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Correlation of Some Tumor Markers Between Serum and Saliva of Patients with Breast Cancer

[1]Huthaifa Tarawneh and [1,2]Omar Atrooz

[1]Department of Medical Laboratory Sciences, Faculty of Applied Medical Sciences, Al-Ahliyya Amman University, Amman, Jordan
[2]Department of Biological Sciences, Faculty of Science, Mutah University, Mutah, Jordan

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Corresponding Author:
Omar Atrooz
Department of Medical Laboratory Sciences, Faculty of Applied Medical Sciences, Al-Ahliyya Amman University, Amman, Jordan
E-mail: omihandd@gmail.com

Abstract: Breast cancer, which affects 36% of all female oncological patients, is the most frequent cancer in the world among women. Since routine laboratory evaluation of serum can be stressful for patients, it is critical to comprehend the features and limitations of salivary test methods in order to achieve an accurate diagnosis of breast cancer. The purpose of the study is to evaluate and correlate tumor markers that are present in the serum and saliva of both healthy controls and breast cancer patients. This cross-sectional study recruited Jordanian females including 40 breast cancer patients and 20 control individuals in Al Basheer Hospital, Amman, Jordan. Data was collected using a questionnaire and laboratory examinations of serum and unstimulated saliva samples. Statistical analyses were performed by PRISM software. To analyze tumor biomarkers (Carcinoembryonic antigen, cancer antigen 15-3, cancer antigen 125, and Alpha-fetoprotein). Pearson's correlation coefficient and One-way ANOVA. Moreover, Python static analysis tools were used to evaluate the diagnostic accuracy of biomarkers, using the Receiver Operating Characteristic curve. All tumor biomarkers showed a significant elevation in breast cancer compared to controls in serum and saliva. CEA appeared robust positive correlations between two biofluid levels in breast cancer patients. CA15-3 represented the optimal Area Under the Curve values. Biomarkers could be useful bioindicators for breast cancer. However, the correlation between two biofluids of CEA levels suggested a reliable diagnosis for BC patients. CA15-3 demonstrated perfect diagnostic performance with an AUC value.

Keywords: Carcinoembryonic Antigen, Cancer Antigens Alpha-Fetoprotein, Saliva

Introduction

Breast cancer, which affects 36% of all female oncological patients, is the most frequent cancer in the world among women. The World Health Organization (WHO) reported that more than two million women globally received a BC diagnosis in 2020 (Nardin *et al*., 2020). The occurrence of this malignant tumor is rising worldwide, but industrialized countries have the highest incidence rates and developed countries account for nearly half of all cases (Bellanger *et al*., 2018). This elevated incidence is attributed to a Western lifestyle characterized by an unhealthy diet, regular moderate/high alcohol consumption, nicotinism, stress, lack of regular physical activity, and night work (Bellanger *et al*., 2018; Smolarz *et al*., 2022). Furthermore, it is occurrence is associated with genetic and hereditary predisposition risk factors (Hong and Xu, 2022). Thus, it's critical to understand that BC ranks second globally in terms of cancer-related mortality among women (Sung *et al*., 2021). After lung and colorectal cancers, breast cancer ranks third in Jordan in terms of cancer-related deaths (Abdel-Razeq *et al*., 2020).

The clinical diagnostic methods of BC mainly involve imaging (Youssef *et al*., 2024), ultrasound, and detection of tumor markers in serum (Luo *et al*., 2023). A tumor marker is a biomarker that indicates the existence and growth of the tumor (Hong *et al*., 2022) and can be detectable in the liquid biopsy techniques, allowing the detection of tumor biomarkers in serum, saliva, and urine, which is liquid biopsy becoming of great attention in the early detection of cancer (Tarek *et al*., 2022). Since BC can be a life-threatening disease, tumor marker detection can aid in early diagnosis and treatment (Hong *et al*., 2022). Critically, an early diagnosis of BC is important for a positive prognosis (Zalloum *et al*., 2022). Whereas patients who are diagnosed with small tumor sizes have a significantly higher chance of survival and reduced chance of the cancer being fatal (Bahjat Heilat *et al*., 2019). The treatments for BC are more effective in the early stages of diagnosis than in the later stages of the disease's initial

tumor burden (Giuliano *et al*., 2017). BC diagnosis is based on radiology, clinical assessment, and confirmation from a biopsy (Tagliafico *et al*., 2020). Conventional screening is considered less-than-desirable sensitivity and specificity and breast biopsy is the reference standard, but invasive and fraught with morbidity risk (Cortadellas *et al.*, 2017).

There are parameters that provide a non-invasive and easily accessible diagnosis that can help identify the disease at an earlier stage and improve treatment outcomes, such as salivary parameters which are considered a potential source of biomarkers, a substitute for serum and other biological fluids (Porto-Mascarenhas *et al.*, 2017). Saliva is a complex body fluid containing metabolites, Deoxyribonucleic Acid (DNA), Messenger Ribonucleic Acid (mRNA), microRNAs, proteins, and microbiota. Saliva-based molecular diagnostics provide physiological body conditions (Rapado-González *et al*., 2020). Studies have identified various salivary biomarkers for cancers such as BC (López-Jornet *et al*., 2021). Therefore, saliva is preferable for clinical diagnostics than traditional blood-based biochemical analyses due to several advantages; non-invasiveness, stress-free collection methods, easy sample collection methods, numerous sampling chances, decreased need for sample pre-processing and restricted risk of contracting infectious organisms (Punyadeera and Slowey, 2019). Providing a non-invasive and easily accessible method for diagnosis can help identify the disease at an earlier stage and improve treatment outcomes. With affordability and convenience, salivary biomarkers could be the key to a future where BC is detected and treated more effectively (Porto-Mascarenhas *et al*., 2017).

Serum tumor indicators play a critical role in the management of patients with various cancer types. To guarantee efficient patient care, it's critical to keep an eye on these indicators (Holdenrieder *et al*., 2016). Serum levels of tumor markers have been used in recent decades to identify tumor activity. The potential uses of serum markers in BC offer a less intrusive, more affordable source of information that is helpful for tracking the course of the disease, estimating prognosis, and helping with treatment planning. Accurate result interpretation requires knowledge of the features and constraints of each test (Kabel, 2017). The American Society of Clinical Oncology (ASCO) has revised its recommendations for using breast tumor markers in the prevention, screening, therapy, and surveillance of BC (Donepudi *et al*., 2014).

Carcinoembryonic Antigen (CEA) CEA is a surface glycoprotein elevated in various cancers, in BC it's overexpressed (Raikwar *et al.*, 2016). In BC cases, elevated CEA is associated with metastatic BC, and preoperative measurements of CEA correlated with the pathological stage of BC and tumor extent, and after treatment continuously increasing CEA indicated either cancer recurrence or no response to treatment (Kabel, 2017). Cancer Antigen 15-3 (CA15-3) is a carbohydrate-containing protein antigen (David *et al.*, 2016), predominantly used in patients with stage IV BC (Li *et al*., 2022). Cancer Antigen 125 (CA125) is a protein, encoded by the MUC-16 gene and has been detected in elevated amounts in the serum of women with BC (Gaughran *et al*., 2020).

Numerous investigations have revealed a correlation between greater BC stage, tumor size positive axillary lymph nodes, and raised serum CA15-3 levels at diagnosis (Shao *et al*., 2015). CEA and CA15-3 widely used as BC-specific biomarkers have prognostic significance in early BC and potentially predict survival for metastatic BC (Wu *et al.*, 2014). Each one as a single marker is not useful for early BC diagnosis due to its limited specificity and sensitivity (Fu *et al*., 2017). In a study conducted by Hasan (2022), evaluated the serum levels of CA15-3 and CEA in breast cancer patients who received adjuvant chemotherapy at both early and late stages. The results showed that BC patients had higher levels of CEA in their serum than healthy controls and that late-stage patients had higher positive serum levels of both markers than early-stage patients, with a preference for CA15-3 over CEA. According to (Fang *et al*., 2017), BC patients' serum levels of CEA, CA125, and CA15-3 were noticeably greater than those of the control group.

Besides serum biomarkers, several studies detect salivary biomarkers. For example, (Bel'skaya *et al*., 2023) found that saliva in British Columbia showed varying concentrations of CA15-3 and CEA, depending on the stage of the disease. At advanced stages of the disease, the concentration increases of both markers were statistically significant, whereas in situ the increases were negligible. Furthermore, (Tarek *et al*., 2022) research demonstrated a significant difference in the expression of CA15-3 between patients with BC and healthy individuals, with salivary CA15-3 expressing more than serum does. This finding suggests that saliva is a more accurate diagnostic tool than serum for the early detection of BC. Furthermore, (Dwivedi *et al.*, 2023) enrolled women with different stages of BC, indicated a statistically significant role of CA125 in BC patients and also found a positive correlation between serum and salivary levels of CA125. (López-Jornet *et al*., 2021) Observed higher salivary levels of the CA125 biomarker in patients with BC, where the CA125 was 2.6-fold higher in the saliva of the patients than in the healthy controls and concluded that the salivary biomarker CA125 revealed to be a promising tool in the diagnosis of BC.

AFP is a serum glycoprotein, that regulates tumor growth by promoting and inhibiting growth (Mizejewski, 2007). Numerous research has indicated high blood levels of AFP are considered a risk factor for BC (Zhao *et al*., 2015; Kassab *et al*., 2013) supposed that the high serum AFP level is responsible for BC development with a useful

prognosis for this disease. He *et al*. (2019) showed BC patients had the highest values in the mean serum AFP levels compared to healthy controls. Moreover, (Luo *et al.*, 2023) revealed that in BC patients the detection of serum CEA, CA15-3, CA125, and AFP was significantly higher than in patients with benign BC. The detection of AFP in saliva has been reported in studies. For instance, (López-Jornet *et al*., 2021) showed no increase in the salivary concentration of AFP in BC patients compared to the control group.

Early detection of BC lowers morbidity, increases survival rates, and lessens the likelihood of illness recurrence. Early diagnosis and treatment are more successful; nonetheless, they call for an intrusive and dangerous confirmatory biopsy as well as radiological screening. Saliva is a non-invasive, readily available biomarker that, in addition to its high specificity and sensitivity, offers promise as a viable substitute for blood and other biological fluids in the early detection of BC. Taking into account that earlier research has shown saliva, a biological fluid, to be a reliable predictor of the results of BC diagnosis and follow-up development. To the best of our knowledge, no research has been conducted in Jordan using saliva samples to measure a patient's biochemical condition.

The study's objective is to analyze the tumor biomarkers alpha-fetoprotein, cancer antigen 15-3, cancer antigen 125, and carcinoembryonic antigen in serum and saliva biofluids from both breast cancer patients and healthy controls. Additionally, to investigate the relationship between patients with BC and control groups' serum and salivary tumor markers.

Materials and Methods

Study Design and Setting

The current study designed a cross-sectional study investigating some tumor biomarkers of BC patients in Al Basheer Hospital breast clinics, in Amman/Jordan.

Study Sample

The current study recruited 40 Jordanian females with BC (as an experimental group) and 20 non-BC females (as a control group). Serum samples and unstimulated saliva in the current study within the period (20 December/2023 to 20 April/2024).

The Inclusion criteria for the BC patients' group involve the Histopathologic diagnosis of BC and All stages of BC. The exclusion criteria for the BC patients' group involve Pregnancy, lactation, or presently undergoing fertility treatment, Patients with active oral/dental disease, and Patients with health conditions (autoimmune disease, impaired renal function, active infection, hepatitis, diabetes, and hypertension).

The control individuals were non-BC female volunteers chosen from the general population.

Before sample collection, a structured interview was used to collect data through a questionnaire. The questionnaire involved Sociodemographic data (Including age, marital status, family members, employment status, income, educational level, and smoking), Anthropometric data (Weight, Height, and BMI), and Clinical data (Including duration of incidence, family history of BC, contraception use, any other diseases, and the BC stage).

In the current study, BC staging was based on the TNM classification as adopted by JBCP, (2019) as follows: stage 0, stage I, stage II, stage III, and stage IV.

Data Collection

Saliva sample: The collection of the unstimulated whole saliva sample involved: Participants were instructed to refrain 2 h before saliva specimen collection from eating, drinking (i.e., on an empty stomach), smoking, and tooth brushing. All samples of participants were collected between 8:00 a.m. and 2:00 p.m. Participants were asked to wash their mouths 3-5 times with water before collecting samples, then sit conveniently in an upright position and slope slightly their heads down to accumulate saliva in the mouth. Over the period of roughly 15 min, each participant spitted 5 mL saliva into a pre-labeled falcon conical tube. The collected samples were refrigerated at a temperature of 4°C for 30 min. Subsequently, saliva samples were transformed into plastic tubes for centrifugation at 3,500-5,000 rpm for 5 min to obtain supernatant without any debris. After centrifugation, the supernatant was carefully put in Eppendorf tubes, labeled, stored at -20°C, and kept in storage pending analysis.

Serum sample: The collection of the serum sample involved: Under complete aseptic circumstances, each participant obtained 5 mL of venous blood and then immediately transferred to the pre-labeled, plain tube with gel. The serum samples of participants were collected promptly after the saliva samples. The collected samples were kept in the refrigerator at a temperature of 4°C for 30 min. After clotting, samples were centrifuged at 3,500-5,000 rpm for 5 min to separate serum and obtain supernatant, which was carefully put in Eppendorf tubes, stored at -20°C and kept in storage until analysis.

The serum and unstimulated saliva participant samples were analyzed after incubation in a water bath at a temperature of 37°C.

Methods

Specific kits (Beckman Coulter Irel and Inc., USA) were used to estimate the levels of tumor markers CA125, CA15-3, AFP, and CEA) assays by chemiluminescent immunoassay (CLIA). The assays were carried out by the Unicel DXI 600 Access immunoassay system (Beckman

Coulter, Ireland) as illustrated by the kit's manufacturer instructions. The principle of each tumor marker kit assay is demonstrated as follows.

CEA: The Access CEA assay employs two mouse monoclonal anti-CEA Antibodies (MAb) that respond with distinct CEA epitopes. It is a two-site immunoenzymatic sandwich assay. The first anti-CEA MAb-alkaline phosphatase conjugate and the second anti-CEA MAb coupled to paramagnetic particles were added to a sample in a Reaction Vessel (RV).

CA15-3: An immunoenzymatic sandwich assay with two sites is the Access CA15-3 Monitor assay. A first mouse monoclonal anti-CA15-3 antigen alkaline phosphatase conjugate and paramagnetic particles coated in a sec mouse monoclonal anti-CA15-3 antigen-antibody were added to the sample in an RV. The conjugate antibody responds with a unique antigenic location on the CA15-3 antigen molecule when the CA15-3 antigen binds to the immobilized monoclonal anti-CA15-3 antigen on the solid phase in the sample.

CA125: The Access CA125 Monitor assay is a two-site immunoenzymatic sandwich test. The sample in an RV was supplemented with mouse monoclonal anti-CA125 antigen alkaline phosphatase conjugate and paramagnetic particles coated with a second mouse monoclonal anti-CA125 antigen antibody. While the CA125 antigen attaches to the immobilized monoclonal anti-CA125 antigen on the solid phase of the sample, the conjugate antibody interacts with a specific antigenic site on the CA125 antigen molecule.

AFP: The access AFP assay is a two-site immunoenzymatic sandwich assay. The sample in an RV was supplemented with mouse monoclonal anti-AFP-alkaline phosphatase conjugate and paramagnetic particles coated with a sec mouse monoclonal anti-AFP antibody. On the solid phase of the sample, the immobilized monoclonal anti-AFP binds to the AFP. The mouse monoclonal anti-AFP-alkaline phosphatase conjugate also reacts simultaneously with several antigenic sites on the AFP sample.

Following incubation in RV, the biomarkers of CEA, CA15-3, CA125, and AFP were separated into materials bound to the solid phase held in a magnetic field, and unbound materials were washed away. Subsequently, the chemiluminescent substrate was inserted into the vessel and the illuminometer measures the light produced by the reaction. The amount of light production is directly proportional to the concentration.

Statistical Analysis

In the current study, statistical analyses were encoded by Excel Microsoft programs and the PRISM software (version 9.2) for statistical analyses for the data collected from all participants who completed the study questionnaire and sample collections. Briefly, the two types of methods used to analyze the sociodemographic data, anthropometric measurements, and health information were Mann Whitney and Chi-squared test analysis, which measured variables between the BC patients and control groups, comparing frequency, percentage, and p-value.

The comparative analysis measured tumor biomarkers (CEA, AFP, CA125, and CA15-3) in the serum and salivary levels of BC patients and the control group using a One-way Analysis of Variance (One-way ANOVA) test, with ($p<0.001$) for CEA and AFP, ($p<0.05$, $p<0.001$) for CA125, CA15-3.

Pearson's correlation coefficient was used for the correlative analysis of tumor biomarkers to find out whether a relationship exists between tumor biomarkers in serum and salivary levels of the control groups and the BC group. The linear relationship between two variables was visualized using a scatter plot (its values): The correlation coefficient 1.0 indicates a very strong positive linear relationship, -1.0 indicates a very strong negative linear relationship and 0.0 indicates no linear relationship.

Additionally, to assess the diagnostic accuracy of tumor biomarkers in diagnosing BC, the Receiver Operating Characteristic (ROC) curve was examined using Python static analysis tools. This analysis showed the trade-offs between sensitivity and specificity across different thresholds. Using this technique, the true positive rate (sensitivity) and the false positive rate (specificity) were plotted against one other to produce an Area Under the Curve (AUC), a measure of a test's overall ability to distinguish between BC patients and controls. Table (1) illustrates this point: An AUC value near 1.0 denotes ideal diagnostic performance, while a value near 0.5 implies no more discrimination than chance (Nahm, 2022)

Ethical Considerations

Ethical approval for the research was obtained from the faculty of Allied Medical Science at Al-Ahliyya Amman University (Approval No. (IRB: AAU/4/5/2023-2024)) and ethical approval was obtained from the Ministry of Health of the Scientific Research Ethics Committee (Approval No. MOH/REC/2024/12)).

Each patient received information that their participation in the current study was voluntary and informed consent had been attained before collecting the saliva and serum samples for laboratory examinations.

Table 1: Interpretation of the AUC values

AUC values	Interpretation
AUC = 1	Perfect test
0.9≤ AUC <1	Excellent test
0.8≤ AUC <0.9	Good test
0.7≤ AUC <0.8	Fair test
0.6≤ AUC <0.7	Poor test
AUC = 0.5	No discriminative ability

Results

Sociodemographic Characteristics

The sociodemographic profile of participants in the current study presents a comprehensive view of the characteristics distinguishing those diagnosed with BC from the control group. Our analysis has elucidated several critical sociodemographic variables that may bear on the diagnosis and management of BC, as shown in Table (2).

Age emerged as a significant differentiator; individuals in the BC group were notably older, with an average age of 54.83 years compared to 32.20 years in the control group. This significant age disparity ($p<0.001$) may highlight the increased BC incidence in advancing age. In contrast, the average height between the two groups showed no significant difference, indicating that stature does not have a discernible role in the risk of BC in our cohort.

When examining body composition, the mean weight and BMI of individuals with BC were found to be higher than those in the control group. The difference in BMI was statistically significant ($p = 0.041$), indicating a potential link between body mass and BC risk. This is further supported by the weight distribution among participants: Nearly half of the BC group were overweight and a fifth were obese, proportions that were markedly different from the control group, where no individuals were classified as obese.

Marital status also exhibited a significant association with the incidence of BC. An overwhelming majority (97.5%) of the BC group were married, compared to 70% in the control group ($p = 0.007$). Employment status accentuated the differences between the two cohorts even further; a substantial 95% of the BC group was not employed, in stark contrast to the control group where only 30% were unemployed ($p<0.001$). These statistics may reflect the impact of BC on individuals' capacity to. Compared to 70% in the control group ($p = 0.007$). Employment status accentuated the differences between the two cohorts even further; a substantial 95% of the BC group was not employed, in stark contrast to the control group where only 30% were unemployed ($p<0.001$). These statistics may reflect the impact of BC on individuals' capacity to work, or alternatively, suggest that employment status could be a factor in BC risk.

The disparity in education levels between the two groups was pronounced. Participants with BC disease were less likely to have attained higher levels of education, with 40% having only primary school education and none with a master's degree. In contrast, 45% of the control group had a bachelor's degree. This difference was statistically significant ($p<0.001$), hinting at a potential correlation between educational attainment and BC prevalence or detection.

Lifestyle factors, such as smoking habits and breastfeeding history, did not exhibit any significant difference between the groups, suggesting that these factors may not be as strongly associated with BC risk in this population.

Within the clinical characteristics of the BC group, the distribution of disease stages ranged from 7.5% in stage I-12.5% in stage IV, indicating a varied progression of the disease among participants. A notable 42.5% reported a family history of cancer, which may suggest a genetic predisposition in this cohort. The majority did not use contraception and participating women were free of other diseases.

All in all, the results point to a complicated interplay of sociodemographic and clinical factors in BC incidence and management. Age, marital and employment status, BMI, and educational attainment have emerged as notable factors differentiating individuals with BC from the control group.

Comparative Analysis of the Levels of the Tumor Biomarkers in Serum and Saliva of Patients with BC and Control Group

In the current study, the four tumor marker levels: CEA, AFP, CA125, and CA15-3 were measured in both serum and saliva to explore the utility of saliva in diagnosing and monitoring these biomarkers.

CEA demonstrated significant elevations in the BC group compared to the control group across both tested mediums. Serum levels of CEA in the BC group averaged 4.86±1.5 ng/mL, markedly higher than the 1.17±0.42 ng/mL observed in the control group, with the difference being highly significant ($p<0.001$). Similarly, salivary CEA levels were also higher in the BC group, averaging 715.4±157.1 ng/mL compared to 353.3±139.6 ng/mL in the control group, with this difference also reaching statistical significance ($p<0.001$).

Furthermore, the salivary levels of CEA in both control and BC groups were significantly higher than the serum counterparts ($p<0.001$). These results suggest that CEA, particularly in saliva, could serve as a reliable biomarker for BC, as demonstrated in Fig. (1).

Table 2: Sociodemographic characteristics of study participants

Variable	BC group (n = 40)	Control group (n = 20)	p-value
Age (Mean ± SD)	54.83±11.57	32.20±6.01	<0.001
Height (Mean ± SD)	160.95±6.18	161.70±4.19	0.58
Weight (Mean ± SD)	69.92±12.81	65.45±7.49	0.094
BMI (Mean ± SD)	26.98±4.65	25.02±2.61	0.041
Weight category (n, %)			0.095
Underweight	1 (2.5%)	0 (0.0%)	
Normal	12 (30.0%)	9 (45.0%)	
Overweight	19 (47.5%)	11 (55.0%)	
Obese	8 (20.0%)	0 (0.0%)	
Marital status (n, %)			0.007
Single	1 (2.5%)	6 (30.0%)	
Married	39 (97.5%)	14 (70.0%)	
Employment status (n, %)			<0.001
No	38 (95.0%)	6 (30.0%)	
Yes	2 (5.0%)	14 (70.0%)	
Number of family members (n, %)			0.154
1-5	17 (42.5%)	7 (35.0%)	
5-10	23 (57.5%)	13 (65.0%)	
Monthly income (n, %)			0.525
Less than 100 JD	18 (45.0%)	5 (25.0%)	
100-400	2 (5.0%)	11 (55.0%)	
More than 400 JD	20 (50.0%)	4 (20.0%)	
Education level (n, %)			<0.001
Not educated	3 (7.5%)	0 (0.0%)	
Primary school	16 (40.0%)	1 (5.0%)	
High school	17 (42.5%)	3 (15.0%)	
Diploma	2 (5.0%)	5 (25.0%)	
Bachelor	2 (5.0%)	9 (45.0%)	
Masters	0 (0.0%)	2 (10.0%)	
Smoking (n, %)			0.666
No	39 (97.5%)	19 (95.0%)	
Yes	1 (2.5%)	1 (5.0%)	
Breastfeeding (n, %)			0.745
No	40 (100.0%)	19 (95.0%)	
Yes	0 (0.0%)	1 (5.0%)	
Stage of BC (n, %)			
I	3 (7.5%)	NA	
II	16 (40.0%)	NA	
III	16 (40.0%)	NA	
IV	5 (12.5%)	NA	
Family history of cancer (n, %)			
No	23 (57.5%)	NA	
Yes	17 (42.5%)	NA	
Contraception (n, %)			
No	39 (97.5%)	NA	
Non-hormonal	1 (2.5%)	NA	
Other diseases (n, %)			
No	40 (100.0%)	NA	

Evaluation of AFP biomarkers showed higher levels in the BC group in both serum and saliva. The serum AFP level was 3.98±1.35 ng/mL in the BC group, significantly higher than the 2.2±0.9 ng/mL found in the control group ($p<0.001$). In saliva, the BC group exhibited an AFP level of 0.56±0.19 ng/mL, almost double the 0.33±0.13 ng/mL in the control group, with this increase also being statistically significant ($p<0.001$). However, in contrast to the CEA, the serum levels of AFP were significantly higher than that of saliva counterparts in both control and BC groups ($p<0.001$), as illustrated in Fig. (2).

CA125 showed a similar pattern between serum and saliva. In serum, the increase in CA125 levels from 17.5±4.76 U/mL in the control group to 22.4±7.26 U/mL in the BC group was statistically significant ($p<0.05$), suggesting that a serum CA125 biomarker may be used in distinguishing between BC and control cases. In saliva, CA125 levels more than doubled in the BC group

(1696.4±509.3 U/mL) compared to the control group (790.5±248.1 U/mL), with this difference being significant ($p<0.001$). Similar to CEA, the saliva samples revealed significantly higher levels of CA125 in both groups compared to the serum samples ($p<0.001$), as shown in Fig. (3).

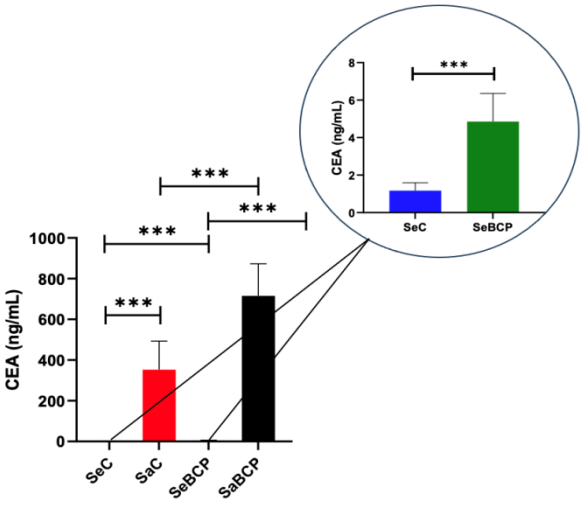

Fig. 1: Comparative analysis of CEA biomarkers in BC patients and the control group. A. Salivary and serum levels of CEA in BC patients compared to the control group. B. Serum levels of CEA in BC patients compared to the control group. ***p<0.001 calculated using One-way ANOVA. Serum Breast Cancer Patients (SeBCP), Saliva Breast Cancer Patients (SaBCP), Serum Control (SeC), Saliva Control (SaC)

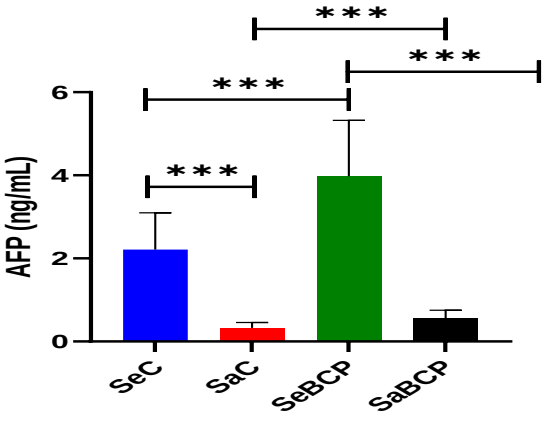

Fig. 2: Comparative analysis of AFP biomarker of salivary and serum levels in BC patients compared to the control group. p<0.001 calculated using One-way ANOVA. Serum Breast Cancer Patients (SeBCP), Saliva Breast Cancer Patients (SaBCP), Serum Control (SeC), Saliva Control (SaC)

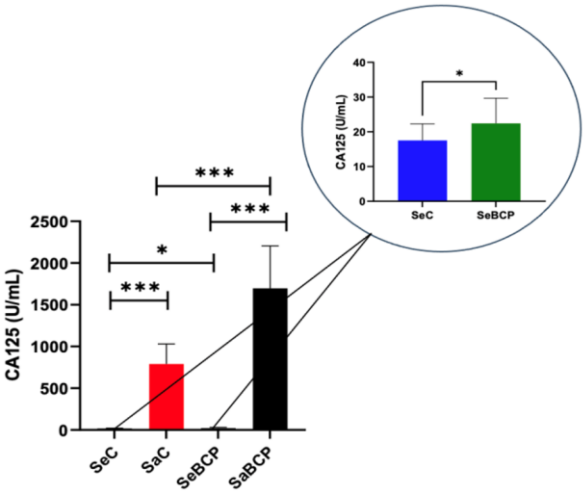

Fig. 3: Comparative analysis of CA125 biomarkers in BC patients and the control group. A. Salivary and serum levels of CA125 in BC patients compared to the control group. B. Serum levels of CA125 in BC patients compared to the control group. p<0.05, ***p<0.001 calculated using One-way ANOVA. Serum Breast Cancer Patients (SeBCP), Saliva Breast Cancer Patients (SaBCP), Serum Control (SeC), Saliva Control (SaC)

CA15-3 presented the most dramatic increases among the biomarkers studied. In the BC group, serum CA15-3 levels elevated to 188.3±42.5 U/mL, substantially higher than the 11±5.7 U/mL observed in the control group, with a highly significant ($p<0.001$). The salivary CA15-3 levels, rose to 176±64.2 U/mL in the BC group compared to 7.4±4.3 U/mL in the control group, also with a highly significant ($p<0.001$). Within the BC group, there was no statistically significant difference between serum and saliva levels. On the contrary, serum levels of CA15-3 were significantly higher than that of saliva within the control group ($p<0.05$), as demonstrated in Fig. (4).

In summary, the measurement of these biomarkers in saliva and serum suggests that salivary assessments, particularly for CEA, AFP, CA125, and CA15-3, could potentially serve as convenient and effective diagnostic tools for BC.

Correlation Analysis of Tumor Biomarkers in Serum and Saliva of Patients with BC and Control Group

The current study explored the correlations between serum and salivary levels of four tumor biomarkers: CEA, AFP, CA125, and CA15-3 in control and BC groups. The results demonstrate varied levels of correlation, shedding light on the interplay between this biofluid and its potential utility in clinical practice.

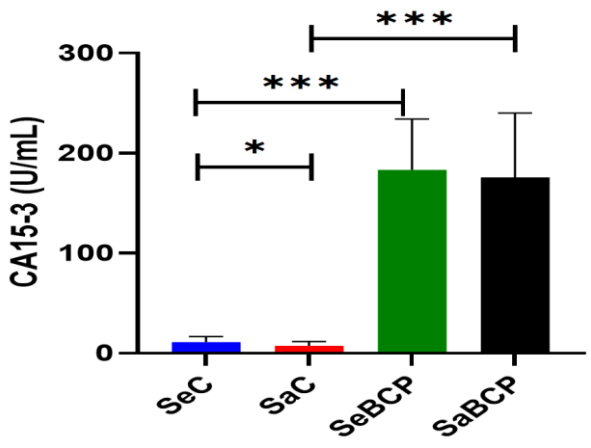

Fig. 4: Comparative analysis of CA15-3 biomarker of salivary and serum levels in BC patients compared to the control group. *p<0.05, ***p<0.001 calculated using One-way ANOVA. Serum Breast Cancer Patients (SeBCP), Saliva Breast Cancer Patients (SaBCP), Serum Control (SeC), Saliva Control (SaC)

For the CEA biomarker, the control group exhibited a very weak positive correlation with a coefficient of 0.16, suggesting a non-significant linear relationship between the serum and salivary levels. This indicates that, in a non-cancerous state, salivary levels of CEA are unlikely to reflect serum levels accurately. In contrast, the BC group presented a very strong positive correlation coefficient of 0.93, indicative of a strong positive correlation, and suggests a more predictable relationship between serum and salivary levels in BC.

The analysis of AFP revealed a different pattern. In the control group, there was a weak negative correlation, with a correlation coefficient of -0.37, indicating a minimal linear relationship between serum and salivary AFP levels, suggesting that this biomarker does not correlate strongly with controls. However, in the BC group, the correlation weakens to a very weak level, with a coefficient of 0.06.

For the CA125 biomarker, the control group showed a very weak positive correlation coefficient of 0.11 pointing to an almost non-existent linear relationship between serum and saliva levels, highlighting the potential challenges of using this marker in saliva. Conversely, BC patients showed a very weak negative correlation with a coefficient of -0.08, indicating a limited relationship.

Lastly, CA15-3 in the control group displayed a very weak positive correlation between serum and salivary levels, with a coefficient of approximately 0.03. Additionally, the BC group revealed a weak negative correlation between serum and salivary levels, where the coefficient plummets to around -0.13.

ROC Curve Analysis

In the current study, ROC curve analyses were performed for serum and saliva biomarkers to assess their efficacy in diagnosing BC. The results of these analyses, along with corresponding ROC curves and tabulated data on AUC, cut-off values, sensitivity, and specificity, are presented to elucidate the diagnostic potential of each marker Table (3).

Figure (5) elucidates the ROC curve of serum and saliva tumor biomarkers. The ROC analysis for serum CEA revealed an AUC of 0.95, indicating excellent diagnostic performance. The optimal threshold for serum CEA was determined to be 2.6 ng/mL, with a sensitivity and specificity of 95%. Similarly, salivary CEA also demonstrated an AUC of 0.95, with an optimal threshold of 602.2 ng/mL. The sensitivity and specificity for salivary CEA were 90 and 95%, respectively.

For serum CA125, the AUC was 0.78, suggesting a moderate diagnostic capability. The optimal threshold for serum CA125 was 19.1 U/mL, yielding a sensitivity of 70% and a specificity of 95%.

In contrast, salivary CA125 exhibited a higher AUC of 0.93, indicating better diagnostic performance. The optimal threshold for salivary CA125 was 1195 U/mL, with a sensitivity of 91% and a specificity of 95%.

Serum AFP demonstrated an AUC of 0.89, indicative of good diagnostic performance. The optimal threshold for serum AFP was 3 ng/mL, with both sensitivity and specificity recorded at 80%. Salivary AFP had a slightly lower AUC of 0.91. The optimal threshold for salivary AFP was 0.4 ng/mL, with a sensitivity of 85% and a specificity of 70%.

Table 3: ROC analysis results of serum and salivary tumor biomarkers

Biomarker	AUC	Optimal Threshold (ng/mL)	Sensitivity (%)	Specificity (%)
Serum CEA	0.95	2.6	95	95
Saliva CEA	0.95	602.2	90	95
Serum CA125	0.78	19.1	70	95
Saliva CA125	0.93	1195	91	95
Serum AFP	0.89	3	80	80
Saliva AFP	0.91	0.4	85	70
Serum CA15-3	1	112.2	100	100
Saliva CA15-3	1	70	100	100

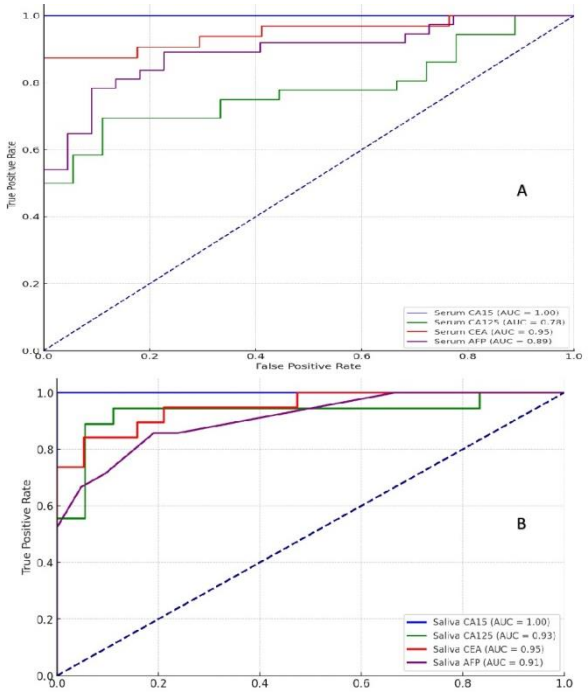

Fig. 5: ROC curves with the corresponding AUC values of tumor biomarkers. A. Serum AUC values of tumor biomarkers. B. Salivary AUC values of tumor biomarkers

Both serum and salivary CA15-3 achieved perfect diagnostic performance with an AUC of 1.0. The optimal threshold for serum CA15-3 was 112.2 U/mL and for salivary CA15-3, it was 70 U/mL. Both biomarkers exhibited 100% sensitivity and 100% specificity, demonstrating their superior diagnostic accuracy.

Discussion

One of the frequent and numerous malignant tumors that affect women is BC. The occurrence of this malignant tumor is rising worldwide, attributed to several modifiable and non-modifiable factors (Łukasiewicz *et al*., 2021). Therefore, knowledge of the risk factors associated with BC patients is important for deeper comprehension of this heterogeneous disease. The incidence of BC has increased globally in women of all ages, specifically in those less than 50 (Lima *et al*., 2021). In Jordanian women, the majority were diagnosed with BC between the ages of 40-59 years (Qatamish and Nusairat, 2018). In the current study, this risk factor emerged as a significant differentiator with an average age of 54.83 years and this agreed with other previous studies (Dwivedi *et al*., 2023). In addition to age, also family history appeared as a major risk factor, suggesting a genetic predisposition to increase BC incidence. In Jordanian study of (Abu-Helalah *et al*., 2020) demonstrated that BC patients diagnosed had a high possibility of familial predisposition. Likewise, the current study found that about half of the patients reported a family history of BC. This illustrated that family history can influence factors that potentially develop BC, considering that family history has clinical significance.

An epidemic of excess weight is considered a main lifestyle-related risk factor in BC patients. Elevated BMI induces chronic inflammation in the breast adipose tissue, this developing BC is directly associated with signals and cells from the obesity-damaged tissue (Devericks *et al*., 2022). Therefore, obesity in BC is established as a risk factor (Andò *et al*., 2019). In this context, the Jordanian study by Ayoub *et al*., (2019) concluded that BC patients impaired from obesity are at increased risk of BC recurrence. Similarly, the current study observed high BMI significantly associated with BC patients. Moreover, some studies revealed that obesity increases the risk of BC in postmenopausal women (Dehesh *et al*., 2023). This cooperates with current study findings, in which the majority of participants were postmenopausal women.

Biomarkers are critically important in evaluating the biological condition of the tumor (Seale and Tkaczuk, 2022). Serum biomarkers are substances released into the plasma in high quantities during tumor evolution (Fernandez-Olavarria *et al*., 2016). Their estimation is crucial to detecting tumor cells and cancer progression (Seale and Tkaczuk, 2022). Moreover, salivary biomarkers are also evaluated to identify BC (Porto-Mascarenhas *et al*., 2017). Therefore, the current study evaluated a diversity of serum and salivary biomarkers.

Several previous studies evaluated these tumor marker levels in BC patients, which showed a prognostic utility in early BC and predicted the prognosis for patients with BC (Tarek *et al*., 2022, Mohammed *et al*., 2022). For occasion, (Farahani *et al*. 2020) observed that serum levels of CEA biomarker were significantly higher in the patients with BC than in the control group, while no significant increase in the levels of salivary CEA, CA15-3, and serum CA15-3 in BC than control and in the BC patients the results showed no significant correlation between the CEA levels of serum and saliva. Assad *et al*. (2020) found a significant correlation between CA15-3 levels in serum and saliva among BC patients. Compared to our results CEA and CA15-3 levels in serum and saliva were significantly higher among BC patients than for the controls. In BC patients, the correlation between CEA levels in serum and saliva indicated a positive correlation, suggesting a more predictable relationship in BC malignancy, and for serum and salivary CA15-3 levels was a negative correlation. The inconsistency between our findings and other studies may be related to TNM stages in BC cases. Concerning CA125 tumor biomarker, which is commonly used in ovarian cancer patients. However, it may also increase in the metastatic BC patients (Yerushalmi *et al*., 2012), so can be used as an indicator for diagnosing and progression of BC (Ma *et al*., 2022). Accordingly, the study by Dwivedi *et al*. (2023) indicated that BC cases had significantly higher CA125 levels in serum and saliva than controls and also positive correlation was

found between serum and salivary levels of CA-125 among BC patients. In comparison, the results of the current study also observed significantly higher CA125 levels in serum and saliva among patients with BC than in controls, whereas BC patients showed a negative correlation. Indicating that the levels of CA125 increase whenever the metastatic cancer increases. Beyond that, AFP biomarkers in serum and saliva are specific for the diagnosis of liver cancer cases (He *et al*., 2019). Whereas fewer studies investigate serum AFP in BC patients (Kumari *et al.*, 2024). Such as the study by Luo *et al.* (2023) revealed that the serum AFP levels among BC patients were significantly higher than in controls. This agreed with the current study findings, suggesting that a high serum AFP level indicates a higher risk of BC. Nevertheless, there was a positive correlation between the levels of AFP in serum and saliva in BC patients.

The AUC is widely used to measure the accuracy of diagnostic tests (Nahm, 2022). Therefore, ROC curve analyses were performed for serum and saliva biomarkers to assess their efficacy in diagnosing BC. Concerning AUC values for serum biomarkers, the previous study by Luo *et al.* (2023) observed that serum CA15-3 is a superior tumor marker for BC to serum CA125, CEA, and the lowest values in serum AFP. This is in accordance with current study findings that serum CA15-3 achieved perfect diagnostic performance, whereas the serum values of the CA125 biomarker demonstrated the lowest values than serum CEA and AFP. These disparities in AUC values resulted from differences in findings of the serum biomarker levels. On the other hand, the diagnostic accuracy of serum biomarkers as a single detection is less than optimal, nevertheless, the combined detection of serum biomarkers improves the accuracy (Seale and Tkaczuk, 2022; Luo *et al.*, 2023). Concerning AUC values for salivary biomarkers, the previous studies indicated that highest AUC values for salivary CA15-3 than CEA (Farahani *et al*., 2020) and high salivary CA125 values (López-Jornet *et al*., 2021). These findings correspond with the current study results. This indicates that these salivary biomarkers can be effective for BC diagnosis.

Conclusion

The socio-demographic profile demonstrated higher BMI, older age, married women, unemployment, and lower educational levels play a significant role in BC risk and incidence. On the contrary, lifestyle factors such as smoking and breastfeeding history did not exhibit any significant correlation with BC risk. The current study supported the utility of salivary tumor biomarkers and parameters as diagnostic and monitoring tools for BC. The biomarkers CEA, AFP, and CA125 levels illustrated a significant elevation of serum and saliva in the BC group compared to the controls. However, very strong positive correlations between serum and salivary CEA levels were observed in BC patients, suggesting a more predictable relationship between the two biofluids. The biomarker CA15-3 presented the most considerable increases in serum and salivary levels in BC patients, which achieved perfect diagnostic performance with an AUC value, indicating that this biomarker may serve as a diagnostic tool for BC. Furthermore, the parameters demonstrated serum LDH, urea, and salivary AST levels were significantly different in BC compared to the control group, indicating potentially valuable for BC monitoring. However, other parameters showed no significant differences between serum and saliva in both groups.

Limitations

The current study's sample was limited due to being from a single center, focused only on Al-Basheer Hospital, which includes all cancer types, not only BC.

The current study was a cross-sectional design. Therefore, could not establish the causality of existing correlations. However, the observed outcomes are valuable and can be used in future investigations.

Through days allotted to BC patients in Al Basheer Hospital were receiving chemotherapy in addition to taking other medications and on these days no collected samples, might have an impact on the data analysis's outcomes.

Recommendations

Evaluating the utility of tumor biomarkers in combination instead of individually may provide rather valuable and reliable diagnostic criteria.

Further studies are demanded to investigate the potential significance of routine biochemical parameters in serum and saliva for BC diagnosis and monitoring.

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Author’s Contributions

Omar Atrooz: Designed, supervised, and structured the project and wrote the paper.

Huthaifa Tarawneh: performed all the experiments and data analysis.

All authors read and approved the final manuscript.

Ethics

This study is completely original and hasn't been submitted or published anywhere else. The authors have all declared that they have no conflicts of interest and have given their approval for the work to be published. All information is contained in the article itself.

Conflict of Interest

The authors declare that there is no conflict of interest.

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