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Clinical Significance of Apoptosis Marker Caspase-3, Tumor Marker Carcinoembryonic Antigen, and Electrolyte Imbalance in Patients with Breast Cancer

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ABSTRACT

Background: Reliable circulating biomarkers remain clinically important in the evaluation of breast cancer. This cross-sectional study assessed serum caspase-3, carcinoembryonic antigen (CEA), and certain electrolyte parameters in a control group of healthy individuals and breast cancer patients at different clinical stages.

Methods: Ninety female participants were divided into Control (n = 30), Newly Diagnosed (G1; n = 20), and Treated (G2; n = 40) groups. Serum caspase-3, CEA, and electrolytes (Zn^{2+}, Ca^{2+}, Na^{+}, K^{+}) were measured. Blood samples in the treated group were collected after completion of the fourth chemotherapy cycle during routine follow-up visits. No participants were excluded after laboratory analysis due to missing biomarker data. Data were analyzed using analysis of variance and receiver operating characteristic (ROC) curves. A *P* value less than 0.05 was considered statistically significant.

Results: Caspase-3 levels were lowest in the newly diagnosed group (G1), whereas treated patients (G2) showed higher values than G1. One-way analysis of variance demonstrated a statistically significant difference among the study groups for caspase-3 ($P = 0.004$). CEA concentrations also differed significantly between groups ($P = 0.002$). Significant differences were observed for Zn^{2+} ($P < 0.001$), Ca^{2+} ($P < 0.0001$), Na^{+} ($P < 0.001$), and K^{+} ($P < 0.0001$). ROC analysis indicated moderate diagnostic performance for CEA (area under the curve = 0.75).

Conclusion: Between-group differences were observed in serum caspase-3, CEA, and selected electrolyte levels.

INTRODUCTION

Among women worldwide, breast cancer ranks as the most frequently identified cancer and remains a major driver of both morbidity and mortality due to malignancy. Early detection and biological characterization remain clinically important for patient assessment.[1] Caspase-3 is a key apoptosis-related enzyme[2], whereas carcinoembryonic antigen (CEA) is a tumor-associated marker used in clinical oncology.[3–5] Electrolyte disturbances may accompany malignant disease and related metabolic changes.[6] The present study evaluated serum caspase-3, CEA, and selected electrolytes in healthy controls, newly diagnosed breast cancer patients, and patients undergoing chemotherapy.

METHODS

Study design and setting

A cross-sectional study was conducted from April 2025 to October 2025 at the Oncology Center, Baqubah Teaching Hospital, Diyala, Iraq.

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Participants

A total of 90 women aged 30 to 60 years were enrolled and classified into 3 groups: healthy controls (n = 30), newly diagnosed breast cancer patients (G1; n = 20), and patients undergoing chemotherapy (G2; n = 40; ≥ 4 cycles). Detailed tumor stage, molecular subtype, and receptor status information were not consistently available in hospital records and therefore were not included in the present analysis. Control participants were screened clinically to exclude known malignancy or active inflammatory disease. All eligible participants attending the oncology clinic during the recruitment period were consecutively enrolled.

Exclusion criteria

Participants were excluded if they had recurrent or metastatic breast cancer, concomitant radiotherapy with hormonal therapy, cardiovascular disease, chronic liver disease, diabetes mellitus, rheumatoid arthritis, thyroid disorders, renal or liver failure, pregnancy, or other autoimmune disease.

Blood sampling

Venous blood (4 mL) was collected from all participants using disposable syringes while seated. Samples were allowed to clot at 37 °C for 15 to 30 minutes, then centrifuged at 4000 rpm for 10 minutes ($g = 1207.6$, $r = 12.5$ cm). The separated serum was aliquoted into 5 Eppendorf tubes and stored at −80 °C until further biochemical analysis.

Biochemical analysis

Serum levels of caspase-3 and CEA were measured using commercially available enzyme-linked immunosorbent assay (ELISA) kits. Total caspase-3 and CEA concentrations were measured according to the manufacturer's ELISA protocol.[7] Electrolytes (Zn^{2+}, Ca^{2+}, Na^{+}, K^{+}) were analyzed using standard clinical chemistry procedures.[8]

Statistical analysis

Normality of data distribution was assessed using the Shapiro-Wilk test. Normally distributed variables were analyzed using one-way analysis of variance with Tukey's post hoc test, whereas non-normal variables were analyzed using the Kruskal-Wallis test. Receiver operating characteristic (ROC) curve analysis was performed to evaluate diagnostic discrimination. Statistical significance was set at $P < 0.05$. Sample size was determined by the number of eligible participants available during the recruitment period, and the study was considered as an exploratory cross-sectional analysis.

RESULTS

Baseline characteristics

Age and body mass index did not differ significantly among the study groups (Table 1).

Table 1. Demographic and Anthropometric Characteristics of the Study Groups

Variable	Control (n = 30)	G1 (n = 20)	G2 (n = 40)
Age, mean (SD), y	46.1 (9.7)	51.1 (7.8)	49.2 (8.7)
95% CI	42.5–49.7	47.4–54.7	46.4–51.9
Body mass index, mean (SD), kg/m^2	24.7 (1.6)	24.7 (0.6)	25.4 (3.0)
95% CI	24.1–25.3	24.4–25.0	24.4–26.3

Caspase-3

Serum caspase-3 concentrations differed across the study groups (Table 2, Figure 1A). Mean values were 241.1 (131.9) pg/mL in controls, 136.0 (63.4) pg/mL in newly diagnosed patients, and 213.1 (105.9) pg/mL in treated patients. The lowest values were observed in newly diagnosed patients. Because the study was cross-sectional, the higher values observed in the treated patients should be interpreted as between-group differences rather than treatment-induced changes.[9,10]

Table 2. Serum Caspase-3 Concentrations

Variable	Control (n = 30)	G1 (n = 20)	G2 (n = 40)
Mean (SD), pg/mL	241.1 (131.9)	136.0 (63.4)	213.1 (105.9)
95% CI, pg/mL	190.9–291.3	106.3–165.7	179.2–247.0
Minimum	77.7	43.7	62.7
Maximum	581.3	250.3	457.0

CEA

Serum CEA concentrations differed across the study groups (Table 3, Figure 1B). Mean CEA levels were 14.1 (5.1) pg/mL in controls, 16.9 (10.6) pg/mL in newly diagnosed patients, and 36.8 (37.7) pg/mL in treated patients. The high variability observed particularly in the treated group may reflect heterogeneity in disease status and treatment

exposure across independently sampled groups. Because the study was cross-sectional, causal treatment effects cannot be inferred. The wide range of CEA values particularly in the control and treated groups is consistent with the known variability and limited specificity of this marker for breast cancer.[11]

Serum electrolytes (Zn^{2+}, Ca^{2+}, Na^{+}, K^{+})

Electrolytes are essential for maintaining ionic homeostasis and physiological function. They play a crucial role in regulating enzyme activity, cellular signaling pathways, and membrane potential.[12] Zn^{2+} levels were lower in newly diagnosed patients than in controls and higher in treated patients. Ca^{2+} showed slight reductions in patient groups compared with controls, while Na^{+} and K^{+} were also lower in newly diagnosed patients. Na^{+} values remained within the physiological reference range.[13] Overall, the observed electrolyte differences suggest broader metabolic alterations associated with breast cancer; however, causal mechanisms cannot be inferred from this cross-sectional design.[6,14]

Table 3. Serum CEA Concentrations

Variable	Control (n = 30)	G1 (n = 20)	G2 (n = 40)
Mean (SD), pg/mL	14.1 (5.1)	16.9 (10.6)	36.8 (37.7)
95% CI, pg/mL	12.2–16.0	12.0–21.8	24.8–48.9
Minimum	6.6	1.0	4.7
Maximum	24.9	39.3	188.1

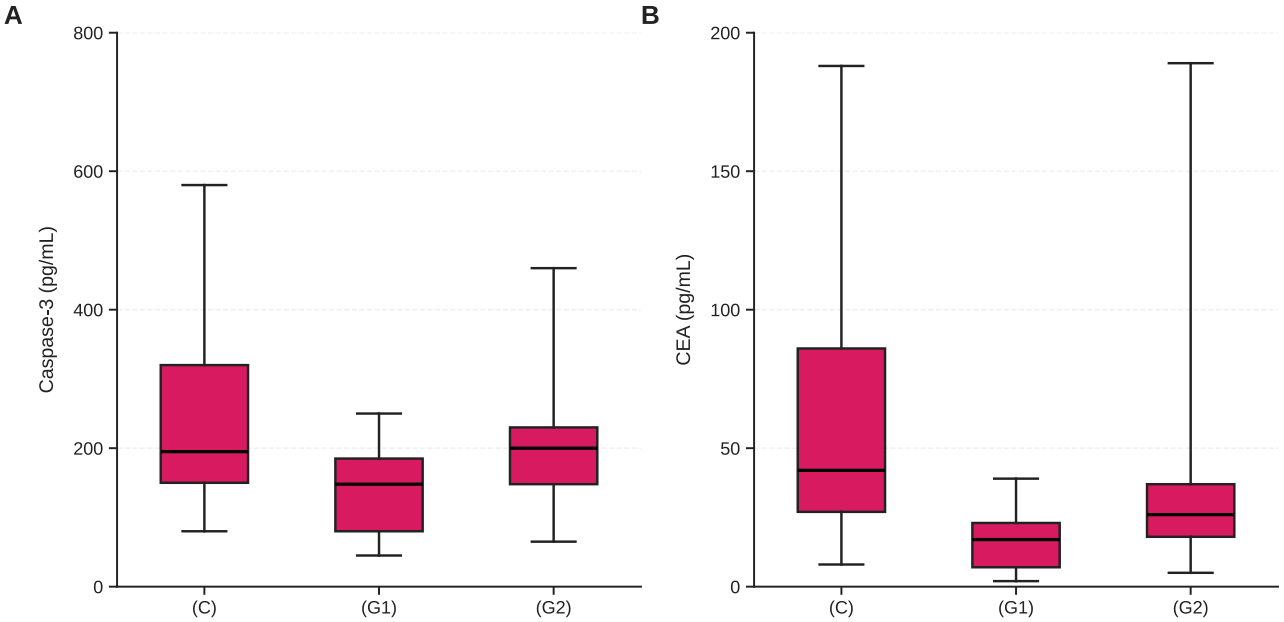

Figure 1. Box-and-whisker plots of serum caspase-3 (A) and carcinoembryonic antigen (CEA) (B).

Table 4. Electrolyte Levels (Zn^{2+}, Ca^{2+}, Na^{+}, K^{+}) in Serum

Parameter	Control (n = 30)	G1 (n = 20)	G2 (n = 40)	*P* value
Zn^{2+}, mean (SD), µg/dL	86.8 (5.7)	80.4 (20.5)	94.7 (7.4)	< 0.001
95% CI	84.7–88.9	70.8–90.0	92.3–97.1	
Ca^{2+}, mean (SD), mmol/L	2.33 (0.30)	2.30 (0.25)	2.29 (0.22)	< 0.0001
95% CI	2.22–2.44	2.19–2.42	2.22–2.36	
Na^{+}, mean (SD), mEq/L	140.1 (2.2)	137.6 (3.0)	138.8 (2.0)	< 0.001
95% CI	139.3–140.9	136.2–139.0	138.2–139.4	
K^{+}, mean (SD), mEq/L	4.28 (0.25)	3.69 (0.29)	3.80 (0.16)	< 0.0001
95% CI	4.19–4.38	3.55–3.82	3.75–3.85	

Reference ranges: Zn (70–120 µg/dL), Ca (2.1–2.6 mmol/L), Na (135–145 mEq/L), K (3.5–5.0 mEq/L).

ROC analysis

ROC curve analysis was performed to evaluate the discriminatory performance of CEA between controls and the combined patient group (G1 + G2) (Table 5, Figure 3). CEA showed an area under the curve (AUC) of 0.75 (95% CI, 0.65–0.86; $P < 0.001$). The optimal cutoff value, determined by the Youden index, was 24.65 pg/mL, with a sensitivity of 58.3% and a specificity of 82.8%. These findings indicate moderate discriminatory performance for CEA in this cohort.[15,16]

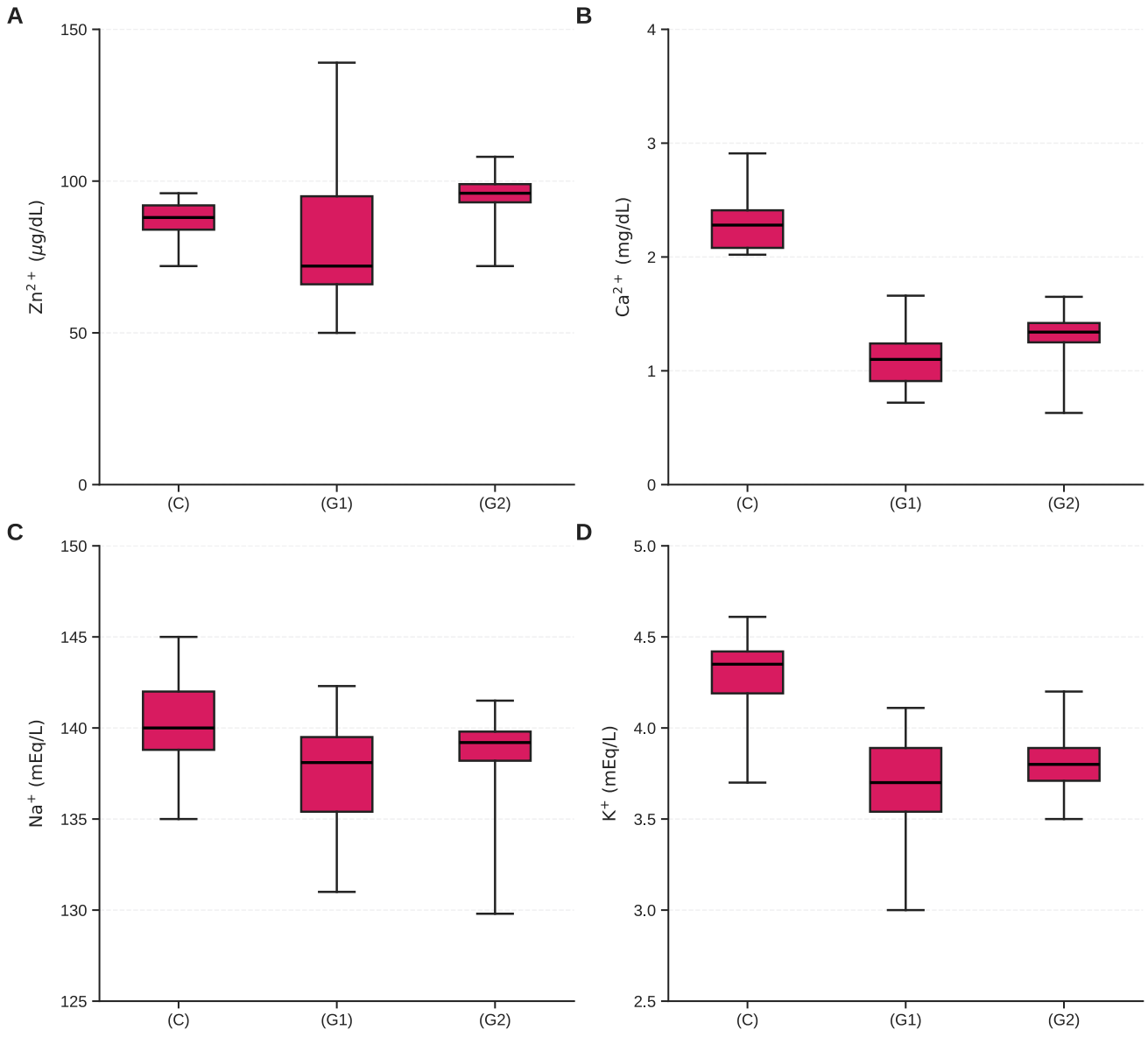

Figure 2. Box-and-whisker plots of serum Zn^{2+}, Ca^{2+}, Na^{+}, and K^{+} across the study groups.

Serum caspase-3 was also evaluated by ROC analysis (Table 5, Figure 3). Caspase-3 showed a limited discriminatory performance, with an AUC of 0.59 (95% CI, 0.47–0.72; $P = 0.15$). The optimal cutoff value was approximately 167.3 pg/mL, with a sensitivity of 40.0% and a specificity of 58.6%. These findings indicate poor diagnostic accuracy when caspase-3 is used alone.[17]

DISCUSSION

This study evaluated serum caspase-3, CEA, and selected electrolytes in healthy controls, newly diagnosed breast cancer patients, and treated patients within a cross-sectional design.[9] Caspase-3 concentrations were lowest in newly diagnosed patients and remained lower in treated patients than in controls, suggesting between-group variation in apoptotic signaling rather than a treatment effect.[10,18,19] ROC analysis further indicated that caspase-3 had limited discriminatory performance when used alone.[11,20]

Table 5. ROC analysis of CEA and Caspase-3 for discrimination between controls and patients (G1+G2)

Biomarker	Area under the curve	95% CI	*P* value	Cutoff	Sensitivity, %	Specificity, %
CEA	0.75	0.65–0.86	< 0.001	≈ 24.65	58.3	82.8
Caspase-3	0.59	0.47–0.72	0.15	≈ 167.30	40.0	58.6

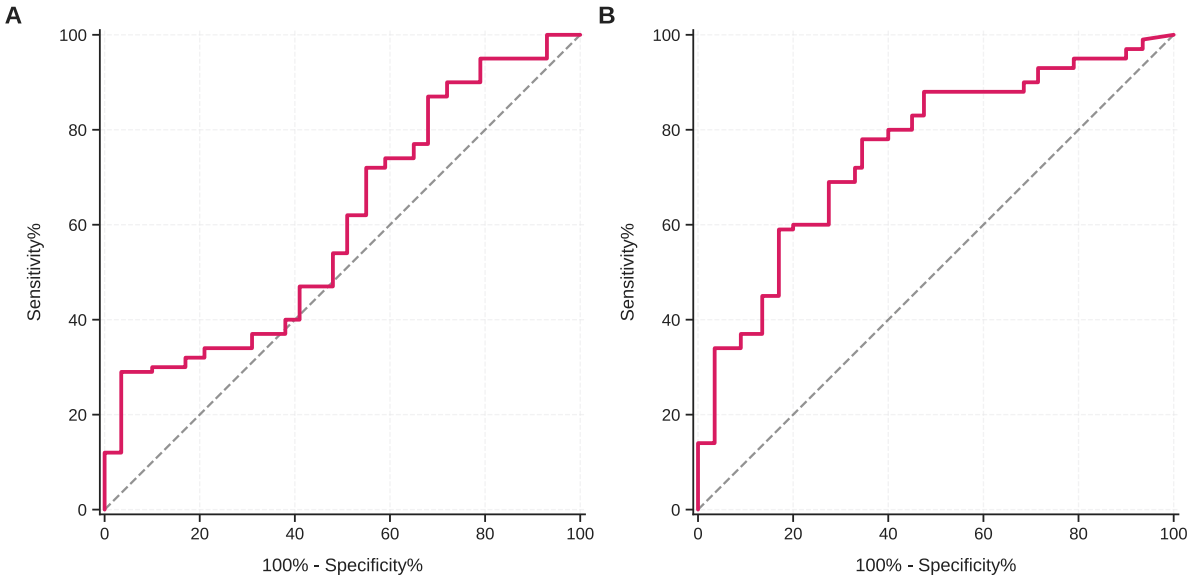

Figure 3. Receiver operating characteristic (ROC) curves for CEA and caspase-3 for discrimination between controls and patients (G1 + G2).

CEA showed higher mean values and greater variability in treated patients than in the other groups. ROC analysis demonstrated moderate discriminatory performance for CEA in the present cohort. These findings should only be interpreted as cross-sectional associations.[21] Electrolyte analysis showed lower potassium and zinc levels in newly diagnosed patients, while sodium remained within the physiological range and calcium showed only slight reductions. These findings may reflect broader metabolic alterations associated with breast cancer.[13,22] However, causal mechanisms cannot be inferred from this design. Because detailed tumor stage, receptor status, and chemotherapy regimen information were not uniformly available, the present findings should be considered exploratory.[23,24]

Limitations

Detailed tumor stage, grade, molecular subtype, receptor status, and chemotherapy regimen information were not uniformly available; therefore, subgroup analyses and treatment-specific conclusions could not be performed. Residual confounding cannot be excluded.

CONCLUSION

In conclusion, this study identified cross-sectional differences in serum caspase-3, CEA, and selected electrolyte levels among healthy controls, newly diagnosed breast cancer patients, and treated patients. Caspase-3 showed limited diagnostic utility, whereas CEA demonstrated moderate discriminatory performance in this cohort. The observed electrolyte differences support the presence of broader biochemical alterations associated with breast cancer. These findings should be interpreted as exploratory cross-sectional observations.

ETHICAL CONSIDERATIONS

Ethical approval was obtained after consultation with the hospital research committee. Written informed consent was obtained from each participant.

DATA AVAILABILITY

Available from the corresponding author.

CONFLICT OF INTERESTS

None declared.

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AI DISCLOSURE

The authors used AI-assisted language support during manuscript preparation. All scientific content, data interpretation, and final revision were performed and approved by the authors, who take full responsibility for the manuscript.

AUTHOR CONTRIBUTION

Ahmed A. Al Shammari: Conceptualization, methodology, investigation, data curation, formal analysis, writing – original draft, writing – review and editing. Wasan Nazhan Hussein: Supervision, methodology, validation, writing – review and editing.

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