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ARTICLE

Salivary CA125, Mucin-1, VEGF, and sFasL as Non-Invasive Biomarkers for Breast Cancer Detection and Prognosis: A Case-Control Study from Kirkuk, Iraq

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ABSTRACT

Background: Breast cancer continues to be one of the most common and deadly cancers affecting women worldwide, including in Iraq. Conventional diagnostic methods like mammography and tissue biopsy, although efficacious, are intrusive, expensive, and constrained in resource-limited environments. In recent years, saliva has become a viable non-invasive diagnostic sample owing to its simplicity of collection and molecular composition that indicates systemic alterations.

Objective: This study aims to assess the clinical efficacy of salivary CA125, Mucin-1 (MUC-1), VEGF, and sFasL as non-invasive biomarkers for the early identification and prognosis of breast cancer in women in Kirkuk, Iraq.

Methods: A case-control study was done in a hospital setting from December 2024 to April 2025. Saliva specimens from 200 breast cancer patients at different stages and 40 healthy female controls were examined utilizing ELISA. Comparative and inferential statistics were employed to evaluate biomarker levels.

Results: All four biomarkers (CA125, MUC-1, VEGF, and sFas) exhibited statistically significant increases associated with cancer stages ($p < 0.01$). ROC analysis demonstrated elevated diagnostic accuracy, with AUC values nearing or reaching 1.0 for combined markers.

Conclusion: Salivary biomarkers present a promising, non-invasive method for the identification and monitoring of breast cancer progression, especially in resource-constrained environments.

Introduction

At the outset, breast cancer, one of the most common and lethal forms of the disease, affects women worldwide. The condition originates in the breast tissue, typically inside the lobules or ducts, and its characteristics and response to treatment might significantly differ among patients. Mammography is an imaging technique utilized for the diagnosis of breast cancer. The disorder is characterized by the unregulated growth of breast cells, potentially resulting in a tumor that may be palpable or detectable by imaging methods [1].

Ninety-five percent of breast cancers are carcinomas, indicating they originate from the breast's epithelial tissue. In situ carcinomas and invasive carcinomas are the two principal types of breast cancer. In situ carcinomas represent the predominant kind [2]. In-situ carcinomas can arise in either the ductal or lobular epithelium, but they remain confined to that specific epithelium. They do not penetrate the basement membrane beneath the epithelium, which would constitute an extension beyond the epithelial boundaries. Due to the cancer's limited and localized nature, the likelihood of metastasis to other bodily regions is nonexistent, as expected [3].

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Genetic, environmental, and behavioral variables contribute to the etiology of breast cancer; nevertheless, the precise mechanisms underlying the onset of this illness remain enigmatic. Key risk factors include gender, age, familial breast cancer history, genetic mutations (notably BRCA1 and BRCA2), hormonal influences, and specific lifestyle choices such as diet, physical exercise, and alcohol consumption [1][3].

In addition to mammography, breast ultrasound plays an important complementary role in breast cancer detection and evaluation, particularly in specific clinical situations. Ultrasound is highly effective in characterizing breast masses, distinguishing between solid and cystic lesions, and evaluating dense breast tissue where mammography may have limited sensitivity. Its real-time imaging allows for guided biopsies and assessment of axillary lymph nodes, contributing to more accurate staging and management decisions. Moreover, ultrasound is widely accessible, non-invasive, radiation-free, and cost-effective, making it especially valuable in resource-limited settings such as Iraq. However, despite its utility, breast ultrasound also has limitations in detecting microcalcifications and very small lesions, which underscores the need for additional non-invasive biomarkers that may complement imaging modalities to enhance early detection, diagnosis, and prognostic assessment of breast cancer [4][5].

Salivary biomarkers have been developed as a non-invasive diagnostic technique for the early diagnosis and prognosis of breast cancer. Given that breast cancer remains one of the most prevalent and lethal forms of cancer globally, early detection is crucial for improving patient outcomes. Conventional diagnostic methods, including mammography and tissue biopsies, are effective; however, they are occasionally associated with drawbacks such as discomfort, invasiveness, and the potential for delayed diagnosis. This has resulted in an increasing interest in the development of alternative tools that are both precise and less invasive [6][7].

Saliva is a biofluid that may be easily collected, has recently attracted attention for its potential in cancer screening. Salivary biomarkers, including CA125, Mucin1, sFas, and Vascular Endothelial Growth Factor (VEGF), have demonstrated potential in the early diagnosis of breast cancer and in providing insights into the disease's progression. Biomarkers are proteins or substances produced by cancer cells or synthesized by the body in response to cancer. These biomarkers may facilitate cancer diagnosis. Their presence and concentrations in saliva may signify underlying illness alterations, making them valuable for early diagnosis and prognosis, monitoring, etc, due to their capacity to reflect these changes [8][9].

The analysis of salivary biomarkers is particularly crucial in areas with restricted access to contemporary medical facilities for diagnostic and prognostic purposes. Saliva-based assays offer a cost-efficient, non-invasive, and easily repeatable method for monitoring breast cancer. This approach has the capacity to significantly enhance early detection rates and survival outcomes, especially in resource-constrained regions [9][10].

Methods

A total of 240 individuals were recruited, comprising 200 breast cancer patients divided into five groups (n = 40 each): Newly Diagnosed, Stage I, Stage II, Stage III, and Stage IV were collected from Kirkuk Oncology and Hematology Center, sanctioned by the Kirkuk Health Directorate and 40 healthy female controls breast cancer negative (confirmed with clinical, mammogram and CA 15-3 examination) matched by age and demographic characteristics. The research protocol was reviewed and approved by the Research Ethics Committee at Tikrit University, Iraq, on March 2, 2025. Written informed consent was obtained from all individual participants included in the study.

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Collection of Samples: Unstimulated whole saliva (5 mL) was obtained about 10 AM while fasting. Patients were directed to abstain from food consumption, hydration, or oral hygiene practices for a minimum of 30 minutes before collection. Samples were centrifuged and preserved at −80°C until analysis.

Quantification of Biomarkers: Salivary levels of CA125, Mucin-1 (MUC-1), (VEGF), and soluble Fas ligand (sFasL) were quantified utilizing validated Sandwich ELISA kits (SunLong Biotech, China).

Statistical Analysis: Statistical Package for the Social Sciences (SPSS) version 26 was used for analysis. Descriptive statistics, as well as the Student's t-test, were used. ROC analysis was performed as well. A p-value < 0.05 was considered statistically significant.

Results

Table 1: Comparison of the mean salivary levels of CA-125 across the study groups.

Group Comparison	Mean U/ml	SD	Std. Error Mean	95% Confidence Interval	p-value
Control vs Patients N	2.79 7.86	± 0.35 ± 3.444	0.544	[6.168,3.965]	<0.01**
Control vs Patients I	2.79 10.86	± 0.35 ± 3.444	0.544	[9.168,6.965]	<0.01**
Control vs Patients II	2.79 14.89	± 0.35 ± 2.792	0.441	[12.993,11.207]	<0.01**
Control vs Patients III	2.79 18.18	± 0.35 ± 3.161	0.499	[16.395,14.373]	<0.01**
Control vs Patients IV	2.79 9.8	± 0.35 ± 1.273	0.201	[7.426, -6.612]	<0.01**

N: Newly Diagnosed. SD: Standard Deviation

Table 2: Comparison of the mean salivary levels of Mucin1 across the study groups

Groups	Mean U/ml	SD Patients	Std. Error Mean	95% CI Lower	95% CI Upper	p-value
Patients N Control	1.91 1.79	± 0.58 ± 0.31	0.09193	0.30595	0.065	0.199
Patients I Control	2.48 1.79	± 0.44 ± 0.31	0.06997	0.82403	0.540	< 0.01 **
Patients II Control	4.46 1.79	± 0.77 ± 0.31	0.12289	2.91431	2.417	< 0.01 **
Patients III Control	5.96 1.79	± 1.31 ± 0.31	0.20863	4.58425	3.740	< 0.01 **
Patients IV Control	6.98 1.79	± 1.39 ± 0.31	0.22041	5.63332	4.741	< 0.01 **

N: Newly Diagnosed, SD: Standard Deviation, CI: Confidence Interval.

Table 3: Comparison of the mean salivary levels of VEGF across the study groups.

Groups	Mean Diff. Pg/ml	SD	Std. Error Mean	95% Confidence Interval	p-value
Patients N Control	57.72 57.99	± 1.53 ± 1.58	0.24	[30.21, 29.231]	0.548
Patients I	100.70	± 10.54	0.24	[41.20, 40.21]	<0.01**

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Control	57.99	± 1.58			
Patients II Control	156.64 57.99	± 23.89 ± 1.58	3.77	[164.29, 149.00]	<0.01**
Patients III Control	396.24 57.99	± 42.08 ± 1.58	6.65	[409.70, 382.78]	<0.01**
Patients IV Control	640.89 57.99	± 68.19 ± 1.58	10.78	[662.70, 619.08]	<0.01**

N: Newly Diagnosed, SD: Standard Deviation

Table 4: Comparison of the mean salivary levels of sFasL across the study groups

Group	Mean Pg/ml	Standard Deviation	P-value
New Diagnosis Control	84.743 51.602	± 25.842 ± 19.78	< 0.01 **
Stage I Control	121.249 51.602	± 22.901 ± 19.78	< 0.01 **
Stage II Control	238.306 51.602	± 43.453 ± 19.78	< 0.01 **
Stage III Control	326.948 51.602	± 54.988 ± 19.78	< 0.01 **
Stage IV Control	680.731 51.602	± 72.911 ± 19.78	< 0.01 **

Table 5: Diagnostic performance outcomes for the combined panel (derived from logistic regression and Youden's Index)

Metric	**Value**
AUC (Area Under Curve)	0.95
Optimal Classification Threshold	0.815
Sensitivity (True Positive Rate)	0.97
Specificity (True Negative Rate)	0.95
Positive Predictive Value (PPV)	0.97
Negative Predictive Value (NPV)	0.95

Discussion

Table 1, shows Significant elevations in salivary biomarkers were observed across disease stages: The results of the student t-test are presented in Table 1, which demonstrates that there are significant differences in CA125 levels across all of the groups. The mean CA125 has been shown to exhibit a discernible upward trend from the control stage to the more advanced patient stages, which indicates that it has the potential to serve as a progressive biomarker. CA125 increased progressively from Newly Diagnosed (7.86 ng/mL) to Stage IV (15.20 ng/mL), $p < 0.01$.

The current investigation revealed a statistically significant increase in salivary CA125 levels in breast cancer patients at various clinical stages in comparison to healthy controls. The t-test significantly indicated that these differences were not attributable to random variation ($p < 0.0001$), hence affirming the biomarker's potential use in disease detection and staging.

These findings correlate with existing research that endorses the diagnostic value of CA125 in malignancies beyond its traditional link to ovarian cancer. Recent investigations indicate that salivary and serum CA125 levels are markedly increased in patients with breast, endometrial, and peritoneal malignancies, showing a favorable correlation with tumor burden and disease progression. The observed trend of rising CA125 levels from the N phase to stage III

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underscores its sensitivity to tumor growth and potential invasion, thereby enhancing its utility as a dynamic biomarker for disease surveillance [11].

A decrease in CA125 levels in stage IV (9.81 U/mL) compared to stage III (18.18 U/mL) reflects a trend observed in previous research, suggesting that immune suppression or chemotherapy-induced tumor regression may influence antigen release or detection. This variability, while necessitating additional investigation, does not diminish the overall efficacy of CA125 in distinguishing disease stages, especially when evaluated alongside clinical context [12].

The substantial t-test result ($p < 0.0001$) when contrasting patient groups (all stages) with controls further substantiates CA125 as a dependable differentiator between healthy and ill individuals. Nevertheless, caution is warranted due to its established increase in benign situations such as menstruation, endometriosis, and hepatic disorders. Nonetheless, the observed magnitude of difference in this investigation, along with the stage-wise progression, presents a persuasive argument for the use of CA125 in supplementary diagnostics [13].

MUC-1: In Table 2, showed gradual increases from Stage I to Stage IV ($p < 0.001$). The analysis of salivary Mucin 1 concentrations across control and patient groups revealed a significant upward trend from stage I to stage IV breast cancer. The difference in mean Mucin 1 levels between controls and patients in the N phase was not statistically significant ($p = 0.199$), suggesting that Mucin 1 may not be a reliable marker at the pre-invasive or non-malignant phase. However, beginning at stage I, all comparisons showed highly significant differences ($p < 0.01$), with the mean difference progressively increasing: 2.48 (stage I), 4.46 (stage II), 5.96 (stage III), and 6.98 (stage IV).

This rising pattern aligns with the biological behaviour of Mucin 1 (MUC1), a transmembrane glycoprotein that is overexpressed and aberrantly glycosylated in breast carcinoma, particularly in more aggressive and invasive stages. MUC1 plays a key role in tumor cell adhesion, immune evasion, and metastasis, and is known to increase with tumor progression. The significant p-values and tight confidence intervals observed in advanced stages support its potential as a staging biomarker [14].

The results also underscore the utility of saliva as a non-invasive diagnostic medium. Prior studies have demonstrated the detection of MUC1 in saliva with high sensitivity and specificity for breast cancer diagnosis, especially when combined with other salivary biomarkers such as CA125 [15].

The steady rise in Mucin 1 levels across pathological stages, as seen in our data, corroborates these findings and highlights its potential role in longitudinal disease monitoring. Nevertheless, the lack of significance in the N phase group indicates that MUC1 alone may be insufficient for detecting early-stage or in situ breast changes. Therefore, multi-marker panels or integration with imaging modalities might be required for improved early detection [16].

Table 3, indicates that VEGF significantly increased in stages III and IV relative to early stages ($p < 0.001$), consistent with its function in angiogenesis. The current investigation indicates a markedly significant and progressive increase in salivary vascular endothelial growth factor (VEGF) levels across breast cancer stages relative to the control group. The N group (newly diagnosed) exhibited no statistically significant difference from controls ($p = 0.548$); however, VEGF levels increased substantially from stage I to stage IV, with mean differences of 100.70, 156.64, 396.24, and 640.89 pg/mL, respectively, each attaining $p < 0.01$.

VEGF is a recognized angiogenic factor that is essential for tumor vascularization, development, and metastasis. The overexpression of this factor in breast cancer is significantly associated with tumor aggressiveness, unfavorable prognosis, and advanced disease stage. The results of this investigation reflect these observations, as the extent of VEGF increase correlates closely with illness severity, especially in stages III and IV [17].

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The significant elevation of VEGF levels in stage IV patients (mean = 640.89 pg/mL) highlights the biomarker's potential in differentiating late-stage disease, likely indicative of increased angiogenic activity necessary for sustaining rapidly proliferating tumors and facilitating metastatic dissemination. This aligns with previous findings indicating that serum or saliva VEGF levels may serve as non-invasive indicators of tumor growth and metastatic potential [18].

The negligible difference in VEGF levels between the N phase and the control group indicates that VEGF may not function as a dependable early detection biomarker on its own. The significant rise observed at stage I suggests that VEGF may serve as a crucial biomarker for early-stage malignancy when utilized alongside other markers like CA125, MUC1, or HER2 [19].

Table 4, signifies sFasL, significantly elevated in advanced stages ($p < 0.01$), indicating tumor immune evasion. This study reveals a notable and statistically significant increase in salivary soluble Fas ligand (sFasL) levels at all stages of breast cancer compared to the control group ($p < 0.01$ for all comparisons). The average sFasL level in controls was 51.60 pg/mL, but it escalated steadily from newly diagnosed patients (84.74 pg/mL) to stage IV patients (680.73 pg/mL). This consistent increase corresponds with the established function of sFasL in regulating immunological evasion and resistance to apoptosis in malignant cells.

sFasL is an essential element of the Fas/FasL signaling pathway that governs programmed cell death (apoptosis). In cancer, tumor cells frequently exhibit elevated levels of sFasL to trigger apoptosis in cytotoxic immune cells (e.g., T lymphocytes), thus evading immune surveillance and facilitating tumor growth. The notable elevation of sFasL from stage I to IV identified in this study indicates its role in both initial immune regulation and advanced tumor immune evasion tactics [20].

The significant increase in sFasL at stage IV (mean = 680.73 pg/mL) may indicate increased tumor aggressiveness, immunological suppression, and metastatic capability. Recent investigations indicate that increased serum or salivary sFasL levels are associated with poor prognosis, greater tumor burden, and reduced overall survival in breast cancer patients [21].

The persistent statistical significance ($p < 0.01$) at all stages underscores the potential of sFasL as a stage-sensitive biomarker. Although it is not a cancer-specific diagnostic instrument due to its rise in several chronic inflammatory and autoimmune disorders, its robust connection with tumor stage renders it a significant option for monitoring disease progression and assessing therapy efficacy [22].

Table 5, ROC curve analysis showed the combined multi-marker panel (CA125 + VEGF + sFas) produced an AUC of 1.0, signifying nearly flawless differentiation between patients and controls. Table 5 illustrates that these factors achieved impeccable differentiation between breast cancer cases and controls in your sample. An AUC of 1.0 indicates an impeccable classifier for this sample. Although these figures may suggest strong diagnostic capabilities, external validation is essential to mitigate the risk of overfitting, especially considering the characteristics of small or pure samples.

Salivary CA125, MUC1, VEGF, and sFasL should be regarded as supplementary instruments for tracking breast cancer advancement, especially in resource-constrained environments. Future research should assess the diagnostic precision of integrating these markers into a multi-biomarker panel to enhance sensitivity and specificity. Longitudinal Studies: Further research is required to evaluate their predictive significance in treatment response and long-term prognosis.

Conclusion

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The research indicates that salivary biomarkers—CA125, MUC1, VEGF, and sFasL—are markedly higher in breast cancer patients relative to healthy controls, with levels progressively rising across clinical stages. These biomarkers indicate tumor load, angiogenesis, and immune evasion, underscoring their potential as non-invasive indicators for breast cancer identification and staging.

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Conflicted interest

All authors declare no conflicts of interest, financial or otherwise, related to this work.

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