Role of Biomarkers ALCAM and CA-15-3 in the Diagnosis of Breast Cancer: A Case - Control Study

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ABSTRACT

Introduction: Biomarkers can serve as an easy and non-invasive method for the diagnosis of breast cancer. They can also be useful in grading and follow-up of these patients. The present study was done to compare the levels of serum Activated leukocyte cell adhesion molecule (ALCAM), carbohydrate antigen 15-3 (CA15-3) and salivary CA-15-3 in breast cancer patients and healthy controls and to correlate the levels of these biomarkers with histological grade and nuclear morphometry of breast cancer patients.

Material and methods: This was case control study done at a tertiary care centre from November 2014 to June 2016. Newly diagnosed patients with a biopsy proven diagnosis of breast cancer and age matched healthy controls were included in the study. The serum levels of ALCAM and serum and salivary CA15-3 were determined by Sandwich Enzyme Linked Immunosorbent Assay (ELISA) with standard protocol. Human ALCAM (Qayee Bio) and the Calbiotech CA15-3 ELISA Kit were used for performing the ELISA. SPSS version 16.0 (Chicago, IL) was used for this purpose. Chi-square or fisher exact was used to compare qualitative variables. Mann-Whitney U test/ Kruskal-Wallis test were used to compare continuous variables as applicable.

Results: The levels of biomarkers serum ALCAM (P<0.001), CA-15-3 (P<0.001) and salivary ALCAM (P<0.001) were significantly higher in the breast cancer patients as compared to the healthy controls. The receiver operation characteristic (ROC) curve showed that these biomarkers have a good accuracy in the diagnosis of the breast cancer. There was a significant positive association between grade of the disease and serum ALCAM levels (P=0.005)

Conclusion: These serum and salivary biomarkers may be useful in the diagnosis of breast cancer. These biomarkers especially serum ALCAM can also predict disease severity. These findings needs to be replicated in larger population.

Keywords: Breast Cancer, Biomarkers, ALCAM, CA-15-3

INTRODUCTION

Breast cancer is amongst the most common cancers in females and it is a major cause of death and disability globally. The definite diagnosis of breast cancer requires biopsy and Histopathological examination. A serum biomarker may be an easier method to make the diagnosis; biomarkers may also be useful during follow up of patients with cancer. Tumor markers like carcinoembryonic antigen (CEA) and carbohydrate antigen 15-3 (CA15-3) have been used in the diagnosis of breast carcinoma since last 40 years. However these biomarkers have low sensitivity and specificity in detecting breast cancer in early stages.[1,2] Role of Activated leukocyte cell adhesion molecule (ALCAM) have been implicated in the genesis of cancer. ALCAM mediates cell to cell clustering through hetrophilic and homophilic interactions. ALCAM is expressed mostly in tissues involved in active growth or migration.[3] Recent evidence suggests that expression of ALCAM may reflect the onset of a cellular program for homeostatic control of growth saturation, which induces either growth arrest or cell migration.[3] Recently conducted studies have found that ALCAM can represent a potential biomarker for the diagnosis of breast cancer.[4,5] Diagnostic sensitivity of ALCAM for diagnosis of breast cancer was found to be good and comparable to CA15-3 and CEA.[5]

Our study was conducted with aims of estimating serum ALCAM levels and serum and salivary CA15-3 in breast cancer patients and to compare them with healthy controls. The other aim of the study was to correlate the levels of these biomarkers with histological grade and nuclear morphometry of breast cancer patients.

MATERIAL AND METHODS

This was a case-control study. The study was performed in a tertiary care institute located in northern India. The study duration ranged from November 2014 to June 2016. A written and informed consent was taken from all the study participants before enrolling them in the study. The study was approved by the institutional ethical committee. Newly diagnosed patients with a proven diagnosis of breast cancer and age matched healthy controls were included in the study.

Inclusion criteria

Consecutive patients with newly diagnosed cases of breast cancer were included in the study. The diagnosis of breast cancer was made on histopathological biopsy presenting in our department. Equal numbers of age matched healthy controls were also included in the study.

Exclusion criteria

Previously diagnosed and treated cases of breast lesions.

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Any patients with double malignancy, immunodeficiency diseases or any other associated chronic debilitating disorder which is likely to interfere with detection of marker, were excluded.

Evaluation

All the included patients underwent detailed history, clinical examination including local and systemic examination. Patients underwent routine haematological and biochemical investigations. We took blood sample from patients of breast cancer for analysis of marker ALCAM and CA15-3. The blood samples were centrifuged at 3000 rpm for 10 minutes to separate the serum. All the serum samples were stored at -20 ^{0} C until further analysis. We also took saliva sample from patients for assessment of marker CA15-3. Stimulated saliva was taken in morning from each participant. Participants were refrained from eating, drinking, or smoking for at least 2 hours before the test.[6] Participants had to rinse her mouth several times and sit 5 minutes before collecting 5 ml of complete stimulated saliva in plastic cups. After collection of saliva 2 drops of protease inhibitor was added and the sample was stored at -20^o C.

Biopsy

Morphometry analysis was done in H&E stained tissue sections. In morphometry we assessed the nuclear size and nuclear diameter.

Serum and salivary markers of breast cancer

The serum levels of ALCAM and serum and salivary CA15-3 were determined by Sandwich Enzyme Linked Immunosorbent Assay (ELISA) with standard protocol. Human ALCAM (Qayee Bio) and the Calbiotech CA15-3 ELISA Kit were used for performing the ELISA.

STATISTICAL ANALYSIS

Statistical analysis was done using SPSS version 16.0 (Chicago, IL, USA). All the categorical variables were expressed as percentages and continuous variables were expressed as median as well as mean ± standard deviation. Chi-square or fisher exact was used to compare qualitative variables. Shapiro-Wilk test was used to test whether the continuous variables were normally distributed or not. As most of the continuous variables were not normally distributed non parametric test Mann-Whitney U test/ Kruskal-Wallis test were used to compare continuous variables as applicable. Receiver operation characteristic curve (ROC) was plotted and area under the curve (AUC) was calculated for the biomarkers. Correlation between the biomarkers was studied using bivariate correlation and spearman correlation coefficient. All p values <0.05 were taken as significant.

RESULTS

Baseline characteristics of cases and controls

The baseline clinical and laboratory characteristics of 25 breast carcinoma patients are shown in Table-1. There was no significant difference in the age and sex distribution of cases and controls. The median age of breast carcinoma patients was 43 years and that of controls was 41 years (P=0.900). 24/25 (96%) of cases were females amongst the case, 100% of controls were females (P=0.312). Amongst the cases invasive ductal carcinoma was the most common diagnosis which was made in 18 out of 25 cases. Lymph node involvement was seen in 5 (20%) cases.

Comparison of biomarkers amongst case and controls

The levels of serum ALCAM was significantly higher amongst cases as compared to the controls (P <0.001). Serum and salivary CA-15-3 levels were also significantly higher amongst the breast carcinoma patients as compared to the healthy controls (P<0.001). The comparison of biomarkers amongst the two study groups is shown in Table-2.

Association of biomarkers with disease severity

There was a positive association between serum ALCAM levels and disease grading. The median ALCAM levels in grade I patients was 91.350, in grade II it was 215.743; the median serum ALCAM levels were highest in grade III 490.773 (P=0.005). There was no significant difference in the levels of serum and salivary CA-15-3 across the three grades. The levels of biomarkers in the three grades are shown in Table-3. As expected the nuclear size and nuclear diameter were also significantly higher in higher grades of

S.No	Variables	Values
1.	Age in years	
	Median (IQR)	43 (15)
	Mean ± SD	44.12±11.461
2.	Sex	
	Females N(%)	24 (96%)
	Males N (%)	01 (4%)
3.	Diagnosis	
	Intra-ductal N (%)	18 (72%)
	Lobular N(%)	02 (8%)
	Invasive solid papillary N (%)	01 (4%)
	Cribriform N (%)	01 (4%)
	Metastatic N (%)	01 (4%)
	Mixed type N (%)	01 (4%)
	Lipid rich N (%)	01 (4%)
4.	Grade I N (%)	18 (72%)
	Grade II N (%)	02 (8%)
	Grade III N (%)	05 (20%)
5.	Lymph node involvement N (%)	5 (20%)
7.	Nuclear diameter	
	Median (IQR)	5.582 (1.455)
	Mean ± SD	5.887±1.388
8.	Nuclear size	
	Median (IQR)	21.572 (15.381)
	Mean ± SD	26.788±12.228
9.	Serum ALCAM	
	Median (IQR)	143.766 (226.042)
	Mean ± SD	244.490±239.958
10.	Serum CA-15-3	
	Median (IQR)	28.598 (33.941)
	Mean ± SD	38.803±31.697
11.	Salivary CA-15-3	
	Median (IQR)	3.470 (2.500)
	Mean ± SD	3.955±1.865

Table-1: Baseline characteristics of breast carcinoma patients (N=25)

breast carcinoma (P=0.001) Table-3.

We also stratified the patients according to presence or absence of lymph node involvement. There was no significant difference in the levels of the biomarkers in the patients with or without lymph node involvement however the levels of serum ALCAM and CA-15-3 were higher in patients with lymph node involvement. The nuclear size and diameter were significantly higher in patients with lymph node involvement as compared to no involvement (P=0.017) Table-4.

Correlation between biomarkers in breast carcinoma patients

There was a significant positive correlation between serum ALCAM levels and nuclear size (Spearman's rho=0.589, P=

Parameters	Cases N=25	Controls N=25	P value
Age Median Mean ± SD	 43.00 (15) 44.12 ± 11.461	 41.00 (14) 44.04 ± 11.998	 0.900
Sex (female)	24 (96%)	25 (100%)	0.312
Serum ALCAM Median (IQR) Mean ± SD	 143.766 (226.042) 244.490 ± 239.958	 29.547 (23.062) 27.106 ± 12.017	 <0.001
Serum CA-15-3 Median (IQR) Mean ± SD	 28.598 (33.941) 38.803 ± 31.697	 8.879 (10.159) 12.267 ± 6.190	 <0.001
Salivary CA-15-3 Median (IQR) Mean ± SD	 3.470 (2.500) 3.955 ± 1.865	 2.039 (0.995) 1.971 ± 0.669	 <0.001

Table-2: Comparison of cases and controls

Parameter	Grade I	Grade II	Grade III	P value
Serum ALCAM Median (IQR) Mean ± SD	 91.350 (143.931) 152.740 ± 130.338	 215.743 (55.751) 215.743 ± 39.422	 490.773 (584.997) 586.287 ± 297.599	 0.005
Serum CA-15-3 Median (IQR) Mean ± SD	 26.055 (31.788) 33.573 ± 28.029	 50.209 (26.204) 50.209 ± 18.529	 38.992 (76.972) 53.069 ± 46.484	 0.379
Salivary CA-15-3 Median (IQR) Mean ± SD	 3.470 (2.261) 3.761 ± 1.628	 4.989 (4.021) 4.989 ± 2.843	 2.789 (4.721) 4.241 ± 2.602	 0.834
Nuclear diameter Median (IQR) Mean ± SD	 5.331 (0.901) 5.238 ± 0.788	 5.822 (5.822 ± 0.107	 8.234 (0.281) 8.250 ± 0.164	 0.001
Nuclear size Median (IQR) Mean ± SD	 19.493 (6.938) 20.266 ± 5.404	 33.740 (33.740 ± 2.457	 49.453 (5.919) 47.485 ± 4.528	 0.001

Table-3: Comparison of markers according to grading

Parameter	Lymph node involvement present N=5	Lymph node involvement absent N=20	P value
Serum ALCAM Median Mean ± SD	 243.618 (455.977) 344.851 ± 316.420	 104.909 (229.237) 219.399 ± 219.865	 0.221
Serum CA-15-3 Median Mean ± SD	 60.243 (70.317) 61.208 ± 43.002	 26.056 (31.096) 33.201 ± 26.732	 0.077
Salivary CA-15-3 Median Mean ± SD	 3.188 (3.789) 4.297 ± 2.039	 3.470 (2.418) 3.869 ± 1.865	 0.683
Nuclear size Median Mean ± SD	 35.477 37.288 ±	 19.866 24.163 ± 11.908	 0.017
Nuclear size Median Mean ± SD	 6.743 (2.408) 6.969 ± 1.222	 5.361 (0. 942) 5.616 ± 1.3162	 0.017

Table-4: Comparison of markers according to lymph node involvement

0.002) and nuclear diameter (Spearman's rho= 0.408, P= 0.043). No significant correlation was found amongst the three biomarkers.

DISCUSSION

Breast cancer is an emerging health problem and biomarkers are being increasingly studied for a prompt and correct diagnosis.[4] So the present study was conducted to ascertain the usefulness of ALCAM and CA15-3 as diagnostic and prognostic markers in breast cancer.

Among the baseline characteristics the mean age of cases was found to be 43 which was consistent with other studies underscoring the rising trend of breast carcinoma in younger age group. Overwhelming majority of cases were females which is also consistent with other studies.[7,8]

In present study mean serum ALCAM levels were significantly higher in patients of breast carcinoma as compared to healthy controls and this was comparable with other studies. Witzel et al. (2012)[9] also observed a significant difference in serum ALCAM levels of breast cancer patients and healthy controls. They reported the median values as 24.2 and 18.9 ng/ml respectively in cases and controls. Similarly mean serum CA15-3 values were higher in breast carcinoma cases compared to controls, which was in concordance with similar studies done in the past. In a study by Ali et al. (2013)[10] mean levels of CA 15-3 were found to be higher in cases as compared to controls. Salivary CA 15-3 values were also higher significantly in cases of breast carcinoma, comparable with other similar study however the results were not statistically significant. Laidi et al. (2014)[11] also found median salivary CA-15-3 levels to be higher in cases as compared to controls but did not find the difference to be significant. This can be attributed to the fact that oral cavity has a dynamic environment and saliva is affected by dietary factors too.

As far as correlation with morphological parameters and grade was considered, the values of serum ALCAM significantly correlated with nuclear size and hence histopathological grade. With higher grade the values of s ALCAM increased proportionately. However same was not observed with values of serum and salivary ca 15-3. Similar to results of present study, Tan et al. (2001)[12] also showed a significant increasing trend of nuclear area and perimeter with increasing histological grade. Similar association was also reported by Ikpatt et al. (2002)[13] in their study.

There was no significant correlation between lymph node status of the patients and levels of these biochemical parameters. Contrary to results of present study Paio et al. (2012)[14] found a significant association of ALCAM expression in breast cancer tissue and lymph node involvement while Park et al. (2008)[15] found a significant association of CA 15-3 levels with lymph node status. This might be attributed to different demographic profile of the cases.

CONCLUSION

The serum and salivary biomarkers may be useful in the diagnosis of breast cancer. But large sample size is needed to validate and generalize the result.

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