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Evaluation of Serum Total and Lipid Associated Sialic Acid as a Tumor Marker in Breast Malignancy

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Abstract: *Carcinoma of the breast is the third most common cancer in the world. Such increasing Global Incidence Emphasises the need to find a tumor marker for detecting and monitoring the response to treatment. Hence estimation of Sialic Acid, which is simple, cheap and no sophistication requiring investigation is undertaken. 50 cases are histologically confirmed breast malignancy, between the age group 20 to 70 years were studied. In addition 8 cases of Benign tumors of breast and 7 cases of breast abscess were also studied. In these cases Total Sialic Acid (TSA) , Lipd Associated Sialic Acid (LASA) and Serum Total Proteins (TP) were analyzed before treatment and after treatment. 50 healthy females are also studied as controls and analysed for the same parameters. TSA and LASA were estimated by spectrophotometric method using Resorcinol and Serum Total Proteins were estimated by HENRY'S method. TSA /TP and LASA/ TP Ratios were calculated. The Serum TSA, TSA /TP, LASA and LASA/ TP values are significantly increased in breast malignancy as compared to healthy controls. Significant decreases occur in Sialic Acid concentration, after treatment indicating usefulness of Sialic Acid as a marker to monitor the progress of disease to effective treatment.*
Key Words: *Total Sialic Acid (TSA), Lipid Associated Sialic Acid (LASA), Total Proteins (TP).*

I. Introduction

Malignancy is an abnormal new growth. The term cancer, neoplasm and malignancy are usually used interchangeably in both the technical and popular literature. The etiology of breast cancer is multifactorial. Risk factors include early menarche, late menopause, nulliparity, late age at first birth, post menopausal obesity, extended use of oral contraceptives, hormone replacement therapy, family history and previous benign breast disease [(1)]. Marked increased risk in first Chromosome 17Q, 21 in a large number of families in whom breast cancer develops at an early age BRCA 1. (Breast Cancer 1) gene. The various tumor markers for evaluation of Breast Cancer are Estrogen Receptors, Progestrone-Receptors, DNA Index, DNA Flow cytometric proliferation analysis, CA15-3, CEA, C-erb, B-2,P53, Cathepsin-D, Mammary Serum Antigen, MAM-6, and Sialic Acid. We attempted to study Sialic Acid, a simple, cheap and no sophistication requiring investigation as a marker for breast malignancy. The Sialic Acid containing Glycoproteins and Glycosphingolipids are ubiquitous membrane components. Malignant Cell surface Glycoproteins and Glycolipids have altered Carbohydrate compositions that may contribute to aberrant Cell – Cell recognition, Cell Adhesion, Antigenicity and Invasiveness demonstrated by Malignant Cells[(2)]. The Glycoproteins and Glycolipids can be released into the Sera through increased secretion and /or shedding and thus they are of considerable interest for their potential diagnostic and prognostic value. Carcinoma Associated alternations O-Glycosylation are reported in Breast Cancer cells and in Breast Cancer Cell lines like T47D[(3)]. Hypersialated Glycan structures in malignancy may depend on altered regulation of one or more Sialyltransferases which occur in Breast Cancer Cells[(4)]. There is decreased branching, increased Fucosylation and changed Sialylation of Alpha-1-Proteinase Inhibitor (API) in Breast Cancer[(5)]. It is also reported that tissue fibronectin and Sialic Acid levels are higher in Breast Tumors than in normal tissues. WINZLER, [(6)] in 1955 is the first to document significant elevations in Protein bound Sialic Acid in patient with cancer. The simplest means to assess Glycoporteins and Glycosphingolipids is by estimation of Total Sialic Acid (TSA) and Lipid Associated Sialic Acid (LASA) we have analysed these parameters and we have studied them along with ratios when they are nomalised to total protein (TSA /TP and LASA / TP) in Breast Malignancies and compared them with those of benign Breast Tumors and Breast abscess. It has been suggested that Glycoprotein bound Sialic Acid is determined predominantly in LASA assays contrary to the name LASA suggesting only are mainly Lipid - bound Sialic Acid measurement. So we normalised LASA also to Serum Total Proteins (LASA / TP).

II. Materials And Methods

50 cases of Histologically confirmed Breast Malignancy, admitted in Govt. Gen. Hospital, Guntur from 01-07-2013 to 28-02-2014 of the age group between 20 to 70 years were studied. Out of these 50 cases, 43 cases were Cancer Breast without Metastasis and 7 cases were Cancer Breast with Distant Metastasis. In these 50 cases, blood samples were analysed for Total Sialic Acid (TSA) and Lipid Associated Sialic Acid (LASA) and

Serum Total Proteins (TP) in these patients blood samples were collected before treatment and 10 days surgical removal and 90 days after Radiotherapy. In addition 8 cases of benign tumors of Breast and 7 cases of Breast Abscess were also studied and in these cases blood samples were analysed for the same parameters. before treatment and 10 days after surgical removal. Blood samples from 50 healthy females were also collected for the same parameters. Total Sialic Acid (TSA) was estimated by spectrophotmetric method using resorcinol. Principle of TSA estimation – Resorcinol is added to chilled sera. Boiled and the resultant chromophore extracted by n-butylacetate / n- butanol mixture. The blue color developed in supernatant was read at 580nm. Principle of LASA estimation – Chloroform / methanol mixture is added to chilled serum. The upper layer is used for extraction of lipid associated Sialic Acid after adding phosphotungstic acid and redisolving the pellets formed with resorcinol and n-butylacetate, n- butanol mixture. Blue colour developed is read at 580nm. Serum Total Proteins (TP) were estimated by HENRY'S Method Principle of TP estimation – Based on Biuret Reaction proteins from violet coloured complex with cuprous ions in alkaline solution which is measured colorimetrically. TSA /TP and LASA/ TP Ratios were calculated.

III. Figures And Tables

Table - 1

The Mean + Standard Deviation Values of Various Parameters.

Sl. No	Group	Total Sialic Acid (TSA) mg/dL	TSA/ TP mg/G	Lipid Associated Sialic Acid (LASA) mg/dL	LASA / TP mg /G	Total Proteins (TP) G/dL
1.	Healthy Controls	50.83 + 13.77	8.60 + 1.86	15.81 + 7.37	2.57 + 1.15	6.08 + 0.53
2. a	Breat Malignancy without Metastatsis	81.42 + 10.25	14.79 + 2.24	24.76 + 6.92	4.49 + 1.34	5.40 + 0.33
b		61.45 + 7.97	11.35 + 1.95	18.03 + 4.01	3.07 + 0.71	5.92 + 0.34
3. a	Breat Malignancy with Distant Metastatsis	90.0 + 11.18	16.48 + 1.80	31.57 + 5.84	5.78 + 1.04	5.28 + 0.29
b		73.75 + 2.5	12.78 + 0.65	21.12 + 8.17	3.40 + 1.16	5.92 + 0.34
4.a	Benign Tumors of Breast	68.63 + 7.44	12.31 + 1.20	18.90 + 7.38	3.40 + 1.41	5.58 + 0.39
b		55.62 + 3.20	9.05 + 0.64	16.25 + 3.47	2.64 + 0.57	6.15 + 0.39
5.a	Breast Abscess	59.28 + 4.49	10.56 + 0.95	16.71 + 3.47	3.07 + 0.71	5.59 + 0.33
b		51.42 + 2.43	10.56 + 0.95	16.71 + 3.47	3.07 + 0.71	5.59 + 0.33

a. Before treatment b. After treatment

Table - 2

Reference Ranges (Mean + 2SD)

Sl. No	Parameter	Reference Range
1.	Total Sialic Acid (T.S.A)	23.29 – 78.37 mg /dL
2.	Total Sialicacid / Total proteins (T.SA/TP)	4.88-12.32 mg /G
3.	Lipid Associated sialic Acid (LASA)	1.07 – 30.55 mg/dl
4.	Lipid Associated sialic Acid / Total proteins (LASA / TP)	0.27 – 4.87 mg /G
5.	Total Proteins (TP)	5.02 – 7.14 G /dl

Table – 3

The 't' and 'p' values of various parameters

Sl.No.	Group	TSA	TSA /TP	LASA	LASA / TP	TP
1	Controls Vs Malignancies	9.82 P<0.001	14.33 P<0.001	7.62 P<0.001	10.66 P<0.001	5.61 P<0.001
2	Controls Vs Benign tumors	5.28 P<0.001	7.72 P<0.001	4.01 P<0.001	2.15 P<0.05	2.16 P<0.05
3	Controls Vs Breast abscess	2.78 P<0.001	4.38 P<0.001	1.31 N.S.	1.51 N.S.	2.07 P<0.05
4.	Malignancies Vs Benign tumors	3.85 P<0.001	5.82 P<0.001	3.85 P<0.001	3.93 P<0.001	2.35 P<0.05
5.	Malignancies Vs Breast abscess	7.93 P<0.001	9.54 P<0.001	7.20 P<0.001	6.86 P<0.001	3.52 P<0.001
6.	Malignancies with Metastasis Vs without metastasis	15.97 p<0.001	1.93 NS	1.18 NS	1.51 NS	0.4 NS
7.	Benign Tumors Vs Breast Abscess	3.32 p<0.01	1.44 NS	0.84 NS	0.71 NS	0.03 NS

Figure – 1

Total Sialic Acid / Total Proteins

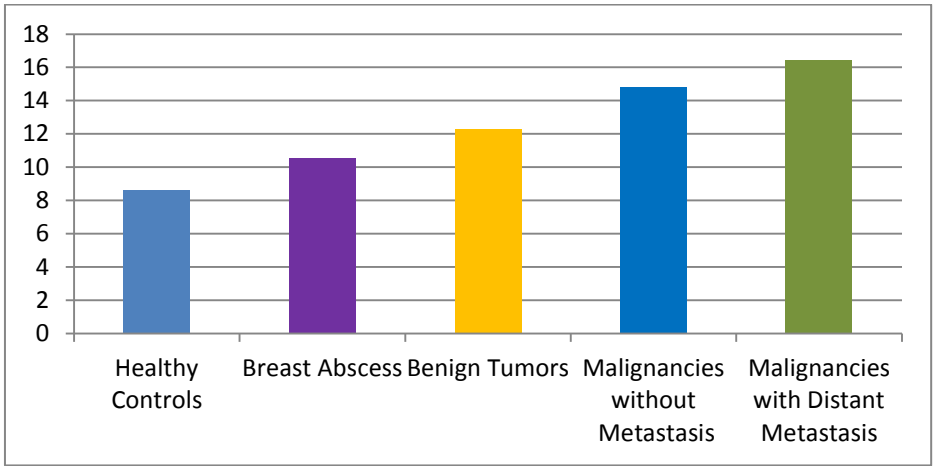
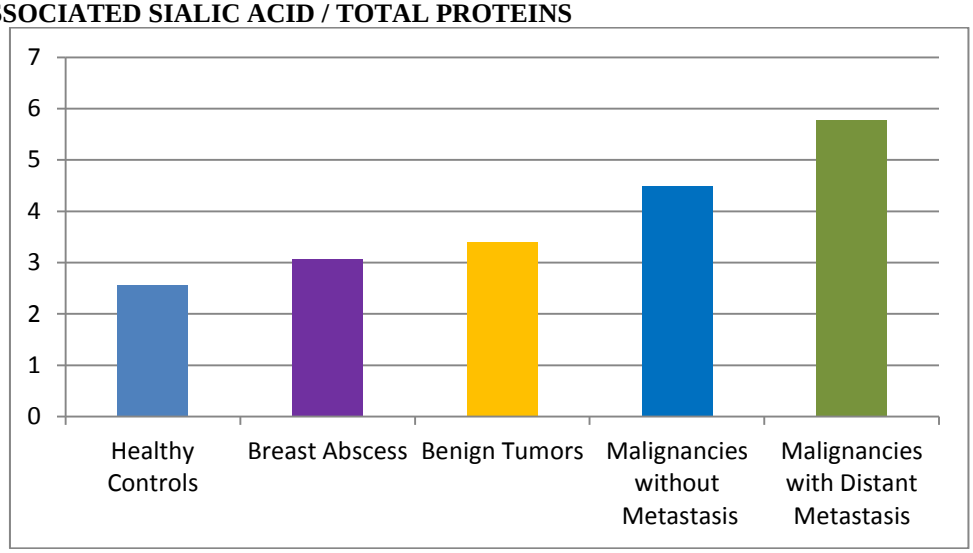


Figure – 2

LIPID ASSOCIATED SIALIC ACID / TOTAL PROTEINS



IV. Discussion

The mean concentration of TSA in all groups of patients studied are higher than those in controls. The highest mean concentration are observed in cases of breast malignancies especially those with distant metastasis.

The mean concentrations of LASA in all groups of patients are studied are higher than those in controls. The highest mean values are observed in breast malignancies with distant metastasis.

The mean concentrations of total proteins are lower in all the groups of patients studied. They are lower in breast malignancies with distant metastasis.

In order to assess the significance of differences observed in mean values of various parameters in different groups studied, the students 't' test is applied and the 't' and 'p' values are calculated. The TSA values in all groups of patients studied i.e., the malignancies, benign tumors and breast abscess are significantly increased when compared to the controls ($t=9.82$ and 5.28 and $p<0.001$ and $t = 2.78$ $p<0.001$) respectively.

In order to assess the diagnostic potential of various parameters the difference ranges (Mean ± 2SD values) for various parameters are calculated from the control values. TSA and TSA /TP values have good Diagnostic potential in identifying breast malignancy.

The diagnostic sensitivities of TSA, TSA / TP, LASA, LASA /TP values are calculated. The TSA and TSA /TP shown better sensitivity (67.34% and 87.75%) than LASA and LASA /TP (46.93%). The TSA, TSA/TP exhibited 95.83% and 85.41% specificity and LASA and LASA/TP showed 89.58% specificity.

Both the Total Sialic Acid and Lipid Associated Sialic acid concentrations in serum are increased in breast malignancies when compared to the controls. In order to the assess correlation among these parameters the correlation coefficient 'r' values are calculated. We find a significant correlation between TSA an LASA both in controls ($r=0.55$ and $p<0.01$) and in malignancies ($r=0.56$ and $p<0.001$).

V. Conclusion

The serum TSA, TSA/TP, LASA and LASA /TP values are significantly increased in malignancies as compared to controls indicating altered metabolism of tumor surface glycoproteins and sialoglycolipids.

The higher increases observed in malignancies to controls and Benign breast conditions indicates that the these are sensitive specific markers for breast malignancy. The TSA and TSA/TP values good diagnostic potential in identifying breast malignancy. LASA and LASA /TP values though highly specific markers are poor sensitive markers. Highest diagnostic sensitivities and specificities can be achieved by use of combination of these markers.

The LASA, LASA /TP and TSA/TP values are significantly higher in malignancies with distant metastasis compared to malignancies without metastasis indicating their usefulness as markers for extent of disease and tumor load.

Significant decreases occur in Sialic Acid concentrations after treatment indicating their usefulness in monitoring the progress of the disease to effective treatment. The increased Sialic Acid concentrations in malignancies though significantly decreased after treatment have not returned to normal during the follow up period.

Significant correlation between TSA and LASA is observed in malignancies and controls.

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