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Research Article

Correlation of Some Biochemical Parameters between Serum and Saliva of Patients with Breast Cancer

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Abstract: Regular breast cancer laboratory examination of serum is a burden on patients, therefore understanding salivary test methods' features and limits is important for diagnosis. The study aims to evaluate the biochemical parameters in the serum and saliva of breast cancer and control individuals. Furthermore, to investigate the correlation between two biofluid levels in both groups. This cross-sectional study recruited Jordanian females including 40 breast cancer patients and 20 control individuals in Al-Basheer Hospital. Data was collected using a questionnaire and laboratory examinations of serum and unstimulated saliva samples. Statistical analyses were performed by PRISM software. The analysis of the biochemical parameters (Total protein, Albumin, Calcium, Alanine transaminase, Aspartate transaminase, Alkaline phosphatase, Lactate dehydrogenase, Gamma-glutamyl transferase, Uric acid, and Urea) used Pearson's correlation coefficient and One-way ANOVA. All serum and salivary biochemical parameters showed no significant differences in breast cancer patients compared to the control group, while salivary AST, serum LDH, and serum urea levels were significantly higher in breast cancer patients compared to the control group. Biomarkers could be useful bioindicators for breast cancer. However, parameters of serum LDH, urea, and salivary AST levels had a potential role in breast cancer monitoring.

Keywords: Malignant Tumor, Genetic Factor, Enzyme Levels, Biochemical Markers, One-Way ANOVA

Introduction

Breast Cancer (BC) is the most common cancer type diagnosed in women worldwide and represents nearly 36% of all female oncological patients. BC is the most common type of cancer among women globally, with approximately over a million women diagnosed with BC worldwide in 2020, as reported by the World Health Organization (WHO) (Nardin *et al.*, 2020). Recent studies show the incidence of this malignant tumor is growing on a global scale; however, industrialized countries have the largest number of incidence rates, and almost half of the cases are in developed countries (Bellanger *et al.*, 2018). The high incidence is due to a Western lifestyle with an unhealthy diet, alcoholic moderate/high consumption of nicotinism, stress, lack of regular physical activity, and night work. Moreover, its occurrence is linked to genetic and hereditary predisposition risk factors (Hong and Xu, 2022). So, it is important for you to understand that BC is the second most significant cause of cancer-related deaths among women worldwide. In Jordan, BC is the third most common cause of cancer death, following lung and colorectal cancers (Abdel-Razeq *et al.*, 2020a).

Studies have identified various salivary biomarkers for cancers, such as a carcinoembryonic antigen, cancer antigen 15-3, alpha-fetoprotein, uric Acid, Urea, total proteins, lactate dehydrogenase, gamma-glutamyl transferase, alkaline phosphatase, alanine transaminase, and aspartate transaminase. 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 BC (López-Jornet *et al.*, 2021). Providing a noninvasive 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).

The potential uses of serum markers in BC offer a less intrusive, more affordable source of information that can be used to assess prognosis, track the course of a disease, and aid in treatment planning. For results to be interpreted accurately, it is essential to comprehend the features and limitations of each test. The guidelines for the use of breast tumor markers in BC prevention, screening, treatment, and monitoring have been updated by the American Society of Clinical Oncology (ASCO) (Kabel, 2017).

BC is classified as invasive or noninvasive based on histopathological outcomes, which rely on the breast cells turning into cancer, where it started, and its relation to the basement membrane. This classification of BC supplied a framework for molecular classification and has long been used extensively with image elucidation. Depending on this classification, BC can be split into numerous subtypes, according to the American Cancer Society (Rasheed and Youseffi, 2024) and the National Breast Cancer Foundation (NBCF, 2024).

About 287,850 female and 2,710 male invasive BC cases were recently diagnosed in the United States of America (USA), with 43,250 female and 530 male deaths, respectively (American College of Cardiology (Rasheed and Youseffi, 2024). As of 2022, there are 28,600 BC cases in Canada (Brenner *et al.*, 2022), and 186,000 new BC cases were reported in Africa in 2020 (McCormack *et al.*, 2020). By 2020, Belgium had the greatest incidence rate of BC globally, followed by France, the Netherlands, and Luxemburg (Sung *et al.*, 2021). According to a previous study, 27 European nations reported a 3.9% decrease in overall BC mortality between 2012 and 2017 (Wojtyla *et al.*, 2021).

In 2019, in the Middle East and North Africa (MENA) region, the age-standardized incidence and death rates of female BC differed considerably between these region country (Mubarik *et al.*, 2023). The countries enrolled with the highest age-standardized incidence rates were Lebanon, Qatar, and Bahrain, and the countries that had the lowest age-standardized incidence rates were Afghanistan, Yemen, and Sudan.

In Jordan, between 1996 and 2017, 16268 cases were registered (JNCCN, 2003). In 2012, according to the Jordanian Ministry of Health, the crude incidence rate was 32.1 cases/per 100,000 females, with the highest age-specific rate in females aged 65-69 years (Nimri, 2018). In 2015, the crude incidence rate was 34.1 cases/100,000 females, with the highest age-specific rate in females found in 60-64 years (Abdel-Razeq *et al.*, 2020a). In 2018, there were 20.8% BC cases among Jordanian males and females, with 24.9% BC mortality in females Jordan Cancer Registry (JCR, 2018). From 1997-2002, a study conducted by Tarawneh *et al.*(2011) consisted of Jordanian females with BC and indicated that stage I 17.7% of the cases were diagnosed, stage II 34.6%, stage III 29.6%, and 16.5% were diagnosed at stage IV. The retrospective study conducted by Obeidat *et al.* (2017) was conducted between 2006-2015 and indicated that 4.8% of cases were diagnosed as ILC, and 74.3% were classified as IDC, 47.42% of cases displayed lymph node metastases, and 6.87% had distant metastases. A retrospective cohort study conducted by Mousa *et al.* (2021) included BC cases between 2011-2014 at Al-Bashir Hospital and the University of Jordan Hospital which indicated that 30.1% had stage III BC diagnosis, and the majority of BC cases had ductal carcinoma diagnosis. Abdel-Razeq *et al.* (2020b) study observed in patients aged 65 years that the predominant pathology identified IDC was 83.2% patients, and ILC was 10.8% patients. A retrospective study conducted by Al Soudi *et al.* (2021) involved BC patients surgically treated at Al-Hussein Hospital and observed that the histological type of breast tumor is IDC nonspecific type in 89% of patients. A cross-sectional study by Al Qadire *et al.* (2021) indicated that women exhibited convergent outcomes between early stages (I, II) and advanced stages (III, IV) of BC, where 50.0% of patients were in the early stages, and 45.3% were in the advanced stages. Nonetheless, stage IV illness is identified in about 15% of BC patients (Abunasser *et al.*, 2023).

The pathophysiology of cancer is influenced by biochemical factors (Rahal *et al.*, 2014), and high serum levels of certain biochemical parameters were linked to an increased risk of cancer (Dovell & Boffetta, 2018). Compared to healthy women, benign and malignant BC patients have significantly increased levels of several biochemical indicators.

Comprehensive research, including BC overview and previous studies, examined a variety of parameters and biomarkers in serum and saliva for diagnosing BC, illustrating the diagnostic aspects of salivary tests and their correlation with serum biomarkers. The current study handles these gaps in the literature by evaluating serum and salivary parameters and biomarkers in BC patients and the control group and investigating their correlation. This reinforces the understanding of the correlation and prepares the way for the development of more valuable, noninvasive diagnostic tools for clinical practices. Thus, the study's objectives were to estimate some serum and saliva biochemical parameters in both BC patients and control subjects. Furthermore, the relationship between the biochemical characteristics of the serum and saliva of BC patients and control subjects should be investigated.

Materials and Methods

Study Design and Setting

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

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 samples were collected from each individual participating in the current study within the period of 20 December/2023 and 20 April/2024.

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

The control individuals were non-BC female volunteers chosen from the general population, for whom BC was excluded by CBE and recurrence screening from six months to one year.

Data Collection

A structured interview was used to collect data through a questionnaire consisting of Sociodemographic data (age, marital status, family members, employment status, income, educational level, breastfeeding, and smoking), anthropometric data (weight and body mass index (BMI), and Clinical data (include duration of incidence, family history of BC, contraception use, any other diseases, and the BC stage).

Saliva Sample

The collection of the unstimulated whole saliva sample. Participants were instructed to refrain two hours 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

Under complete aseptic circumstances, 5ml of venous blood was obtained from each participant 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. The DXC 700 AU Clinical chemistry analyzer (Beckman Coulter, Ireland) was used for biochemical parameters analysis.

Biochemical Markers

The current study explored serum and unstimulated saliva examinations of biochemical parameters including total protein (g/L), albumin (g/L), calcium (mg/dl), ALP (U/L), LDH (U/L), ALT (U/L), AST (U/L), GGT (u/L), uric acid (UA) (mg/dl), and Urea (mg/dl). All kits were obtained from Beckman Coulter Ireland Inc., Ireland. All procedures regarding the kit protocols are followed.

Statistical Analysis

In the current study, statistical analyses were encoded by Excel Microsoft programs and the PRISM software (version 9.2) for statistical analyses from data collected from all participants who completed the study questionnaire and sample collections.

The two 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 of biochemical parameters (total protein, albumin, calcium, ALT, AST, ALP, LDH, GGT, UA, and Urea) in the serum and salivary levels of BC patients and the control group used the One-way ANOVA test, with (p-values <0.001) for total protein, albumin, calcium, ALT, AST, ALP and GGT, (p-value <0.01, p-value <0.05) for LDH, (p-value <0.01, p-value <0.001) for UA, (P-value <0.05) for Urea.

The correlative analysis of biochemical markers was performed to find out whether a relationship exists between biochemical markers in serum and salivary levels of the control groups and the BC group. The Pearson's correlation coefficient measures the presence of the linear relationship between two variables, can be visualized using a scatter plot, its values can be interpreted as described in Table (1) (Sedgwick, 2012).

Table 1: Interpretation of Pearson's correlation coefficient values

Coefficient values	Interpretation
0.9-1.00	Very strong correlation
0.70-0.89	Strong correlation
0.50-0.69	Moderate correlation
0.30-0.49	Weak correlation
0.00-0.29	Very weak correlation

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).

One important differentiator was age; the BC group's average age was significantly higher at 54.83 years, whereas the control group's average age was 32.20 years. This notable age difference ($P < 0.001$) might draw attention to the higher incidence of BC as people age. However, there was no noticeable difference in the average height between the two groups, suggesting that stature is not a significant factor in our cohort's risk of BC.

When looking at body composition, people with BC had higher mean weights and BMIs than people in the control group. The statistical significance of the BMI difference ($p = 0.041$) suggests that body mass and BC risk may be related. 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 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.

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	

Breast Cancer (BC); Body Mass Index (BMI); Mean ± Standard Deviation (Mean ± SD); Not Applicable (NA); p-value = 0.05

Within the clinical characteristics of the BC group, the distribution of disease stages ranged from 7.5% in stage I to 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.

Generally, 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 Biochemical Parameters Levels in Serum and Saliva Across Control and BC Groups

The results for total protein measurements revealed striking differences between serum and saliva within each group. In the control group, the mean serum total protein levels were 70±3.5 g/L, while the mean salivary total protein levels were significantly lower at 1.46±0.7 g/L ($P < 0.001$). The BC group exhibited comparable serum total protein levels to the control group, with a mean of 68.95±5 g/L. The salivary total protein levels in the BC group were 1.68±0.9 g/L, slightly higher than the salivary total protein in the control group and lower than total protein serum levels in BC ($p<0.001$). Substantial disparities were found in total protein concentrations between serum and saliva. The data demonstrates the similarity in serum total protein levels between the control and BC groups. In contrast, salivary levels were consistently and significantly lower within both groups, as illustrated in Fig. (1).

Albumin exhibited significant differences between serum and saliva within each group. In the control group, mean salivary albumin levels were 0.25±0.09 g/L, significantly lower than serum counterparts ($p<0.001$). In the BC group, salivary levels of albumin were 0.35±0.22 g/L, slightly higher than salivary levels of albumin in the control group. Salivary albumin levels were still much lower than the serum albumin levels ($p<0.001$).

The control group had serum albumin levels of 48.33±2.25 g/L, and the BC group had slightly lower serum albumin levels at 43.7±4 g/L, this difference was not statistically significant, highlighting that the serum albumin concentrations were relatively consistent between the control and BC groups (Figure 1).

Liver enzymes such as ALT showed significant differences between serum and saliva within each group. In the control group, serum ALT levels averaged 14.6±3.4 U/L, significantly higher than salivary ALT levels averaged 6.53±2.75 U/L ($p<0.001$), reflecting a clear distinction between the enzyme levels in saliva and serum. In the BC group, serum ALT levels averaged 13.2±4 U/L, significantly higher than the salivary ALT levels averaged 4.95±2.5 U/L ($p<0.001$).

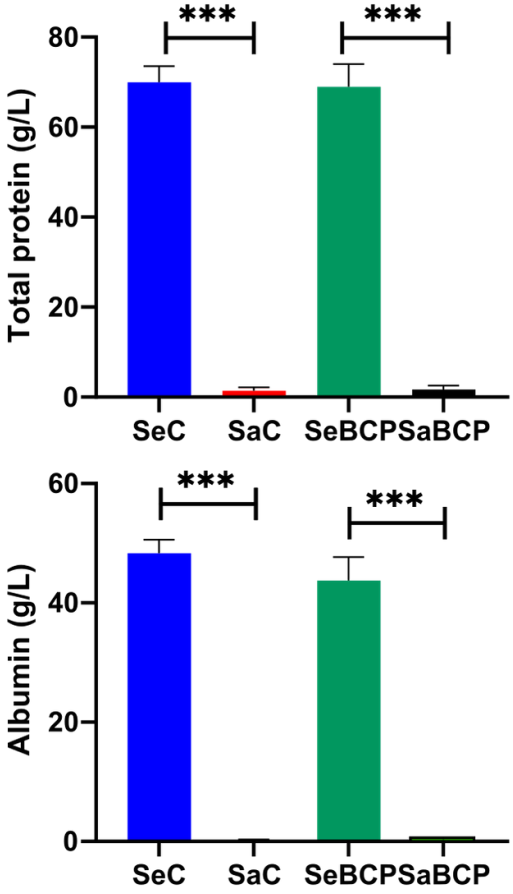

Fig. 1: Comparison of the concentrations of albumin and total protein in serum and saliva between the BC and control groups. ***P<00.001 was determined by one-way ANOVA. Serum Breast Cancer Patients (SeBCP), Saliva Breast Cancer Patients (SaBCP), Serum Control (SeC), Saliva Control (SaC)

However, no significant differences were found in serum ALT levels between the control and BC groups, nor salivary ALT levels between the two groups, as illustrated in Figure (2).

ALP showed differences between serum and saliva in each group. In the control group, serum ALP levels averaged 67.3±15.87 U/L, significantly higher than the salivary ALP levels ($p<0.001$). In the BC group, serum ALP levels at 68.55±20.85 U/L, significantly higher than salivary ALP levels at 13.1±7.8 U/L ($p<0.001$). These findings underscore the clear difference between serum and saliva for ALP levels, as depicted in Figure (2). However, no significant differences were found in serum ALP levels between the control and BC groups, nor salivary ALP levels between the two groups.

For liver enzymes AST, the control group's serum levels averaged 17.9±4.64 U/L compared to their salivary levels at 22.4±7.5 U/L; observing this difference was statistically insignificant between serum and salivary AST. In contrast, in the BC group, serum AST levels averaged 21.2±7.45 U/L, significantly lower than salivary levels averaged 46.04±11.8 U/L ($p<0.001$). Interestingly, the salivary levels of AST exhibited a significant difference between the control and BC groups. However, no significant differences were found in serum AST levels between the control and BC groups, as demonstrated in Figure (2).

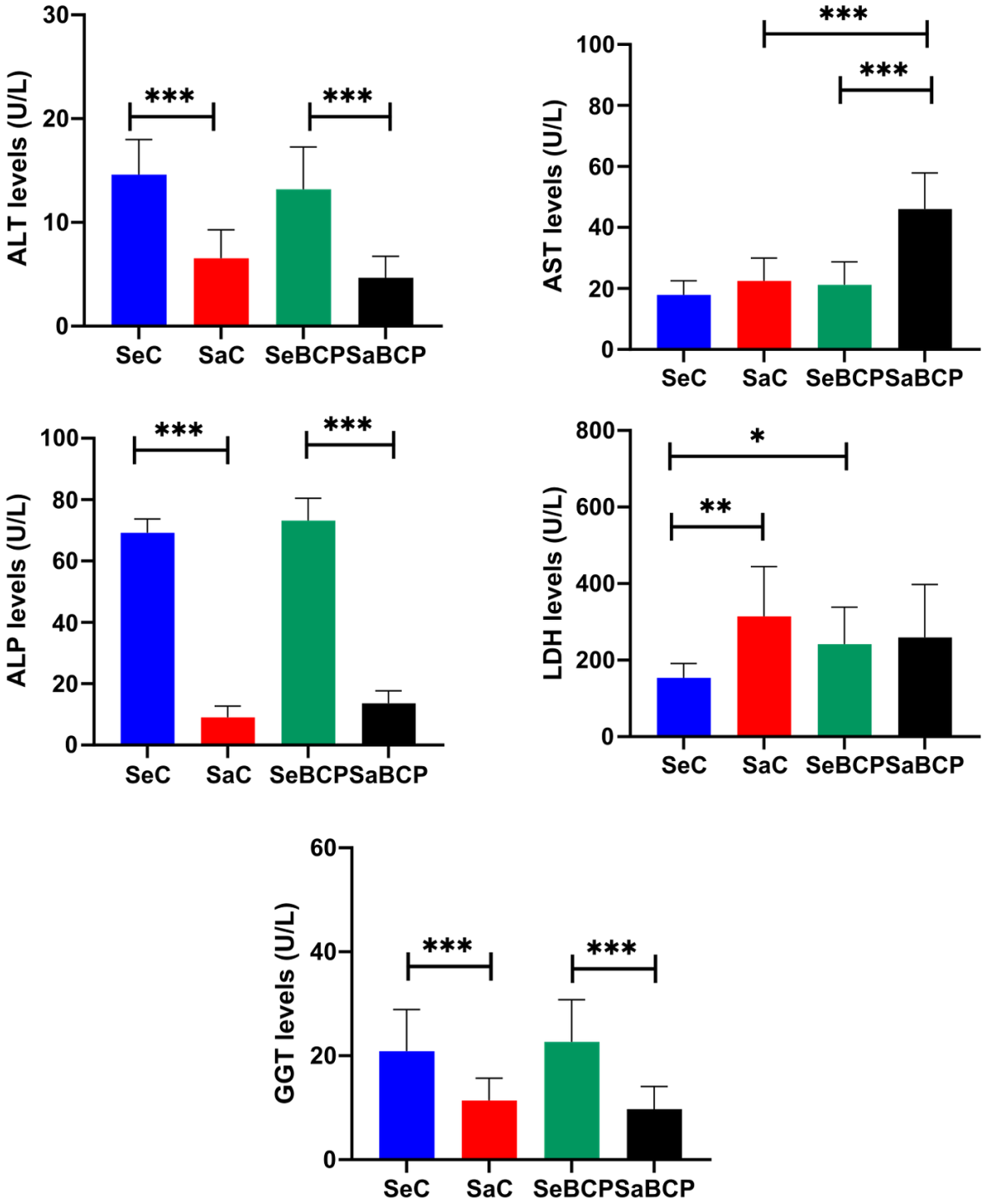

Fig. 2: Comparative analysis of the liver enzymes ALT, AST, ALP, LDH, and GGT in serum and salivary levels across control and BC groups. ***P<0.001 calculated using one-way ANOVA. Serum Breast Cancer Patients (SeBCP), Saliva Breast Cancer Patients (SaBCP), Serum Control (SeC), Saliva Control (SaC)

For LDH, the control group outcomes, there were significant differences between serum and saliva; the serum LDH levels were 153.36±37.7 U/L, significantly lower than the salivary LDH levels at 314.45±129.6 U/L ($p<0.01$). In the BC group, serum LDH levels were 241.4±97 U/L, and salivary LDH levels were 258.86±139 U/L, indicating the variation was not statistically significant. Intriguingly, the levels of serum LDH were significantly higher in the BC group compared to the control counterpart ($p<0.05$), as demonstrated in Figure (2).

GGT levels showed significant discrepancies between serum and saliva in each group. In the control group, serum GGT levels were 209±8 U/L, significantly higher than the salivary GGT at 114±43 U/L ($p<0001$) In the BC group, serum GGT levels were 227±8 U/L, significantly higher than the salivary GGT at 972±434 U/L ($p<0001$) However, no significant differences were found in serum GGT levels between the control and BC groups, nor salivary GGT levels between the two groups, as illustrated in Figure (2).

A similar pattern to GGT was observed for UA. In the control group, the serum UA levels were 3.8±0.6 mg/dL, significantly higher than /the salivary UA at 2.23±1.3 mg/dL ($p<0.01$). In the BC group, the serum UA levels were 4.65±1.4 mg/dL, significantly higher than the salivary UA at 2.48±1.4 mg/dL ($p<0.001$). However, no significant differences were found in serum UA levels between the control and BC groups, nor salivary UA levels between the two groups, as shown in Fig. (3).

Unlike other tested parameters, urea levels showed no statistically significant variation between serum and saliva within each group. In the control group, serum urea levels were 21±5.4 mg/dL, and salivary urea levels were 26.5±7.7 mg/dL. In the BC group, serum urea levels were 30±9.2 mg/dL, and salivary urea levels were 34.6±12.8 mg/dL. However, the serum urea levels in the BC group were significantly higher than the serum of the control group ($p<0.05$), as demonstrated in Figure (3).

Calcium displayed significant differences between serum and saliva within each group. In the control group, serum calcium levels averaged 9.66±0.3 mmol/L, significantly higher than the salivary calcium levels averaged 5.29±1.3 mmol/L ($p<0.001$), indicating a notable difference between serum and saliva. In the BC group, serum calcium levels were 9.7±0.46 mmol/L, significantly higher than the salivary calcium levels, averaging 4.29±1.5 mmol/L ($p<0.001$), indicating the substantial difference between serum and saliva was evident.

Nevertheless, the results showed neither the salivary calcium levels nor the blood calcium levels of the BC patients and the control group differed significantly, indicating that calcium levels were consistent across both groups. Figure (3) illustrates variations of serum and salivary calcium levels that are significantly different within each group.

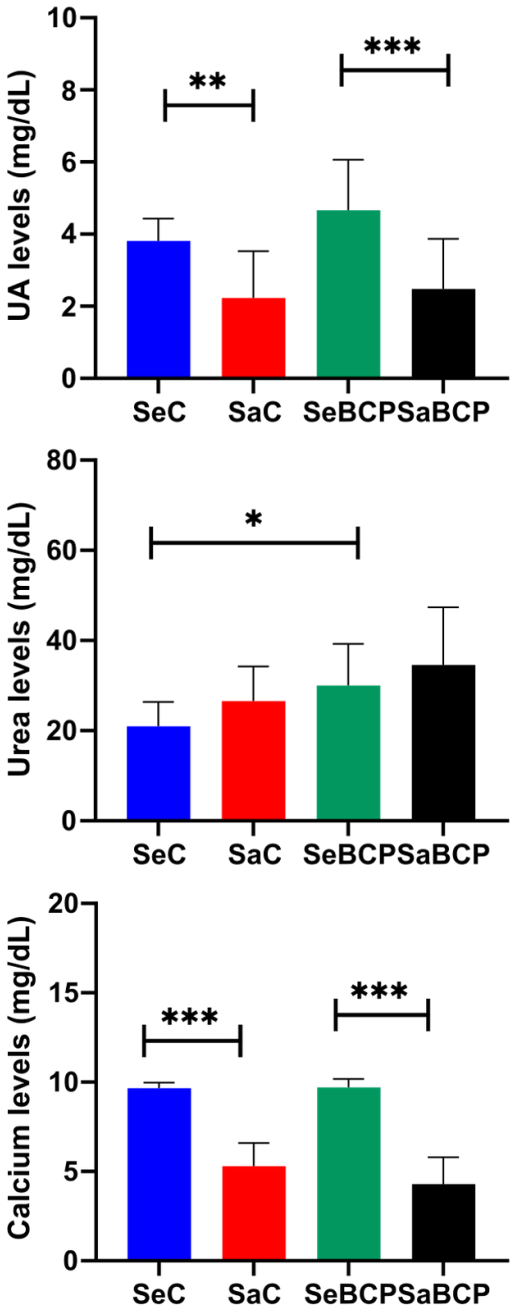

Fig. 3: Comparative analysis of UA, Urea, and calcium in serum and salivary levels across control and BC groups. **P<0.01, ***P<0.001 calculated using one-way ANOVA. Serum Breast Cancer Patients (SeBCP), Saliva Breast Cancer Patients (SaBCP), Serum Control (SeC), Saliva Control (SaC)

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. 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, family history also appeared to be a major risk factor, suggesting a genetic predisposition to increase BC incidence. A Jordanian study by 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 that high BMI was 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. Biochemical Parameters in Control and BC Groups.

Considering serum biochemical parameters. The development of metastasis lymph nodes induced the increase in serum urea levels, which is mostly dependent on the increasing invasion of BC cells (Zhan *et al.*, 2015).

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 that high BMI was 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. Biochemical Parameters in Control and BC Groups.

Considering serum biochemical parameters. The development of metastasis lymph nodes induced the increase in serum urea levels, which is mostly dependent on the increasing invasion of BC cells (Zhan *et al.*, 2015). In this context, Tinfash *et al.* (2022) demonstrated that the BC patients' serum levels of Urea and UA were significantly higher compared to the control group. However, the current study found significant differences in serum urea levels in the BC patients compared to the control group, whereas UA was not significant. It's possible that this is because the variables play a significant role in these disparities.

A variety of liver serum tests are widely used as first investigations for many clinical manifestations. Therefore, there is increasing concern in evaluating these tests as independent indicators of non-liver diseases associated with increased risk of mortality among cancer patients, including BC patients (Liu *et al.*, 2015; Mehdi *et al.*, 2018). This is consistent with the current study observations, demonstrating that the elevation of serum LDH levels is associated with BC recurrence and mortality. Furthermore, studies demonstrated that in BC patients, a progressive elevation of serum ALP levels is an indication of metastasis (Mahmood *et al.,* 2023). In this context, the current study observed no significant differences in serum ALT and AST levels between BC patients and controls. This is not consistent with Alkindi and Alhashemi (2022) which illustrated that there was a significant increase in their levels in BC patients compared with healthy subjects. The reason for their elevation in the BC patient might be a result of inflammation or injury to other organs. Additionally, the current study had shown no significant differences in serum GGT levels between BC patients and controls. Compared to another study by Zhang *et al.* (2024) revealed a significantly increased level of serum GGT in premenopausal BC patients, whereas there were no significant differences in the cases of postmenopausal BC patients. The argument of these findings may be due to the potential impact of menopausal status. Numerous previous studies have shown that serum LDH levels in BC patients are significantly higher than in controls (Liu *et al.*, 2015). In this context, the current study revealed there were no significant differences in levels of serum ALP and serum calcium between BC patients and controls. Compared to another study, (Mahmood *et al.*, 2023) observed there was a significant increase in serum ALP levels in BC patients compared to the control, while there was no significant difference in the serum levels of calcium between the two groups. Since most BC patients were in advanced BC stages, thus elevated serum ALP levels may result.

Since albumin is a component of serum total protein, the changeable increase in serum albumin levels might be due to oxidative stress associated with cancer development. The current study found serum albumin levels high in BC patients and controls with no significance, which is consistent with the previous study (Al-Mohtaseb, 2014). Moreover, increases in serum total protein levels are a consequence of physiological events; as the plasma circulates across the tissues, it assembles the proteins and releases them from their main sites,

causing an increase of protein in blood circulation. The current study revealed that the serum total protein levels were high in BC patients and controls with no significance, compared to the Jordanian study by AL-Muhtaseb (2014), which showed that the mean serum levels of total protein were significantly higher in BC patients compared to the healthy individuals, this may due to a combination of systemic inflammatory responses, increased tumor-related protein synthesis, and physiological alterations associated with cancer.

Concerning salivary biochemical parameters. The capability of salivary parameters in other cancers has been studied, providing an establishment for their potential use in BC diagnosis (Porto-Mascarenhas *et al.*, 2017). Therefore, the current study evaluated the levels of various salivary parameters and revealed that in BC patients, there was an increase in the levels of salivary total protein, ALP, UA, and Urea and a decrease in the levels of salivary LDH and GGT compared to the control group. At the same time, the study by Bel'skaya *et al.* (2023) revealed that levels of some salivary parameters were changed significantly in BC patients, including an increase in the levels of salivary LDH, ALP, GGT, and Urea; in contrast, a reduction in the levels of salivary total protein and UA as compared with the control group. The variability in results between the current study and another study spotlights the potential affected factors on salivary parameters such as population and sample variability. Despite these differences, the consistent increase in parameters such as ALP and Urea indicates that these could be salivary indicators for BC monitoring, suggesting more research to correspond to these differences in salivary analyses to augment saliva reliability as a diagnostic fluid for BC.

Although the current study findings did not exhibit any significant differences in the BC patients' salivary ALT, calcium, and albumin levels as compared with the control group, there was a significant increase in the levels of AST in the BC group compared to the control group, the absence of statistically significant findings in some parameters should not diminish the importance of establishing a baseline insight and recognizing its valuable contributions to BC diagnosis. The comprehensive analysis of evaluating salivary biochemical parameters among BC patients compared to the control group in the current study is pioneering and innovative in Jordan. Several previous studies focused on examining serum investigations in diagnosing and monitoring BC often overlook the potential role of salivary parameters. The absence of previous studies in this context emphasizes the novelty and relevance of this study for establishing a baseline for more investigation.

Conclusion

The sociodemographic 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. Furthermore, the parameters demonstrated serum LDH, urea, and salivary AST levels were significantly different in BC compared to the control group, indicating potential value for BC monitoring. However, other parameters showed no significant differences between serum and saliva in both groups. Further studies are demanded to investigate the potential significance of more routine biochemical parameters in serum and saliva for BC diagnosis and monitoring.

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Author's Contributions

Huthaifa Tarawneh: All experiments and data analysis were carried. In addition to writing the paper.

Omar Atrooz: Designed, supervised, and organized the project. All writers read and approved the final manuscript.

Author's Statement

The authors declare that this study is completely unique and hasn't been published or submitted for publication anywhere else. The article's publication has been approved by all authors, who also declare that they have no conflicts of interest. The article itself contains all of the data.

Ethics

The study received ethical approval from the Ministry of Health's Scientific Research Ethics Committee (Approval No. MOH/REC/2024/12) as well as the Al-Ahliyya Amman University Faculty of Allied Medical Science (Approval No. (IRB: AAU/4/5/2023-2024).

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.

Conflict of Interest

According to the authors, there are no competing interests.

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