The efficacy of salivary biomarkers versus serum biomarkers in diagnosis of breast carcinoma

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Background/aim
The diagnosis of breast cancer depends mainly on mammography or histopathology, but recently, salivary biomarkers have proved to be a promising tool as a noninvasive diagnostic technique. Cancer antigen (CA)15-3 is a widely used prognostic serum breast cancer biomarker. Cytokines have been used as biomarkers in research for prognosis and have been related with symptoms and adverse outcomes in breast cancer. The present study aimed to assess the accuracy of CA15-3 and interleukin (IL)-1β expression in saliva versus serum of patients with breast cancer.

Patients and methods
This study enrolled 26 patients with breast cancer from El Demerdash Hospital, Cairo, Egypt. In addition, 16 healthy individuals served as a control group. Saliva and blood samples were collected from all participants. Saliva was collected in the morning at least 2 h after the last intake of food. CA15-3 and IL-1β expressions were measured in saliva and serum using the enzyme-linked immunosorbent assay technique.

Results
The present results indicated that there were significant differences ($P<0.05$) in the expression of CA15-3 between patients with breast cancer and healthy individuals. Moreover, a significant difference was found in the expression of IL-1β between patients with breast cancer and healthy individuals in both serum and saliva samples. There were higher expressions ($P<0.05$) of CA15-3 in saliva than in serum as well as a higher expression ($P<0.05$) of IL-1β in saliva than in serum.

Conclusion
Saliva can be used as a diagnostic tool in early detection of breast cancer with high accuracy in comparison with serum.

Keywords:
breast cancer, salivary biomarkers, cancer antigen 15-3 and interleukin-1β

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Introduction

In the past few years, liquid biopsy became of great interest in the early detection of cancer, identifying recurrent disease and monitoring therapeutic resistance. Liquid biopsy techniques allow the detection of tumor biomarkers in blood, saliva, and other body fluids [1,2]. These tumor biomarkers will allow the diagnosis of cancer before any clinical symptoms and/or radiological changes appear. It may also help in detecting any residual tumor and their clinicopathological significance [3,4]. Lately, saliva has evolved as one of the liquid biopsies used for diagnosis and prognosis of tumors [5,6].

Saliva is a combined body fluid that is clear and slightly acidic; it is secreted from salivary glands into the oral cavity. It consists of proteins, DNA, mRNA, microbiota, and metabolites [2]. Salivary biomarkers have been detected in different types of cancers such as head and neck [7], salivary gland [8], oral [9], lung [10], ovarian [11], breast [12], and gastric cancers [13]. Several studies have identified many salivary biomarkers, which opened a way in the field of salivary diagnostics [14,15].

Owing to its ease and noninvasive convenience, along with its plenty of biomarkers such as genetic material and proteins, saliva has been broadly studied as a possible diagnostic tool recently. Saliva has many advantages in diagnosis as it is noninvasive, and its collection process is rapid and easy with high potential for diagnosis. In addition, saliva is used in monitoring the disease as it provides dependable clinical data and cellular information related to the biologically active molecules that are used to monitor the disease process

[16,17]. There are ~27% of proteins in saliva common with blood plasma, but when compared with blood or tissue biopsies, storage of saliva is easier, and it can be collected periodically without annoying the patients, so it could be a dependable way to check tumor markers and monitor the effectiveness of treatment [18,19].

One of the most common types of cancer in women is breast cancer, which has high mortality rate worldwide. However, its early diagnosis offers better therapy protocol. Moreover, late diagnosis is associated with delayed stage of the disease and poor prognosis. Recently, the mortality rate of breast cancer has decreased, owing to the early diagnosis and the modified treatment protocol using chemotherapy, target therapy, and hormonal therapy. Thus, detecting tumor markers, which are substances present in the circulation of patients having malignant tumors, could help in all stages of cancer treatment [20].

Cancer antigen 15-3 (CA15-3) is used to monitor response to breast cancer treatment and disease recurrence in saliva of patients with breast cancer [20]. CA15-3 represents an immunodominant epitope in the extracellular part of the protein. It is detected in benign and malignant breast ductal epithelium It is one of the glycoprotein family encoded by the mucin 1 cell surface-associated gene [21].

CA15-3 is shed into the blood and can be detected by many monoclonal antibodies. CA15-3 is one of the most commonly used serum marker to detect breast cancer and monitor treatment of patients. Several studies have detected that the diagnostic levels of CA15-3 have a significance in the outcome in early diagnosed patients with breast cancer [22].

Cytokines regulate body responses as differentiation, proliferation, immune activation, and cell death. They are synthesized by stromal and immune cells. Tumor cells activate cytokines to induce cell migration and transformation, which are essential processes for tumor formation and metastasis [23,24].

Dysregulation of interleukin-1 (IL-1) is associated with tumorigenesis and tumor progression, and its increase is associated with many tumors [25]. The increase in IL-1 agonists (IL-1α and IL-1β) enhances tumor invasion and metastasis [26]. Various cell types within a tumor can produce and secret IL-1β as cancer cells or immune cells. IL-1β is well known as a potent promoter of carcinogenesis [26,27].

The aim of this study was to detect the expression of serum and salivary CA15-3 and IL-1β in patients with breast cancer and healthy individuals and to assess the accuracy of these salivary biomarkers in diagnosing breast cancer versus serum.

Patients and methods

Patients

Patients with breast cancer and healthy individuals were selected from El Demerdash Hospital, Cairo, Egypt. Inclusion criteria were patients with recent proven histopathologic diagnosis of breast cancer and did not receive any treatment yet. The patients should not be pregnant or lactating, should not have any active oral/dental disease or previous neoplasia, and should have no alterations of renal function, congestive heart failure, active infection hepatitis, or HIV. Healthy asymptomatic patients were selected without any breast diseases history or detectible abnormalities by self-examination. However, the exclusion criteria were any case with benign tumors or already treated, and in the healthy group, anyone with breast disease history and detectible abnormalities by self-examination.

Sample size calculation

The sample size was calculated depending on a previous study [28] as a reference. According to this study, the minimally accepted sample size was 16 per group, when the response within each patient group was normally distributed with SD of 1.08, the true mean difference was 1.09, when the power was 80%, type I error probability was 0.05.

Study design

This study was conducted on two groups of patients. The first group (breast cancer group) consisted of 26 patients with breast cancer from El Demerdash Hospital, Cairo, Egypt, and the second group (control group) consisted of 16 healthy asymptomatic women. Patients and healthy volunteers were asked to sign a consent form approved by the Medical Research Ethics committee. Patients were selected in the age range from 18 to 58 years.

Ethical approval

The present study was conducted with the Code of Ethics of the World Medical Association, according to the principles expressed in the Declaration of Helsinki.

This study has been approved by the local ethics committee of National Research Centre, Cairo, Egypt with approval number 13132032021. A written informed consent was provided by each participant before their inclusion in the study.

Methods

Blood samples

An aliquot of 5 ml of venous blood was taken from each individual under complete aseptic conditions and kept in the refrigerator as described in previous reports [29]. After clotting, the specimen was centrifuged at 5000 rpm to separate serum. Sera was stored at freeze (−80°C) until use.

Saliva sample

Saliva samples were collected from all patients before the administration of any adjunct therapy or surgery. The collection protocol has been described in previous reports [30,31]. In brief, unstimulated saliva was collected in the morning at least 2 h after the last intake of food. The mouth was rinsed three times with physiological saline immediately before the beginning of the collection and then, the patients were requested to swallow first, tilt their head forward and then expectorate all saliva into sterile centrifuge tube for 5 min without swallowing. The samples were centrifuged at 5000 rpm for 20 min. The clarified supernatants were drawn off and immediately frozen at −80°C until use in the enzyme-linked immunosorbent assay (ELISA).

Cancer antigen 15-3 and interleukin-1β quantification

The quantification of the markers was done using ELISA. The ELISA kits of CA15-3 and IL-1β were purchased from Sunlong Biotech (Zhejiang, China) with catalog number (SL0383Hu and SL0984Hu, respectively). Determination of the level of the selected markers was carried out according to the manufacturers' instructions. In brief, after preparing all needed buffers and reagents, serum and saliva samples was added to microwells and then antibodies of the selected markers were added according to the manufacturer's instruction and then the reaction was ended by adding a stop solution. Subsequently, absorbance was read on an ELISA reader.

Statistical analysis

All data were expressed as mean±SD. Data were analyzed by SPSS 16 (Statistical Package for Scientific Studies, IBM, Chicago, IL, USA), GraphPad prism, Microsoft Excel to correlate between the level of the markers in serum and saliva. Differences among means were considered statistically significant at *P* value less than 0.05. Correlation between serum and saliva was performed using Pearson's correlation coefficient.

Results

In patients with breast cancer, the expression of CA15-3 in saliva and serum was higher than its expression in saliva and serum of healthy individuals, and also the expression of IL-1β in saliva and serum of patients with breast cancer was higher than healthy individuals, where the serum level of CA15-3 in the breast cancer group was 24.53±6.24, whereas in the control group was 8.62±0.84, and the saliva level of CA15-3 in the breast cancer group was 29.74±6.14, whereas in the control group was 8.74±0.66, as presented in Table 1. Moreover, the serum level of IL-1β in the breast cancer group was 31.93±5.55, whereas in the control group was 18.73±2.95. Moreover, the saliva level of IL-1β in the breast cancer group was 37.26±7.37, whereas in the control group was 19.28±3.37, as presented in Table 2.

Exploration of the given data was performed using Shapiro–Wilk test and Kolmogorov–Smirnov test for normality, which revealed that the significance level (*P* value) was insignificant, as *P* value more than 0.05, which indicated that alternative hypothesis was rejected, and the concluded data originated from normal distribution (parametric data) resembling a normal Bell curve. Moreover, comparison between two different groups was performed by using independent *t* test.

Table 1 Cancer antigen 15-3 markers in serum and saliva samples among control and breast cancer groups

	Breast cancer (*N*=26)	Controls (*N*=16)	*P* value
Serum	24.53±6.24	8.62±0.84	<0.001a
Saliva	29.74±6.14	8.74±0.66	<0.001a
P value	0.020b	0.65	

All data are expressed as mean±SD. [a]Significant difference between control and breast cancer group at *P* value less than 0.05, using independent *t* test. [b]Significant difference between serum and saliva at *P* value less than 0.05, using independent *t* test.

Table 2 Interleukin-1B marker in serum and saliva samples among control and breast cancer groups

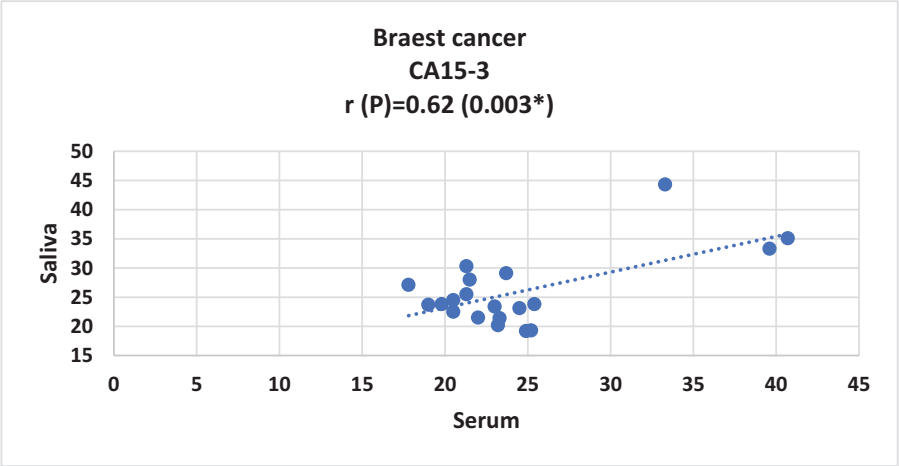
	Breast cancer (*N*=26)	Controls (*N*=16)	*P* value
Serum	31.93±5.55	18.73±2.95	<0.001a
Saliva	37.26±7.37	19.28±3.37	<0.001a
P value	0.010b	0.620	

All data are expressed as mean±SD. [a]Significant difference between control and breast cancer group at *P* value less than 0.05, using independent *t* test. [b]Significant difference between serum and saliva at *P* value less than 0.05, using independent *t* test.

Comparison between both groups revealed that the breast cancer group was significantly higher than the control group, as *P* value less than 0.05. In the breast cancer group, comparison between serum and saliva revealed that saliva (29.74±6.14) was significantly higher than serum (24.53±6.24) regarding CA15-3, as *P* value less than 0.05. However, in the control group, comparison between serum and saliva revealed insignificant difference between them, as *P* value more than 0.05, as presented in Table 1. Moreover, in the breast cancer group, comparison between serum and saliva revealed that saliva (37.26±7.37) was significantly higher than serum (31.93±5.55) regarding IL-1β, whereas in the control group, the comparison between serum and saliva revealed insignificant differences between them, as *P* value more than 0.05, as presented in Table 2.

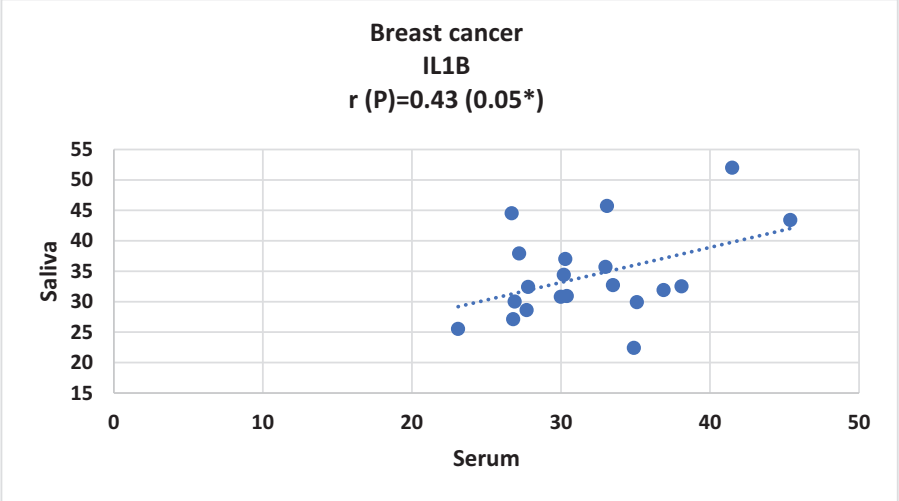
Pearson's correlation coefficient was performed to investigate the relation between serum and saliva regarding CA15-3 expression, and it revealed a moderate, positive significant correlation in the breast cancer group, as presented in Fig. 1. The test was done as well regarding IL-1β expression, revealing a moderate, positive significant correlation in the breast cancer group, as presented Fig. 2.

Figure 1



Correlation between serum and saliva CA15-3 expression in the breast cancer group as determined by Pearson correlation coefficient. CA, cancer antigen.

Figure 2



Correlation between serum and saliva IL-1β expression in the breast cancer group as determined by Pearson correlation coefficient. IL, interleukin.

Discussion

Salivary biomarkers are considered a promising approach in the efficiency of detecting multiple types of cancer [32–34]. Cancer cells cause the secretion of specific tumor biomarkers, and these biomarkers can be used in detecting tumors or monitoring the status of the tumor [35,36].

The sampling of saliva is easier than serum, and when needed, it can be sampled repeatedly by the patient himself and can be stored at home. Moreover, the clinical assessment of salivary biomarkers are technically easier, which may be owing to its low fat content [37–39].

Previous studies have stated that unstimulated saliva was used in the detection of the biomarkers because it does not have any change in composition and concentration of the detected tumor biomarkers [40,41].

This study was formulated to study the accuracy of salivary biomarkers in the diagnosis of patients with breast cancer. Our results detected the expression of tumor biomarkers (CA15-3 and IL-1β) in the serum and saliva of patients with breast cancer. The biomarkers were expressed significantly highly in patients with breast cancer than the healthy individuals.

Similar studies detected these markers in early stages of breast cancer and found significant difference between patients with breast cancer and healthy volunteers [16,42,43]. Regarding the detection of CA15-3 in patients with breast cancer, a study by Laid *et al.* [44] found no significant difference in CA15-3 between patients with breast cancer and healthy individuals. The cause of this conflict may be the difference in methodology, sample size, and using nonstandardized assays [44–46].

CA15-3 is the most reliable marker of breast cancer. The mechanism by which CA15-3 is expressed in saliva may be the active transport of proteins by salivary glandular epithelium, and this explain the presence of large molecules of membrane-bound proteins in the saliva [36]. This large transmembrane glycoprotein is overexpressed glycosylated in cancer [46]. So, it is used as serum marker to diagnose breast cancer and monitor treatment [39].

Our results reveal a significant difference in the expression of CA15-3 between saliva and serum of patients with breast cancer. The level of CA15-3 was expressed in saliva significantly higher than in serum.

Our results are in accordance with similar studies, which assessed the correlation between saliva and serum CA15-3 and detect them in patients with breast cancer and healthy ones. The authors found a significant difference between the salivary and serum CA15-3 in diagnosing breast cancer and allowing the use of saliva in initial detection [47]. Another study assessed the expression of CA15-3 in patients with breast cancer, patients with breast benign tumor, and healthy individuals. They found elevated CA15-3 expression in patients with breast cancer patients than in patients with benign tumor and healthy individuals [48]. Fatna *et al.* [28] suggested that saliva could be used in monitoring breast cancer as an alternative to serum. Another study found significantly higher level of CA15-3 in different subtypes of breast cancer and suggested the future use of CA15-3 in clinical trials [49]. A moderate correlation was found by Daniele *et al.* [50] between serum and salivary CA15-3 in patients with breast cancer using ELISA.The study by Laid *et al.* [44] found no significant difference in CA15-3 between patients with breast cancer and healthy individuals, but they found a statistically significant difference between saliva and serum levels of CA15-3 in patients.

In patients with breast cancer, there are overexpressed bioactive proteins due the accelerated and abnormal growth of the tumor, and this may affect the salivary glands and saliva in turn [51].

IL-1β is a cytokine that is secreted in association with cancer progression. It can act alone or in association with other cytokines in inflammation. It is released upon activation of inflammasome, which is a protein pathogen complex [52–54].

Regarding IL-1β expression, our study reveals a significant high expression of IL-1β level in the breast cancer group than in the control group as well as in saliva more than serum of patients with breast cancer, and this agreed with other studies [41,55,56], which stated that IL-1β expression is increased in 90% of invasive breast carcinoma and that it is localized to both tumor cells as well as stromal cells.

In addition, other studies indicated that elevated IL-1β levels in breast cancer are associated with more aggressive phenotype and high tumor grade. Moreover, IL-1β is an important mediator of the severe phase reaction to infections and tissue

damage. These findings show that inflammasome mediators such as IL-1β play an important role in the progression of breast cancer [16]. Our results may confirm the diagnostic accuracy of saliva and its use as a useful tool in the diagnosis of breast cancer.

Conclusion

The present study supports the utility of CA15-3 and IL-1β as diagnostic salivary biomarkers for breast cancer and confirms their accuracy in diagnosis. In addition, this study confirmed that saliva can be used as a diagnostic tool in early detection of breast cancer with high accuracy in comparison with serum.

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Nil.

Conflicts of interest

The authors have no competing interests to declare that are relevant to the content of this article.

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