

RESEARCH ARTICLE

Significant Correlation between Salivary and Serum Ca 15-3 in Healthy Women and Breast Cancer Patients

Fatna Laidi^{1*}, Amal Bouziane², Amina Lakhdar³, Samira Khabouze³, Mariam Amrani⁴, Brahim Rhrab³, Fatima Zaoui¹

Abstract

The tumor marker CA 15-3 is one of the most important reliable for metastatic breast cancer monitoring. While it is generally assessed in serum of patients, blood sampling is an invasive method compared to saliva sampling which is simple and could be an alternative to blood according to many studies. The aim of this investigation was to assess the relationship between serum and salivary concentrations of the protein CA 15-3 in patients with breast cancer and healthy asymptomatic volunteers. A case-control study was conducted with 60 women: 29 breast cancer patients from the Maternity Hospital Souissi Rabat (Morocco) and 31 healthy asymptomatic women. The CA 15-3 concentrations in saliva and serum samples were assessed using an enzyme immune assay (EIA kits) and comparison between cases and controls was made by the Mann-Whitney test. The correlation between serum and saliva CA 15-3 concentration was tested using Pearson correlation. The comparison result of CA 15-3 concentration in saliva and serum level in cases and controls was not statistically significant ($p > 0.05$). However, the correlation between salivary and serum CA 15-3 concentration was positive and statistically significant ($r = 0.27$, $p = 0.03$). In conclusion, the positive correlation between salivary and serum expression found in our study suggests that saliva could be an alternative to blood sampling to help breast cancer monitoring.

Keywords: Breast cancer - tumor markers - CA 15-3 - serum - saliva - diagnostic approaches

Asian Pac J Cancer Prev, 15 (11), 4659-4662

Introduction

Breast cancer is the most common cancer and the second cause of mortality in women worldwide (Jemal et al., 2011; Begum et al., 2012; Ray, 2012). Early diagnosis allows better therapy choice (Mcintyre et al., 1999). In addition, delayed diagnosis often involves an advanced stage of the disease with poor prognosis (Tjemslanda and Soreide, 2004). Breast cancer mortality level decreased and this has been attributed to: the early diagnosis (characterization of prognosis and diagnosis factors) and the treatment with adjuvant chemotherapy, hormonal therapy and target therapy (Xiaoqiang, 2013). Despite the advanced testing methods, like mammography, these procedures may produce a large percentage of false positives and false negatives, especially in women with dense breast tissues (Bigler et al., 2002; Dilhuydy, 2009; Tarhan et al., 2013). Consequently, studies have big interest in tumor markers, substances detected in the circulation of patients with malignant tumors, which could be used in all stages of cancer care (screening, diagnosis, treatment and metastasis prediction) (Porika et

al., 2010; Maric et al., 2011). CA 15-3 is a good example of these markers (Frenette et al., 1994). Several studies have shown that this marker and others are present in saliva. Thus, its use as a diagnostic fluid could have many advantages comparing to serum: saliva collection is noninvasive, simple, can be collected repeatedly without discomfort of patients and could be a reliable way to detect tumor markers and thus contribute to monitoring the effectiveness of chemotherapy (Navazesh and Christensen, 1982; Streckfus and Bigler, 2005).

The aim of this study was to assess the relationship between serum and salivary concentration of the protein CA 15-3 in patients with breast cancer and healthy asymptomatic volunteers.

Materials and Methods

Study design

This case-control study was conducted with 2 groups of women. The first group consisted of 29 patients with breast cancer from the Maternity Hospital Souissi Rabat (Morocco) and the second consisted of 31 healthy

¹Oral Biomechanics and Biotechnology Research Unit, Faculty of Dental Medicine, ²Department of Periodontology Faculty of Dental Medicine, Biostatistical, Clinical and Epidemiological Research Laboratory, Faculty of Medicine and Pharmacy, Mohammed 5th Souissi University, ³Department of Obstetrics and Gynecology, Ibn Sina University Hospital Rabat, ⁴Pathology Department, National Institute of Oncology, Rabat, Morocco *For correspondence: laidi.fatna@gmail.com

asymptomatic women selected by a questionnaire. Patients and healthy volunteers were asked to sign a consent form and answer a short questionnaire before the test. The study protocol was approved by a local ethics committee for biomedical research (CERB).

Inclusion criteria

Patients: with breast cancer, candidate to mastectomy, or in chemotherapy session and without metastasis.

Volunteers: without breast diseases history or detectible abnormalities by self-examination and matched with the age of patients.

Exclusion criteria

Patients: with benign tumors or already treated.

Volunteers: with breast diseases history and detectible abnormalities by self-examination.

Blood and saliva sampling

Peripheral venous blood and stimulated saliva, by chewing gum, were taken simultaneously in the morning from each participant. Participants were refrained from eating, drinking, or smoking for at least 2 hours before the test (Navazesh and Christensen, 1982; Navazesh, 1993).

Each participant had to rinse her mouth several times and seat 5 minutes before collecting 5ml of complete stimulated saliva in plastic cups. The peripheral venous blood was collected in 5ml serum tube.

Blood and saliva samples were then centrifuged at 2000rpm for 10min. Then the aliquoted sera and saliva supernatant were stored at -80°C for later determination of CA 15-3.

CA 15-3 quantification

The quantification of the marker CA 15-3 was done using enzyme immune assay (EIA kits) (Affymetrix Panomics, Southern CA, USA). This kit is designed to quantify the serum CA15-3 concentration.

CA 15-3 protein was detected by a sandwich reaction (protein trapped between two antibodies), the first is attached to the ELISA plate and the second is conjugated to the enzyme horseradish peroxidase. After washing and addition of the enzyme substrate, the color development is proportional to the concentration of CA 15-3 in saliva and serum, the absorbance was then measured by a spectrophotometer at 450 nm. After each test the CA 15-3 concentration in samples was deduced from a standard curve constructed using the Excel software by the TREND function.

Statistical analysis

Statistical analysis was performed by the software SPSS Version 13.0 (Chicago; USA). Variables: CA15-3 concentration and the age of participants and duration of menopause were expressed in median and interquartiles, the age was expressed in mean and standard deviation and the toxic habits and hormone usage were expressed in percentage. The comparison between cases and controls was made by the Mann-Whitney test. The correlation between serum and saliva CA 15-3 concentration was tested using Pearson correlation. A p <0.05 was considered

statistically significant.

Results

The data collected from cases and controls included information on age, toxic habits (smoking and alcoholism), menopause and hormone usage (HRT, oral contraceptives) is summarized in Table 1.

The comparison of CA15-3 concentration in saliva and serum level is summarized in Table 2. There was no significant difference between serum CA 15-3 in patients comparing with healthy women p=0.13. Also, the

Table 1. Demographic Data Based on Participants Questionnaire

	Women with breast cancer n=29	Healthy women n=31
Age M(SD)	47.24±9.52	43.45±14.72
Toxic habits (%):		
<Smoking	0	3.22
<Alcoholism	0	0
Menopause (%)	41.4	32.3
Duration of menopause M(IQ)	0(0;1.5)	0(0; 5)
Hormone usage (%)		
Oral contraceptives	65.5	54.8
Hormone replacement therapy	0	0

*M: mean; SD: standard deviation

Table 2. Serum and Salivary CA 15-3 Concentration Based on Presence or Absence of the Disease

	Women with breast cancer n=29	Healthy women n=31	p
Serum CA 15-3 U/ml M(IQ)	13.7(9.91; 16.52)	16.7(8.84; 24.12)	0.13
Salivary CA 15-3 U/ml M(IQ)	4.77(1.21; 12.26)	2.71 (1.18; 7.69)	0.32

*M: median; IQ: interquartiles

Table 3. Serum and Salivary CA 15-3 Concentration Based on Lymph Node Status

	Healthy women + tumors with negative lymph node status (n=31+11)	Tumors with positive lymph node status	p
Serum CA 15-3 U/ml M(IQ)	15.5(8.24; 20.27)	13.9(10.96; 17.64)	0.9
Salivary CA 15-3 U/ml M(IQ)	2.6(0.86; 6.69)	5.29(1.81; 17.86)	0.1

*M: median; IQ: interquartiles

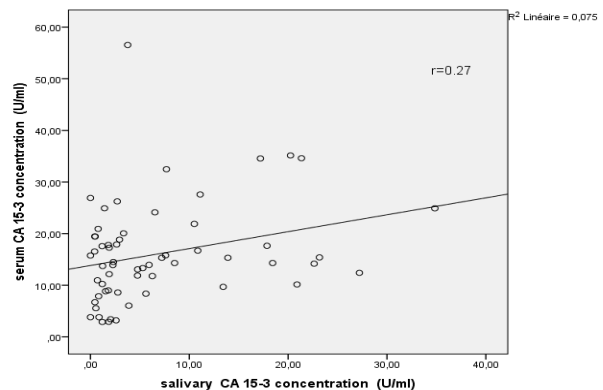


Figure 1. Correlation Curve of Salivary and Serum Marker CA 15-3 (r=correlation coefficient)

difference between the salivary CA 15-3 concentration of cases and controls was not significant ($p=0.32$).

The CA 15-3 concentration in saliva and serum level according to the lymph node status did not show significant difference. The serum and saliva CA 15-3 concentration in women with positive and negative lymph node statue was respectively $p=0.9$ and $p=0.1$ (Table 3).

Discussion

In our study, the data found, did not reveal a significant difference in the early stages of the breast cancer. Considering saliva and serum CA15-3 concentration in healthy women and patients, the difference was not statistically significant. In addition, the CA 15-3 concentration in serum and saliva according to the lymph node status, the extension of the breast cancer, did not show difference between patients with positive status and healthy volunteers considered as negative statue additionally to patients with negative status. This data is opposite to the results found in similar studies (Colomer et al., 1989; Streckfus et al., 1999; Agha-Hosseini et al., 2009) whom found a significant difference in the early stages between patients and healthy volunteers. However, a positive and significant correlation was found between the salivary and serum CA 15-3 concentration ($r=0.27$, $p=0.03$). The correlation found in our data is low comparing to the moderate correlation found in previous works (Streckfus et al., 1999; Agha-Hosseini et al., 2009).

The use of saliva for the assay represents many advantages. From a clinical point of view, the assay of molecules in saliva is technically easier. This is due to its low fat content and harmful molecules found in the plasma (Lac, 1998). In addition, saliva sampling is easier than blood collection especially when volunteers are invited to make samples collection repeatedly. Saliva is simple noninvasive and can be taken by the person itself and stored at home (Lac, 1998; Hofman, 2001).

In practice, salivary markers represent a promising approach for its detection efficiency of many diseases like multiple cancer types (Streckfus and Bigler, 2005; Mandel, 1993). This is the case of CA 15-3, it is considered the most reliable marker in breast cancer. The expression of CA15-3 increases significantly in most breast carcinomas (Frenette et al., 1994; Toth et al., 2008; Moazzezy et al., 2014). It was also shown that the rate of CA15-3 is correlated with tumor size, which reflects the stage of the disease (Clinton et al., 2003; Nicolini et al., 2008; Atoum et al., 2012; Zhang et al., 2013).

Our data may confirm the possibility of using saliva as diagnostic fluid. However the sample size and the lack of metastatic cases in our work limit this confirmation. Investigations in the future should include a large number of patients and healthy women additionally to metastatic cases. Also, the use of CA 15-3 in saliva for early diagnostic to monitor breast cancer must be associated to other tumor-markers then assessed by advanced technology like biosensors (multi-protein chips) known by their performance and precision and this in order to determine the upper limit of normal range.

In conclusion, the positive correlation between salivary

and serum expression found in our study suggests that saliva could be an alternative to blood for monitoring breast cancer or help breast cancer monitoring.

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