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Correlation of serum and salivary CA15-3 levels in patients with breast cancer

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Abstract

Objectives: The aim of this study was to assess the relationship between serum and saliva levels of cancer antigen (CA) 15-3 and to compare them between women with and without breast cancer. Study design: A case-control study was carried out on 61 women aged 28-69 years, including women with and without breast cancer (26 as part of the case study and 35 as part of the control group) conducted at the Emam Khomeini Hospital, Tehran University of Medical Sciences. CA15-3 levels were assayed in serum and unstimulated whole saliva by EIA. Unpaired t-test, one-way ANOVA and Pearson correlation were used as statistical analysis. Results: The salivary and serological levels of CA15-3 in the cancer patients were significantly higher ($P < 0.01$) than the salivary and serum levels of healthy controls. They were also higher in stage 2 than in stage 1 in cancer patients. However, the saliva flow rate was significantly lower in the cancer patients ($P < 0.01$). There was a significant positive correlation between serum and saliva CA15-3 concentration ($r = 0.614$) and also between serum concentration and saliva output of CA15-3 ($r = 0.541$). Conclusion: The results of the study suggest that salivary CA15-3 may have potential use in the initial detection of breast cancer in women.

Key words: *Unstimulated whole saliva, serum, CA15-3, breast cancer.*

Introduction

Breast cancer is the most common type of cancer occurring in women. Approximately 1 woman in every 10 will develop breast cancer in her lifetime (1). Therapy for advanced breast cancer has not improved significantly in recent years, remaining strictly palliative in nature and intent (2). The diagnosis of breast cancer at an earlier stage allows a woman more choice in the selection of treatment option (3).

While physical examination and mammography are useful screening procedures for the early detection of breast cancer, they are also labor intensive and require health professionals who are highly trained and experienced. Also, despite efforts to provide accurate diagnosis, these screening procedures can produce a substantial percentage of false positive and false negative results, especially in woman with dense parenchymal breast tissue (1).
Tumor markers are substances identified in the circula-

tion of patients with malignant disease, which may be used in diagnosis (early detection and differential diagnosis), prognostic evaluation and follow–up (therapeutic monitoring and diagnosis of recurrence) (4).

CA 15-3 is a mucinous glycoprotein that has been detected in elevated amounts in serum of women with breast cancer. Longitudinal studies of healthy woman have demonstrated the statistical significance of this marker to monitor breast cancer patients (3).

Several studies show that cancer markers often detect metastasis before a clinical manifestation (5,6). It has been determined that a number of tumor markers are present in saliva therefore its use as a diagnostic fluid could have significant diagnostic and logistical advantages when compared to serum (1). As diagnostic medium, saliva has several advantages , including collection of saliva is safe , non invasive , and simple , and it may be collected repeatedly without discomfort to the patient, and could also be a cost-effective means to monitor the effectiveness of chemotherapy (1,7).

The aim of this study is to assess the relationship between serum and saliva levels of cancer antigen (CA) 15-3 and compare them between women with and without breast cancer.

Materials and Methods

- Subjects:

Sixty one women participated in a case–control study, conducted at the Emam Khomeini Hospital, Tehran University of Medical Sciences from summer 2007 to summer 2008. Case group (n=26) included women with untreated breast carcinoma (mean age 42.6, ranging from 31 to 63 years) who were candidate for surgery of breast cancer. An oncologist and physician approved the breast tumor and its stage after biopsy. None of case group was metastatic breast cancer. Control group included 35 healthy women (average age 43.6 ranging in age from 28 to 69 years). Majority of participants in control group were relative of patients in case group. Health status was determined by questionnaire, and each participant received a through physical examination and was evaluated for carcinoma of the breast by a physician. The groups were matched in age, menopausal status, duration of menopause and contraceptive usage.

All participants were administered a brief questionnaire at the time of signing consent form. These data were collected by interview and included information concerning age, tobacco usage, and pharmacological and medical histories (Table 1).

The Ethics Committee of TUMS, Iran, approved the study protocol. Informed consent was obtained from all participants.

- Sample collection:

Venous blood and unstimulated whole saliva were collected simultaneously from each participant in the morning. Unstimulated whole saliva was collected by the spitting method. All subjects refrained from eating, drinking and smoking for 2 hours prior to the sampling. Each individual rinsed her mouth thoroughly with water, was seated for approximately 5 minutes, before 5 ml of unstimulated saliva was collected into a plastic cup over a period of about 15 minutes.

Blood specimens were obtained by venipuncture, collected in 10 ml glass vacuum tubes without additive, and allowed to clot. Then blood and saliva were centrifuged (2000 rpm, 10 min) and the serum and supernatants of saliva were separated. Immediately after collection of saliva and serum, the specimens were stored at -70 °C for later determination of cancer antigens CA15-3.

The saliva-filled cups were weighed and the weight of the cups subtracted. The flow rate was calculated in g/min, which is almost equivalent to ml/min (8). Saliva CA15-3 output was calculated as saliva CA15-3 concentration multiply saliva flow rate.

- CA15–3 assays:

CA15-3 tests were performed by using a two-site solid phase enzyme immunoassay (EIA) kits (CanAg Diagnostics AB; Gothenburg, Sweden). The molecules of CA15–3 are "sandwiched" between two monoclonal antibodies. The first one is attached to the ELISA solid phase and the second one linked to the horseradish peroxidase (enzymatic conjugate). After washing, the enzymatic reaction develops a color proportional to the amount of CA15–3 present in the assay. Absorbance is read at 405 nm (horseradish peroxidase) using a spectrophotometer and the concentration are calculated from a standard curve constructed from known concentrations of the ligand. The CA15-3 EIA kit is designed to assay serum specimens.

- Statistical analysis:

Statistical analyses were performed using the SPSS statistical software package. The data are presented as a mean ± SEM. Comparisons between the case and control groups were made with the unpaired t-test. One-way ANOVA (analysis of variance) followed by Student-

Table 1. Demographic data obtained from the questionnaires for the two groups of women.

	Healthy women	Women with breast cancer
n	35	26
Age (years, mean (range))	43.6(28 - 69)	42.6 (31 - 63)
Tobacco usage (n)	0	0
Menopause (n)	16	13
Duration of menopause (range years)	1-13	1-12
Contraceptive usage (n)	9	6

Newman-Keuls post-hoc test was performed when comparisons between the three groups were made. The Pearson correlation analysis was used to identify any correlation between serum and the salivary CA15-3 components. $P < 0.05$ was considered statistically significant.

Results

The mean unstimulated whole saliva flow rate, saliva CA15-3 concentration and its output and serum CA15-3 concentration are shown in Table 2. Student's t- test showed that unstimulated whole salivary flow rate was significantly higher in control group than case group ($P<0.01$), but serum CA15-3 concentration ($P<0.001$), saliva CA15-3 concentration ($P<0.001$) and salivary CA15-3 output ($P<0.01$) were significantly higher in case group.

A one-way ANOVA indicated that saliva flow rate [$F_{2,58}= 3.2$; $P<0.05$], serum CA15-3 concentration [$F_{2,58}= 88.3$; $P<0.001$], salivary CA15-3 concentration [$F_{2,58}= 23.5$; $P<0.001$] and its output [$F_{2,58}= 5.9$; $P<0.01$] were significantly differ between healthy women, women with breast cancer in stage 1 and in stage 2 (Table 2). Post-hoc analysis showed that saliva and serum CA15-3 concentrations were significantly higher in both women with breast cancer in stage 1 and 2 than healthy individuals and also higher in stage 2 than stage 1. Saliva flow rate and salivary CA15-3 output were not significant between stages of 1 and 2.

The correlations coefficients revealed a significant moderate association between serum and salivary CA15-3 concentration at $r = 0.614$ ($P < 0.001$; Fig. 1a). There was also a significant association between serum CA15-3 concentration and salivary CA15-3 output ($r = 0.541$, $P = 0.001$; Fig. 1b).

Table 2. Mean and SE values for saliva flow rate and salivary and serum CA 15-3.

	Healthy women (n=35)	Women with breast cancer (n= 26)	Women with breast cancer, Stage 1 (n=15)	Women with breast cancer, Stage 2 (n= 11)
Saliva flow rate (ml/min)	0.55±0.06	0.33±0.04 *	0.34±0.06	0.32±0.05 *
Serum CA15-3 (ng/ml)	8.68±0.51	18.56±1.94 *	12.49±1.27 *	28.67±1.91 *#
Saliva CA15-3 (ng/ml)	1.02±0.09	2.92±0.45 *	2.17±0.35 *	4.24±0.96 *#
Saliva CA15-3 output (ng/min)	0.55±0.07	0.94±0.15 *	0.77±0.13	1.27±0.36 *

* P<0.05 vs healthy women and # P<0.05 vs women with breast cancer in stage 1

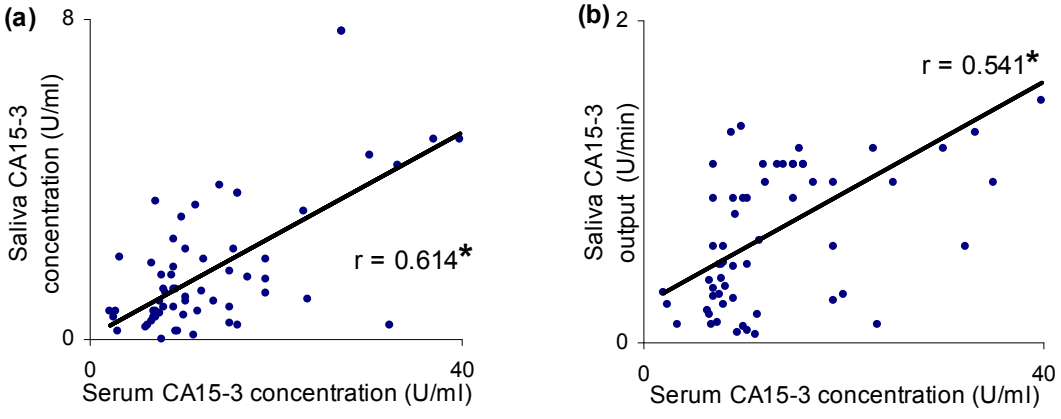

Fig.1. Relationship between serum and unstimulated whole saliva CA15-3 concentrations (a), and between serum CA15-3 concentration and saliva CA15-3 output (b) in women as determined by Pearson correlation coefficient, * P< 0.05.

Discussion

Saliva as a diagnostic specimen can give not only the same information as serum testing, but also additional or new information that cannot be obtained from serum (9). From a logistical perspective, the collection of saliva is safe, noninvasive, and simple, and it may be collected repeatedly without discomfort to the patient. Because of these significant characteristics, finding biomarkers in saliva for the detection of serious systemic illnesses, such as cancer, is of great interest for most salivary researchers (7,10)). CA15-3, a high-molecular-mass mucin-like glycoprotein, is expressed at the luminal surface of most secretary epithelial (11). Of the tumor markers examined in breast cancer, it is the best and the most extensively used one as its expression greatly increases in most breast carcinomas (12). It was also shown that levels of circulating CA15-3 correlated with tumor size, thus reflecting disease stage (13,14). Our data showed that saliva and serum levels of CA15-3 were significantly correlated with tumor stage. It is in agreement with other studies.

Our results also indicated that the mean serum CA15-3 was significantly higher in untreated breast cancer group than in healthy women. It is in agreement with other reports (4,12,15-19).

Our results also illustrated that the mean unstimulated whole saliva CA15-3 concentration and its output were significantly higher in breast cancer group than control women. These results confirm with the results of other reports about stimulated whole saliva CA15-3 levels in breast cancer (17,18). To our knowledge there was no report about the unstimulated whole saliva CA15-3 in breast cancer.

The serum concentration of CA15-3 revealed significant correlation with unstimulated whole saliva CA15-3 concentration and output. Results suggest that salivary CA15-3 may have a role in monitoring and in management of breast cancer.

The results of this study demonstrated that the unstimulated salivary flow rate of the healthy women was significantly higher than breast cancer group. As salivary flow rate decreased in stress, saliva flow rate decreasing may be due to the stress of cancer in women with breast cancer.

In conclusion, the results of this study show that there are positive correlations in CA15-3 between serum and unstimulated whole saliva levels, and salivary out put. It seems that salivary CA15-3 may have a role in monitoring and in management of breast cancer.

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