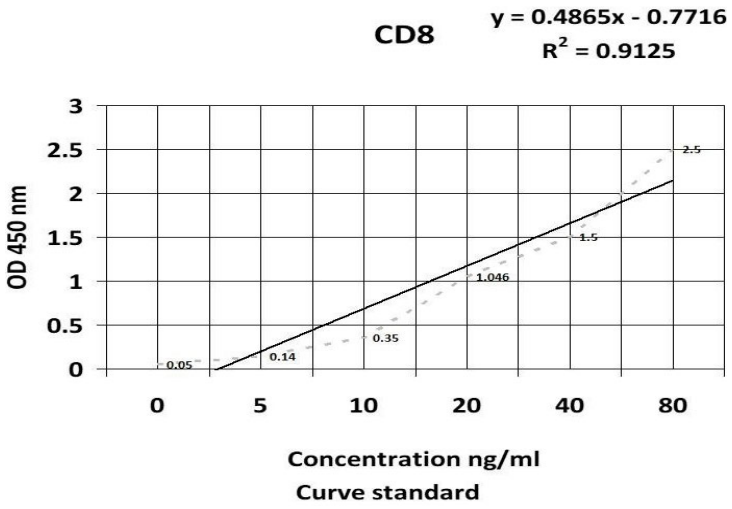

Figure 1: Standard Curve of CD8

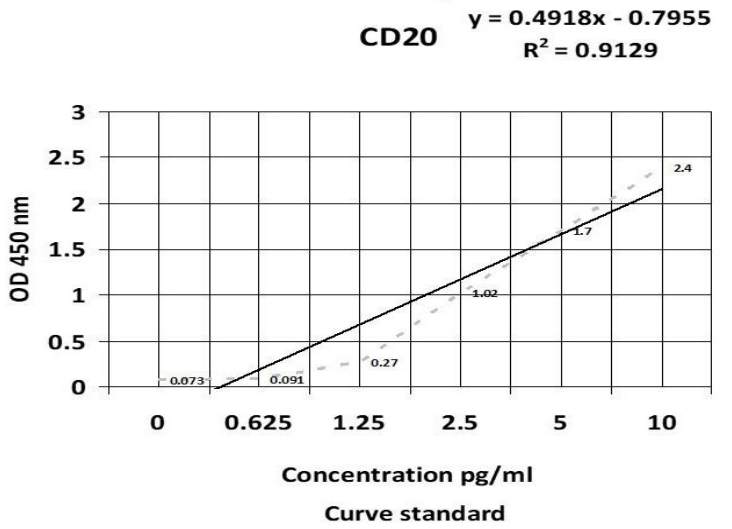

Figure 2: Standard Curve of CD20

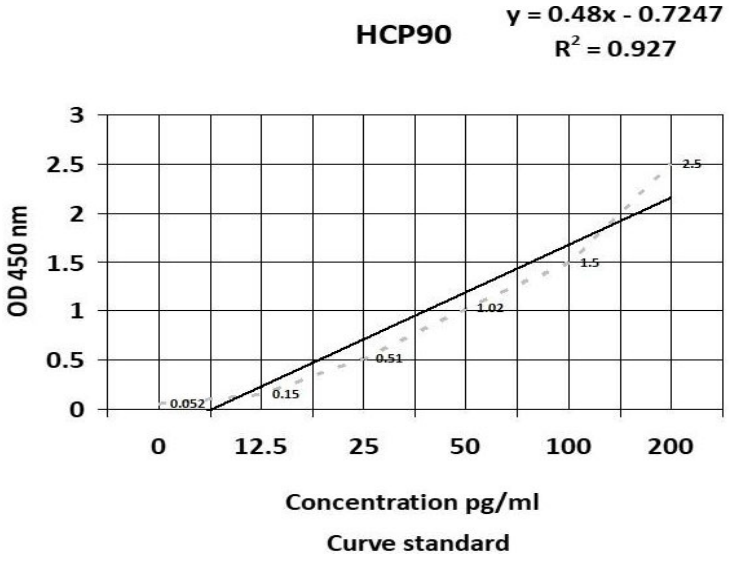

Figure 3: Standard Curve of HSP-90

Evaluation of HSP-90 Level in the Serum of Study Groups

The Sandwich-ELISA principle is applied by the ELISA kit used. An antibody specific to Human HSP-90 has been pre-coated onto the micro-ELISA plate included in this kit.

Elabscince/U.S.A. Human HSP-90 ELISA kit to assess their levels. A Combiwash hs/Germany (Automated ELISA washer) and an Hs-human reader/Germany (Automated ELISA reader) were used to record the data.

Figure 3 shows the HSP-90 Standard Curve.

RESULTS

Demographic Data

The study participants ranged in age from 19 to 85 years. The majority of the sample was in the 46-60 age group. The majority of breast cancer cases were also in this age group, as was the majority of cases in the control group. These mean differences were statistically significant (t = -2.748, df: 138, p = 0.007).

Nonetheless, 89.3% of the women in the research were married, with widows and single women coming in second and third, respectively, at 5.7 and 5%. The distribution of study samples' marital status, however, was not statistically significant (p>0.05), which is consistent with the matching goal of sample collecting (Table 1).

However, with the exception of the controls group, women with breast cancer diagnoses had a duration of less than three months (36; 25.7%) compared to those with a diagnosis of three months or more (34; 24.3%) (Figure 4).

In addition, stage II was the most common stage among women in the breast cancer group of cases sample, followed by stage III, stage I and stage IV (Figure 5).

Paraclinical Characteristics among the Study's Groups

Blood Parameters among Study Groups

Comparison of Immunohistochemistry Parameters Among Case Groups: According to the study's assessment of the Immunohistochemistry (IHC) parameters of the Estrogen and Progesterone Receptors (ER, PR) and the expression of the human epidermal receptor protein-2 (HER-2) among the cases sample of women with breast cancer, the cases group's ER and PR receptors were significantly more positive than the HER2 group's (each 46; 56.7% Vs 23; 32.9%) (Figure 6).

Comparison of Immunological Parameters among Study Groups

The immunological parameter of Heat Shock Protein-90 (HSP-90) was compared between the study groups and significant differences were found. The mean value of HSP-90 was significantly higher among women with breast cancer in the cases group than among those without the disease in the controls group, with a significant mean difference (t = -27.058, df: 138, p = 0.000), respectively (Table 2 and Figure 7).

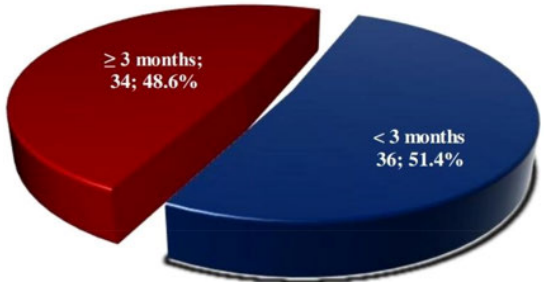

Figure 4: Distribution of breast cancer cases according to duration of diagnosis (n = 70)

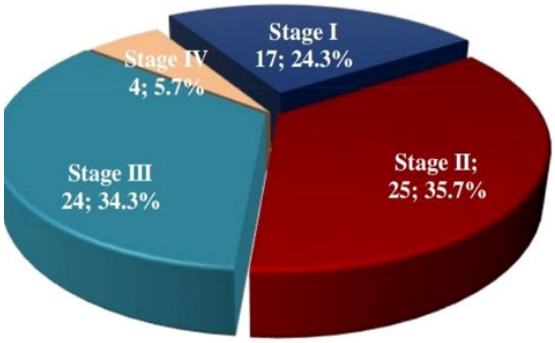

Figure 5: Distribution of Breast Cancer Cases According to Stage of Cancer (n = 70)

Table 1: Baseline Characteristics of the Study's Sample (n = 140)

Characteristics	Study groups			
	Cases (n = 70)	Control (n = 70)	Total (n = 140)	Significancy
Age (years)				
Mean±SD	52.74±12.454	46.97±12.393	49.86±12.713	t = -2.748, df: 138, p = 0.007[a]
Range (min-max)	61 (24-85)	52 (19-71)	66 (19-85)	
Age (In groups)				
<30	2 (2.9)	9 (12.9)	11 (7.9)	x^2: 6.396, df: 4, p = 0.17[b]
30-45	18 (25.7)	18 (25.7)	36 (25.7)	
46-60	33 (47.1)	32 (45.7)	65 (46.4)	
61-75	16 (22.9)	11 (15.7)	27 (19.3)	
>75	1 (1.4)	-	1 (0.7)	
Marital status				
Single	6 (8.6)	1 (1.4)a	7 (5.0)	Likelihood Ratio: 4.162, df: 2, p = 0.125[c]
Married	60 (85.7)	65 (92.9)	125 (89.3)	
Others	4 (5.7)	4 (5.7)	8 (5.7)	

[a]:Unpaired T-Test [b]:Chi-Square Test [c]: Likelihood Ratio: (Alternative Chi-Square Test)

Table 2: Mean Comparison of Immunological Parameters of Heat Shock Protein-90 (HSP-90) among the Study's Groups (n = 140)

Immunological Parameters (Mean±SD)	Study groups (n = 140)			Significance[a]
	Cases (n = 70)	Control (n = 70)	Mean differences	
Heat Shock Protein 90 (HSP-90)	47.85257±3 8.893877	14.57260±5.176012	-33.279971	t = -27.058, df: 138, p = 0.000

[a]: Unpaired T-Test

Table 3: Mean Comparison of Immunological Parameter of Cluster of Differentiation 8 (CD8) among the Study's Groups (n = 140)

Immunological Parameters (Mean±SD)	Study groups (n = 140)			Significance[a]
	Cases (n = 70)	Control (n = 70)	Mean differences	
Cluster of Differentiation 8 (CD8)	18.93541±4.743529	4.82896±1.858044	-14.106457	t = -23.167, df: 138, p = 0.000

[a]: Unpaired T-Test

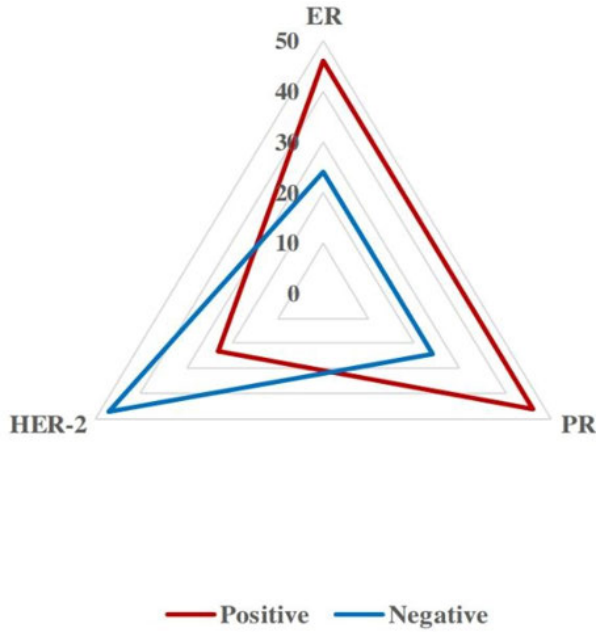

Figure 6: Distribution of Immunohistochemistry (IHC) Parameters (Positivity Expression) among Cases Group of Study Samples (n = 70)

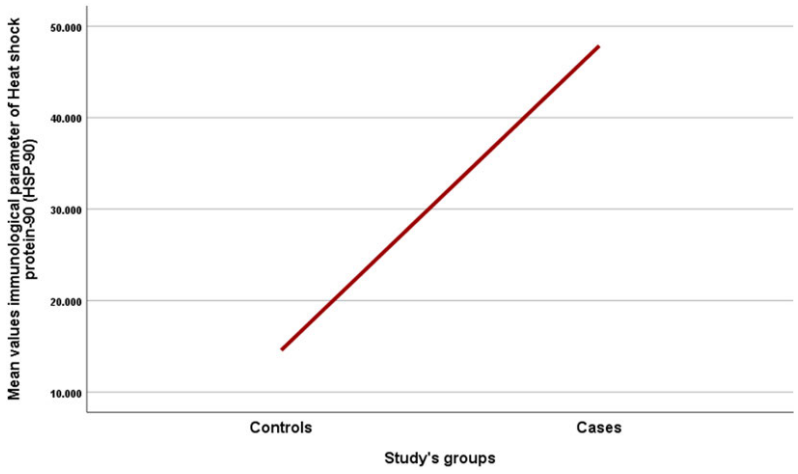

Figure 7: Comparison of Immunological Parameters of Heat Shock Protein-90 (HSP-90) among the Study's Groups (n = 140)

Similarly, women with breast cancer in the cases group had significantly higher mean levels of the immunosuppressive molecule of Cluster of Differentiation 8 (CD8) than women without breast cancer in the controls group, with a significant mean (t = -23.167, df138, p = 0.000) (Table 3 and Figure 8).

However, women with breast cancer in the cases group had significantly higher mean levels of immune suppressive molecule Cluster of Differentiation 20 (CD20) than women in the controls group, with significant mean differences (t= -22.483, df138, p = 0.000) respectively (Table 4 and Figure 9).

Table 4: Mean Comparison of Immunological Parameter of Cluster of Differentiation 20 (CD20) among the Study's Groups (n = 140)

Immunological Parameters (Mean±SD)	Study groups (n = 140)			Significance[a]
	Cases (n = 70)	Control (n = 70)	Mean differences	
Cluster of differentiation 20 (CD20)	1.71293±0.212769	0.91524±0.206988	- 0.797686	t = -22.483, df: 138, p = 0.000

[a]: Unpaired T-Test

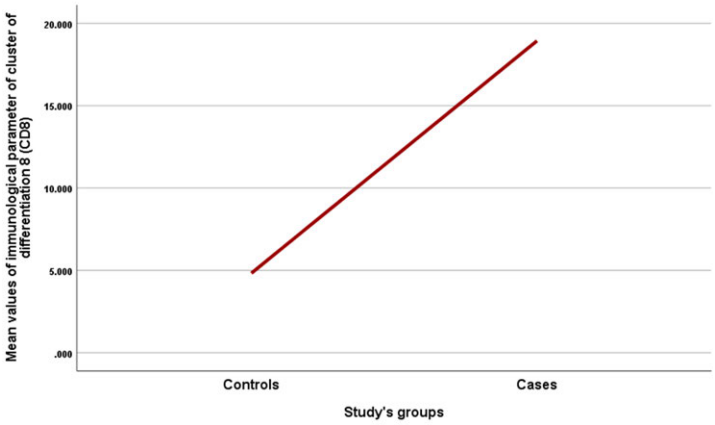

Figure 8: Comparison of Immunological Parameters of Cluster of Differentiation 8 (CD8) among the Study's Groups (n = 140)

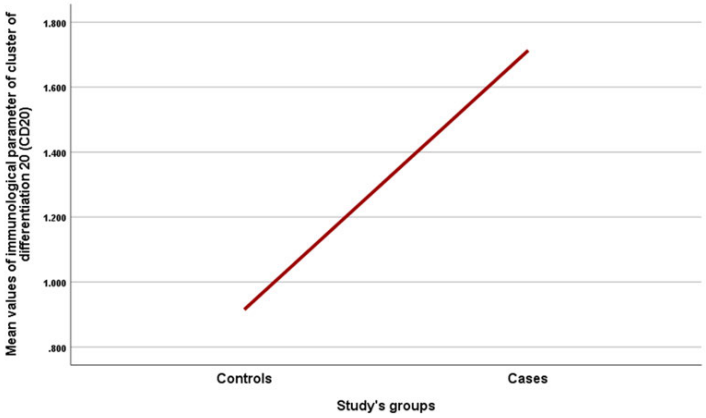

Figure 9: Comparison of Immunological Parameters of Cluster of Differentiation 20 (CD20) among the Study's Groups (n = 140)

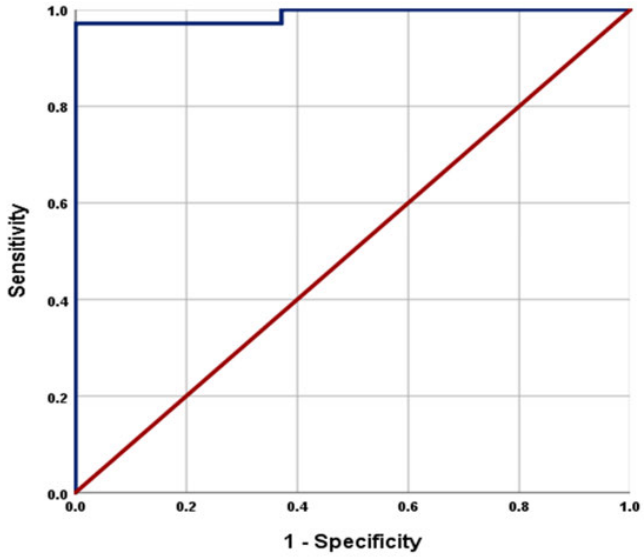

Figure 10: ROC Curve of Breast Cancer Risk Development Predicted by Immunological Parameter of Heat Shock Protein-90 (HSP-90) among Study Samples (n = 140)

Table 5: Predictive Value of Heat Shock Protein-90 (HSP-90) as a Marker for Developing Breast Cancer (n = 140)

Parameter	Validity of the model				
	Sensitvity (Sn)	Specificity (Sp)	Accuracy	Area Under the Curve (AUC)	Significance (p-value)
Heat shock protein-90 (HSP-90)	97.1	100	98.6	0.989	0.000

Heat Shock Protein-90 (HSP-90) as a Predictive Diagnostic Marker for Developing Risk of Breast Cancer

The best cutoff value for Heat Shock Protein-90 (HSP-90) among 140 study samples was 30.63100, with a sensitivity of 97.1% and a specificity of 100%. The regression model accurately predicted this value to be 98.6%, with an excellent area under the ROC Curve (AUC) of 0.989±0.008 (p = 0.000) (Table 5 and Figure 10).

DISCUSSION

The age group most affected by breast cancer in the present study was 46–60 years (46.4%). This finding is consistent with a large epidemiological analysis conducted between 1990 and 2019, which reported the highest incidence and mortality rates among individuals aged 45–59 years across 21 countries in the Middle East and North Africa [9]. This agreement indicates that middle age remains a critical period for breast cancer development in this region.

Regarding sex distribution, all samples in the present study were from female patients, reflecting the global pattern in which breast cancer predominantly affects women. However, one male case was identified during sample collection. This observation is consistent with global statistics showing that male breast cancer accounts for approximately 1% of all cases [1].

$CD8^+$ T cells are recognized as key mediators of antitumor immune response. Peripheral $CD8^+$ T-cell counts have been reported as non-invasive predictors of response to neoadjuvant therapy. A 2022 study demonstrated that peripheral $CD8^+$ T cells, along with $CD3^+$ T cells and NK cells, were independent predictors of pathologic complete response in patients receiving neoadjuvant treatment [10]. In addition, increased levels of $CD8^+$ tumor-infiltrating lymphocytes have been associated with improved overall survival and disease-free survival [11]. Peripheral lymphocyte profiling, including $CD8^+$ cells and their ratios, has also been linked to better survival outcomes and treatment response, particularly in triple-negative breast cancer [12]. These findings are consistent with the results of the present study and support the potential prognostic value of $CD8^+$ expression in breast cancer patients.

CD20, a marker of B lymphocytes, has also been associated with favorable clinical outcomes. Increased CD20 expression in sentinel lymph nodes and tumor tissues has been linked to improved disease-free survival, particularly in HER2-positive breast cancer [13]. Similarly, an Egyptian study reported significantly higher levels of CD20 and CD8 in breast cancer patients compared with controls, suggesting their potential as early predictive and monitoring markers [14]. These findings are in agreement with the results of the present study, indicating that elevated CD20 expression may have prognostic significance.

Heat Shock Protein 90 (HSP90) is frequently overexpressed in breast tumors and is considered a potential therapeutic target. Its role in stabilizing oncogenic proteins contributes to tumor growth, metastasis and treatment resistance [15,16]. A recent study involving patients with HER2-positive breast cancer showed that higher HSP90 expression was associated with significantly improved progression-free survival when triple therapy was used instead of chemotherapy alone [17]. The elevated HSP90 levels observed in the present study are therefore consistent with previous research, supporting its potential role as a prognostic and predictive biomarker in breast cancer.

CONCLUSION

The serum levels of CD8, CD20 and HSP-90 were significantly higher in patients with breast cancer than in healthy women in control groups; they may be predictive and prognostic biomarkers of breast cancer disease.

Recommendation

Although CD8, CD20 and HSP90 appear to have clear prognostic potential based on their biological roles and observed associations, the cross-sectional nature of the current study limits definitive conclusions regarding their prognostic value. Consequently, further prospective studies incorporating long-term patient follow-up are warranted to elucidate and confirm the true prognostic significance of these markers.

Limitations

The current study has some limitations, including a single-center design, which may affect the generalizability of the findings. Furthermore, the study lacked functional validation for the investigated markers and was only able to assess peripheral indicators.

Ethical Statement

The Ethical Approval Committee of College of Medicine/Al-Iraqia University) Conducted in accordance with Tuqa A. Nuaman, Prof: Dr Hayfaa Mh-sen (MSc/PhD) and Dr Rafid Munir Shaker (PhD…), stressing compliance with all ethical regulations as for research on humans.

REFERENCES

[1] World Health Organization. “Breast Cancer.” *World Health Organization*, 2024, https://www.who.int/news-room/fact-sheets/detail/breast-cancer

[2] The United Nations Office at Geneva. “Breast Cancer Cases Projected to Rise by Nearly 40 Per Cent by 2050, WHO Warns.” 2025, https://www.ungeneva.org/en/news

[3] Kuroda, H. *et al.* “Tumor-infiltrating B cells and T cells correlate with postoperative prognosis in triple-negative carcinoma of the breast.” *BMC Cancer*, vol. 21, no. 1, 2021. https://doi.org/10.1186/s12885-021-08009-x

[4] Jääskeläinen, M.M. *et al.* "The prognostic and predictive role of tumor-infiltrating lymphocytes (FoxP3+ and CD8+) and tumor-associated macrophages in early HER2+ breast cancer." *Breast Cancer Research and Treatment*, vol. 201, no. 2, 2023, pp. 1–10. https://doi.org/10.1007/s10549-023-07017-8

[5] Yazaki, S. *et al.* "Integrative prognostic analysis of tumor-infiltrating lymphocytes, CD8, CD20, programmed cell death-ligand 1 and tertiary lymphoid structures in early-stage triple-negative breast cancer without adjuvant chemotherapy." *Breast Cancer Research and Treatment*, vol. 197, no. 2, 2022, pp. 287–297. https://doi.org/10.1007/s10549-022-06787-x

[6] Lin, T. *et al.* "Heat shock protein 90 family isoforms as prognostic biomarkers and their correlations with immune infiltration in breast cancer." *BioMed Research International*, vol. 2020, 2020, pp. 1–15. https://doi.org/10.1155/2020/2148253

[7] Birbo, B. *et al.* "Role of HSP90 in cancer." *International Journal of Molecular Sciences*, vol. 22, no. 19, 2021, p. 10317. https://doi.org/10.3390/ijms221910317

[8] Hong, L. *et al.* "HSP90 promotes tumor-associated macrophage differentiation during triple-negative breast cancer progression." *Scientific Reports*, vol. 14, no. 1, 2024. https://doi.org/10.1038/s41598-024-73394-9

[9] Safiri, S. *et al.* "Burden of female breast cancer in the middle East and North Africa Region, 1990–2019." *Archives of Public Health*, vol. 80, no. 1, 2022. https://doi.org/10.1186/s13690-022-00918-y

[10] Feng, J. *et al.* "Peripheral blood lymphocyte subtypes as new predictors for neoadjuvant therapy efficacy in breast cancer." *Cancer Medicine*, vol. 11, no. 15, 2022, pp. 2923–2933. https://doi.org/10.1002/cam4.4666

[11] Sun, Y.P. *et al.* "Prognostic value of CD8+ tumor-infiltrating T cells in patients with breast cancer: A systematic review and meta-analysis." *Oncology Letters*, vol. 25, no. 1, 2022. https://doi.org/10.3892/ol.2022.13625

[12] Li, M. *et al.* "Predictive and prognostic role of peripheral blood T-cell subsets in triple-negative breast cancer." *Frontiers in Oncology*, vol. 12, 2022. https://doi.org/10.3389/fonc.2022.842705

[13] Luque, M. *et al.* "Tumor-infiltrating lymphocytes and immune response in HER2-positive breast cancer." *Cancers*, vol. 14, no. 24, 2022, p. 6034. https://doi.org/10.3390/cancers14246034

[14] Hamed, M.M. *et al.* "Evaluation of PD-L1, CD8 and CD20 as early predictive and tracking markers for breast cancer in Egypt." *Heliyon*, vol. 8, no. 5, 2022, e09474. https://doi.org/10.1016/j.heliyon.2022.e09474

[15] Rastogi, S. *et al.* "An update on the status of HSP90 inhibitors in cancer clinical trials." *Cell Stress and Chaperones*, vol. 29, no. 4, 2024, pp. 519–539. https://doi.org/10.1016/j.cstres.2024.05.005

[16] Falcone, I. *et al.* "HER2-driven breast cancer: Role of the chaperonin HSP90 in modulating response to trastuzumab-based therapeutic combinations." *International Journal of Molecular Sciences*, vol. 26, no. 14, 2025, p. 6593. https://doi.org/10.3390/ijms26146593

[17] Cheng, Q. *et al.* "Amplification and high-level expression of heat shock protein 90 mark aggressive phenotypes of human epidal growth factor receptor 2-negative breast cancer." *Breast Cancer Research*, vol. 14, no. 2, 2012, R62. https://doi.org/10.1186/bcr3168