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Research Article

EVALUATION OF SERUM AND SALIVARY CA-125 IN BREAST CANCER PATIENTS – AN ANALYTICAL STUDY

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ABSTRACT

Objective: Breast cancer in females is very frequent nowadays both in developed and developing countries. Many tests both invasive and non-invasive are available for the diagnosis of cancer breast. Tumor markers are the substances that are associated with tumors and help in the detection and follow-up. Cancer antigen 125 (CA-125) is one of the tumor markers, the level of which increases in many tumors including breast cancer.

Methods: The present study was carried out to quantitatively measure its level by enzyme-linked immunosorbent assay in the serum and saliva of known breast cancer patients and comparing it with control groups.

Results: Statistically significant role of CA-125 in cases of breast cancer was ascertained over the control group and also positive correlation was found between serum and salivary values of CA-125.

Conclusion: We conclude that CA-125 can be used as tumor markers in breast cancer patients for evaluation and salivary samples are equally effective as serum samples and also are easy and safe to obtain.

Keywords: Cancer antigen 125, Breast cancer, Enzyme-linked immunosorbent assay, Tumor markers.

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INTRODUCTION

According to the World Health Organization at the end of 2020, breast cancer was the most frequent cancer worldwide affecting approximately 7.8 million women in the past 5 years [1]. There are many invasive and non-invasive pathological and radiological tests that are used to detect breast malignancy apart from physical examination and mammography that are used for early screening. Detection of tumor markers is one of the tests that is used to detect cancer in many organs of the human body. Tumor markers are substances that are identified in association with a variety of tumors and help in diagnosis, prognosis, and follow-up. Cancer antigen 125 (CA-125) or carbohydrate antigen 125 also known as mucin-16 or MUC-16 is a protein that in humans is encoded by the MUC-16 gene [2]. MUC-16 is a member of the mucin family glycoproteins. It has been detected in elevated amounts in the serum of women with breast cancer. Many studies have demonstrated the statistical significance of these markers in breast cancer patients mainly CA-15-3 and carcinoembryonic antigen (CEA) and that too in serum samples. In this study, we examined the serum and in addition saliva levels of CA-125 in breast cancer patients and compared serum and saliva levels of this antigen with the control group.

METHODS

It was a descriptive-analytic study of a 1-year duration carried out in a tertiary care institute in the capital city of Uttar Pradesh, India, after taking the approval of the institutional ethical committee and proper informed consent from the patients. All the females who came positive for breast malignancy on either fine needle aspiration cytology or histopathology were included in the study. After obtaining physical, clinical, and demographic data both serum and saliva samples were collected simultaneously. Patients were kept nil by mouth for 2 h and then unstimulated whole saliva was collected by spitting method in a sterile plastic container. A serum sample was collected in a serum tube. Both serum and saliva were kept at −20°C till final testing. The same protocol was applied to the control group that was matched with age and menopausal status.

Estimation of serum and saliva CA-125

It was done by quantitative sandwich enzyme-linked immunosorbent assay (ELISA).

Samples were diluted with distilled water as per protocol from the manufacturer and both samples and reagent were brought to room temperature before testing. The procedure for ELISA was followed manually on a microplate and one by one reagent was added, mixed, incubated, and washed with a wash buffer. Finally stopping reagent was added to stop the reaction marked by the development of yellow color. The optical density of each well on the microplate was determined by an ELISA reader at 450 nm.

Calculation of results

A standard curve was created by reducing the data using computer software capable of generating a four-parameter logistic curve fit. The corresponding concentration of CA-125 in U/mL was determined using the mean absorbance value for each sample from the standard curve.

Statistical analysis

The statistical analysis was done using Statistical Package for the Social Sciences Version 15.0 Statistical Analysis Software. The values were represented in Number (%) and Mean±SD.

The following statistical formulas were used:

Mean: To obtain the mean, the individual observations were first added together and then divided by the number of observations. The operation of adding together or summation is denoted by the sign Σ. The individual observation is denoted by the sign X, the number of observations is denoted by n, and the mean is by $\bar{X}$.

$$\overline{X}=\frac{\Sigma X}{No.of\ observations(n)}$$

Standard Deviation: It is denoted by the Greek letter Σ. If a sample is more than 30 then,

$$\sigma=\sqrt{\frac{\Sigma(X-\overline{X})^2}{n}}$$

When the sample in <30 then,

$$\sigma=\sqrt{\frac{\Sigma(X-\overline{X})^2}{n-1}}$$

The Kolmogorov–Smirnov test (Chakravati, Laha, and Roy, 1976) was used to decide if a sample comes from a population with a specific distribution. The Kolmogorov–Smirnov test is based on the empirical distribution function (ECDF). Given N ordered data points Y_1, Y_2, Y_3, Y_N, the ECDF is defined as

E_N=n (i)/N

Where n (i) is the number of points less than Y_i and Y_i is ordered from smallest to largest value. This is a step function that increases by 1/N at the value of each ordered data point.

RESULTS

A total of 39 women with different stages of breast cancer were enrolled in the study. The age-wise distribution showed that the majority of the female was in between 40 and 60 years of age (26 out of 39, 66.6%) while none of the females below 30 years of the age group was involved. (Table 1) 23 females (59%) were postmenopausal and 16 females (41%) were in the premenopausal state. (Table 2) analysis of CA-125 in 39 cancer patients and nine demographically matched controls showed the following results. Serum CA-125 levels ranged from 44 to 180 U/mL with a mean value of 83.68±33.54 U/mL. On the assessment of the distribution for normality using the Kolmogorov-Smirnov test, the distribution was observed to be symmetric and normal (p=0.200). Serum CA-125 level was significantly higher among cancer patients (83.68±33.54, p<0.0001) as compared to controls (6.92±2.39). In the control group, the serum CA-125 values ranged from 4.4 to 12 U/mL with a mean value of 6.92±2.39 U/mL. On checking the normality of the distribution using the Kolmogorov–Smirnov test, the data were found to be symmetric and normal (p=0.200) (Table 3). Saliva CA-125 levels ranged from 420 to 886 U/mL with a mean value of 550.13±109.24 U/mL. In the control group, the saliva CA-125 values ranged from 100.8 to 206 U/mL with a mean value of 191.5±27.09 U/mL. On checking the normality of the distribution using the Kolmogorov–Smirnov test, the data were found to be symmetric and normal (p=0.200). Saliva CA-125 level was significantly higher among cancer patients (550.13±109.24, p<0.0001) as compared to controls (135.95±34.21) (Table 4). There was a positive significant correlation (r=0.36, p=0.03) between serum CA-125 and saliva CA-125 among cancer patients. The regression equation revealed that if there will be one unit increase in serum CA-125, the saliva CA-125 increased by one unit (Fig. 1).

DISCUSSION

Many studies previously have been performed on quantitative assessment of tumor markers in cancer patients of breast specially CEA, and CA 15–3 [3-5]. CA-125 has been studied comparatively less as opposed to the above-mentioned tumor markers in breast cancer patients. The present study was done to evaluate the effectiveness of serum and saliva CA-125 as a tumor marker in known breast cancer patients by comparing its value with the control group and also to establish the diagnostic role of salivary CA-125 over serum CA-125. Various studies done previously suggested that CA-125 serum levels have a potential role as a diagnostic marker in breast cancer besides other gynecological malignancies such as ovarian cancer, endometrial cancer, fallopian tube cancer, lung cancer, and gastrointestinal cancer [6-9].

Saliva as a diagnostic specimen can give the same information as serum and also the collection of saliva is safe, non-invasive, and simple.

In the present study, we observed that majority of the females were in between 40 and 60 years of age group and also were postmenopausal.

Table 1: Distribution of patients by age

Age	Cancer patients (n=39)		Controls (n=9)	
	No.	%	No.	%
<30	0	0.0	0	0.0
31–40	8	20.5	2	22.2
41–50	13	33.3	4	44.4
51–60	13	33.3	3	33.3
>60	5	12.8	0	0.0
Mean±SD	49.51±9.36		47.00±7.23	

Table 2: Distribution of patients by menopausal status

Menopausal status	Breast cancer patients (n=39)		Controls (n=9)	
	Number	%	Number	%
Pre-menopausal	23	59.0	7	77.8
Post-menopausal	16	41.0	2	22.2

Table 3: Distribution of CA 125 in serum of cases and control

Serum CA 125 (U/mL)	Minimum	Maximum	Mean	SD	p-value
Cases	44	180	83.68	33.54	≤0.0001
Control	4.4	12	6.92	2.39	

Table 4: Distribution of CA 125 in the saliva of cases and control

Saliva CA 125 (U/mL)	Minimum	Maximum	Mean	SD	p-value
Cases	420	886	550.13	109.24	≤0.0001
Control	100.8	206	191.5	27.09	

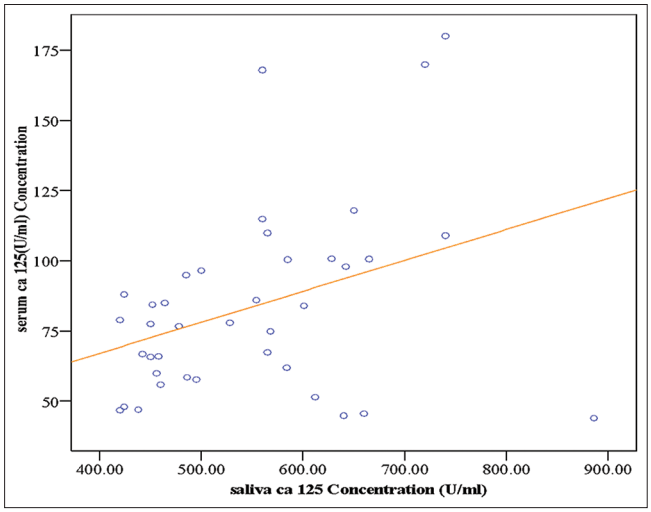

Fig. 1: Correlation between serum and salivary CA 125 levels. n=39, r=0.36, p=0.03

A similar result was reported by Agha-Hosseini *et al.* (2009) with a mean age of 42.6 years and 50% were post-menopausal [10].

In our study, serum and salivary CA-125 and saliva CA-125 were found to be significantly higher in cases of cancer breast with than in healthy controls. The difference was found to be statistically significant ($p<0.0001$). However, in different studies done previously, they did not get significant differences and this slight difference can be due to different races of study groups and differences in sample sizes [11,12].

We found a positive correlation between serum and salivary CA-125 levels and a modest positive correlation was also present in the study of Agha-Hosseini *et al.* [10].

CONCLUSION

CA-125 is one of the useful tumor markers for early detection of patients with carcinoma breast and its high levels in saliva provide easy, safe, and alternate non-invasive means to accurately screen these patients.

AUTHORS CONTRIBUTION

Each author has contributed in either research work or manuscript preparation.

CONFLICTS OF INTERESTS

There are no conflicts of interests.

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