

## **BREAST CANCER; ESTIMATION OF TOTAL SERUM SIALIC ACID AND ITS COMPARISON WITH CA 15-3 AS TUMOR MARKER**

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ABSTRACT... mukhtardr2000@yahoo.com Cancer is a group of diseases with uncontrolled cellular proliferation. Breast cancer accounts for 25% of all cancer deaths in females worldwide. Monoclonal antibodies are used for the detection of tumor markers in order for rapid diagnosis and understanding of the nature of cancer at molecular level. On co-fetal antigens like carcinoembryonic antigen 15-3 (CA 15-3) are a new generation of clinically useful tumor markers. Elevated levels of CA 15-3 are related to stages in primary breast cancer, tumor size and nodal status. Significantly elevated sialic acid concentrations have been found in breast cancer patients and show correlation with tumor stage. Although total sialic acid has been used as general cancer marker but its role as diagnostic and prognostic marker has not been reported. Objective: This project was designed to study the sensitivity and specificity of sialic acid in breast cancer patients and was compared to CA 15-3, which is one of the most specific markers of breast cancer. Study design: Comparative study. Setting: Shaikh Zayed Medical Complex. Material and Methods: 66 breast cancer patients and 30 normal individuals (controls) were included in the study to compare serum sialic acid with CA 15-3 as tumor marker. Sialic acid was estimated by colorimetric method while CA 15-3 by Enzyme-Linked Immunosorbent Assay. Results: The sensitivity and specificity of CA 15-3 in breast cancer patients was 62.5% and 80% while that of sialic acid was 62% and 76% respectively. The sensitivity of CA 15-3 and sialic acid in patients with ductal breast carcinoma was 62.5% and 54.16% whereas the specificity of CA 15-3 and sialic acid was 80% and 76% respectively. The sensitivity of CA 15-3 and sialic acid in

patients with lobular breast carcinoma was 83.33% and 75% whereas the specificity of CA 15-3 and sialic acid was 80% and 76% respectively. Conclusion: It was concluded that sialic acid can be used as a tumor marker in breast cancer patients and has sensitivity and specificity similar to CA15-3. However, CA 15-3 appears to have better predictive value.

Key words: Breast cancer, Sialic acid, Carcinoembryonic antigen 15-3.

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## **INTRODUCTION**

Cancer is a group of diseases with common aspect that sustained and downhill course result in death of patients if not properly treated and managed. In medical terms it is an uncontrolled cellular proliferation<sup>1</sup>. The rate of incidence and death rate in females due to breast cancer is 25% of all cancer deaths. Risk factors of breast cancer are old age of mother at childbirth, early menstruation, late menopause, alcohol consumption, family history and hormone replacement therapy<sup>2</sup>. Tumor markers are one of the methods of investigation of cancer<sup>3</sup>. Tumor markers are the biological substances which are synthesized and released by cancer cells or produced in the host in response to cancer tissue<sup>4</sup>.

With the development of monoclonal antibody and their subsequent application to detection of onco-fetal antigens like CA125, CA15-3 and CA549 have led to rapid understanding of nature of cancer at molecular level. These markers represent a new generation of clinically useful tumor markers<sup>5</sup>. Tumor markers have many potential uses in the management of cancer patients but CA15-3 and sialic acid also have role in monitoring response to therapy<sup>5,6,7,8</sup>.

CA15-3 is a carbohydrate antigen and currently widely used prognostic marker for breast cancer. CA15-3 assay detects a high molecular weight glycoprotein mucin expressed by mammary epithelium known as episialin<sup>9</sup>. Elevated levels of CA15-3 in breast cancer are related to stages in primary breast cancer, tumor size and nodal status. In patients with benign breast cancer the

concentration of CA15-3 is 16.5±9 U/ml and in malignant disease its concentration is greater than 25U/ml<sup>10</sup>.

Sialic acid (N-acetyl neuraminic acid) has been found to be a major component of every cell membrane glycoproteins<sup>11</sup>. Degradation of the glycoproteins is preceded by desialation by neuraminidase<sup>12</sup>. Sialic acids are thus partly contributed factors to the survival of glycoprotein in plasma membrane and cellular integrity<sup>13</sup>. Cells transformation results in alternation of surface charges and pattern of complex of glycoproteins<sup>14</sup>. There is increased shedding of these glycoprotein containing sialic acid which are more concentrated on cell surface<sup>15</sup>. Significantly elevated sialic acid concentrations were found in breast cancer and show correlation with tumor stage<sup>6,16</sup>.

Although total sialic acid has been used as general cancer marker but its role as diagnostic and prognostic marker has not been reported. This project was designed to study the sensitivity and specificity of sialic acid in breast cancer cases and was compared to CA15-3 which is one of the most specific markers of breast cancer.

## **MATERIAL & METHODS**

The study comprised of 30 (thirty) normal individuals and 66 (sixty six) breast cancer patients. 30 normal individuals comprised control group including both males and females from Shaikh Zayed Hospital staff, who were free from any symptoms of ailment and healthy on physical examination. The patient group comprised 66 (2

males & 64 females) histopathologically diagnosed breast cancer patients from Shaikh Zayed Hospital, INMMOL and Mayo Hospital, Lahore.

From control and cancer cases 5 ml of venous blood was collected with disposable syringes under aseptic conditions. Samples were allowed to clot at room temperature for about an hour and then centrifuged at 3000 rpm for 10 minutes. The clear serum was then divided into aliquots and stored at -20°C until assayed. All the samples were thawed to room temperature and tested for both total sialic

acid and CA15-3. Sialic acid was estimated by Ehrlich's reagent method as described by Shamberger<sup>15</sup>. CA15-3 was estimated by enzyme-linked immuno-sorbent assay (CA15-3 ELISA, Kat. / Cat #EIA-1556, DRG).

**RESULTS**

Control and patient group showed a good match for age and sex distribution, with (n=22) 73.33% and (n=45) 68.19% of the individuals fell in age group of 25-50 years whereas (n=8) 26.67% and (n=21) 31.81% fell in age group of 51-85 years (Table I).

Healthy person (n=39)	Age group (yrs)	No. of cases	Males	Females
	25-50	22 (73.33%)	1 (33.33%)	21(77.78%)
>51	8 (26.6%)	2 (66.67%)	6(22.22%)	
Total	30(100%)	3(100%)	27(100%)	
Breast cancer patients (n=66)	25-50	45(68.19%)	1(50%)	44 (68.75%)
	>51	21(31.81%)	1(50%)	20(31.25%)
	Total	66(100%)	2(100%)	64(100%)

The Mean±SEM of CA15-3 was 29.326±6.564 and that of sialic acid was 38.143±2.549 in control group.

The Mean±SEM of CA15-3 was 83.558±3.45 U/ml and that of sialic acid was 63.341 ±10.046 in breast cancer patients. Both CA15-3 and sialic acid were significantly greater (p<0.01) and (p<0.02) as compared to controls (Table II).

In subjects belonging to age group 25-50 years, mean value of both CA15-3 and sialic acid were significantly greater (p<0.01) in patient group as compared to controls. Whereas in subjects

belonging to age above 50 years, the value of CA15-3 was more significant (p<0.01) as compared to the value of sialic acid (p<0.02) in comparison to controls (Table III).

Group	CA1 5-3 (U/ml) Mean±SEM	Sialic acid (mg/dl) Mean±SEM
Control	29.326±6.564	38.143±2.549
Patients	83.558±3.45*** (p<0.01)	63.341±10.046** (p<0.02)
* shows significance of P value		

Table-III. Comparison of CA15-3 and sialic acid concentration in different age groups

Age	CA1 5-3 (U/ml) Mean±SEM	Sialic acid (mg/dl) Mean±SEM
Control (25-50 y)	24.359±3.95	36.026±2.343
Control (>50 y)	46.031±22.187	48.468±6.081
Breast cancer (25-50 y)	93.89±1 3.73*** (p<0.01)	68.729±4.51 5*** (p<0.01)
Breast cancer (>50 y)	66.083±1 3.81 6*** ((p<0.01)	52.309±4.194** (p<0.02)

The patients of breast cancer were also grouped as having ductal carcinoma and lobular carcinoma. Out of 64 female breast cancer patients, 24 belonged to ductal carcinoma group while 12 had lobular carcinoma. 28 patients fell in none of these two groups.

The Mean±SEM of CA15-3 in ductal carcinoma was 90.108±17.213 while that of sialic acid

65.536±5.724.

The Mean±SEM of CA15-3 in lobular carcinoma was 111.461 ±23.682 and that of sialic acid 60.576±6.965 (Table IV).

The sensitivity and specificity of CA15-3 and sialic acid in ductal and lobular breast carcinoma patients is also given in Table IV.

Table-IV. Levels, Sensitivity and Specificity of CA1 5-3 and Sialic acid

Group	CA15-3(U/ml) Mean±SEM	Sialic acid (mg/dl) Mean±SEM	Sensitivity		Specificity	
			CA15-3	Sialic acid	CA15-3	Sialic acid
Ductal carcinoma (n=24)	90.108±17.213	65.536±5.724	62.5%	54.16%	80%	76%
Lobular carcinoma (n=12)	111.461±23.682	60.576±6.965	83.33%	75%	80%	76%

**DISCUSSION**

In present study, a nonspecific tumor marker serum sialic acid was estimated in 30 healthy persons and 66 breast cancer patients to study its sensitivity, specificity and efficacy as a diagnostic and prognostic indicator and was compared with the standard tumor marker carbohydrate antigen 15-3 (CA 15-3) which was also determined in same

control and breast cancer patients as a reference marker. The results of the comparative study are given in Tables I to IV.

This study included total 66 breast cancer patients out of whom 64 were females and 2 males (Table I). Literature shows that rate of incidence of breast cancer in males is only 1 per cent (1 %) and similar

incidence has also been reported by other workers<sup>17</sup>.

Our study shows that the incidence of breast cancer is 68.75% in 25-50 years age group and 31.25% in 50-80 years age group. This is comparable to the results of other studies reporting that breast cancer in females is > common in age group of 35-50 years<sup>18</sup>.

Level of sialic acid in controls ranged from 15-45mg/dl: having a mean of 38.143mg/dl. This data is comparable to previous studies for non-cancer controls with levels of sialic acid were reported to be 40.70-41.70 mg/dl and 36.11-77.91 mg/dl<sup>15,19</sup>.

In our study the range of CA 15-3 in controls was 2.5-30 U/ml (Mean=31.202). These results are comparable to those of other studies reporting 31- and 30 U/ml of CA 15-3 in normal controls<sup>20,21</sup>. In another study the cutoff value of CA 15-3 in breast cancer patients has been reported 38 U/ml. These results correlate with our findings of the levels of CA 15-3 (mean = 83.558 U/ml) in breast cancer patients (Table II). Our data is also comparable with the findings of many workers showing very high percentage (97%) of breast cancer patients having abnormal CA 15-3 levels<sup>22,23,24,25</sup>.

The sensitivity and specificity of CA 15-3 in breast cancer patients was 62.5% and 80% respectively. The sensitivity and specificity of sialic acid in breast cancer patients was 62 % and 76% respectively. This data is comparable to the previous reports from various workers<sup>26,27</sup>.

The level of CA 15-3 in breast cancer patients having ductal carcinoma was 90.108±17.213 U/ml. Previous data shows the high level of CA 15-3 in patients of ductal carcinoma of breast<sup>28</sup>. The level of CA 15-3 in lobular carcinoma was 111.461 ±23.682 U/ml. The level of sialic acid in ductal and lobular carcinoma was 65.536±5.724 mg/dl and 60.576±6.965 mg/dl respectively. Noncomparable

data is available.

The sensitivity of CA 15-3 and sialic acid in patients with ductal breast carcinoma was 62.5% and 54.16% respectively whereas the specificity of CA 15-3 and sialic acid was 80% and 76% respectively.

The sensitivity of CA 15-3 and sialic acid in patients with lobular breast carcinoma was 83.33% and 75% respectively whereas the specificity of CA 15-3 and sialic acid was 80% and 76% respectively.

Thus it may be concluded that sialic acid could be used as a tumor marker in breast cancer patients and has sensitivity and specificity similar to CA 15-3. However CA 15-3 appears to have better predictive value. These findings call for further studies to evaluate diagnostic and prognostic significance of these tumor markers in breast cancer patients in Pakistan.

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