Evaluation of Total Sialic Acid and Lipid Associated Sialic Acid in Serum and Tissue in Patients with Breast Tumor

Jameel Hazza’a Al-Bayati*  
F.I.C.M.S  
Khalid Karim Rajab**  
F.I.C.M.S  
Fadel Habeb Taher***  
F.R.C.S

Abstract:

Background: Total serum sialic acid (TSA) and lipid associated sialic acid (LSA) are found to be increased in different neoplastic diseases. The present study was undertaken to study the changes in the concentration of TSA and LSA in serum and tumor tissue of patients with breast tumors.

Methods: TSA and LSA levels have been estimated in serum and tissue of patients with breast tumors (n=28) in addition to healthy controls (n=34).

Results: Data analysis reflects a significant increase (p<0.05) in the TSA and LSA in the sera and malignant tumor tissue of patients as compared with healthy controls.

Conclusions: TSA reflects the development of malignancy and can be considered as supporting tumor marker in malignant breast tumors.

Keywords: Breast tumor; cancer; sialic acid; lipid-associated sialic acid.

Introduction:

Sialic acids are the predominant carbohydrate of cell membrane. Sialic acids are acetylated derivatives of neuraminic acid and it is a carbohydrate derivative found as a common terminal saccharide of the cell surface constituents (glycoprotein and glycolipids)\textsuperscript{[1]}\textsuperscript{,}[2] Several evidences indicate that different changes of structural components of the neoplastic cells have been carried out. It has been found that many glycoproteins and glycolipids are increased in sera and malignant tissues of patients with various types of cancers. The relevance of sialic acids to the tumor cell is apparent from the increased sialylation and sialyltransferase activity observed in many cancer cells\textsuperscript{[2]}.

In sera and tissues, sialic acid appeared to be found in two forms, bound sialic acid and free sialic acid. In the former, sialic acid was bound to glycoproteins and glycolipids. Thus lipid-associated sialic acid (LSA) has significant roles in different diseases including malignances\textsuperscript{[3]-[6]}.

Sialic acid containing glycosphingolipids could be a microglial activator\textsuperscript{[7]}\textsuperscript{,} and it modulates cell-cell and cell–matrix interactions \textsuperscript{[8]}. Also glycosphingolipids (containing sialic acid) expressed in cancer cells have implicated in modulation of tumor cell growth through their interaction with transmembrane signaling molecules such as growth factor receptors. For glycosphingolipids to interact with growth factor receptors, the presence of sialic acid seems to be essential\textsuperscript{[9]}.

Sialic acid level can be used as diagnostic marker to assess the stage of cancer and can be used as prognostic markers during therapy of some types of cancer \textsuperscript{[10]-[11]}. The objective of this work is to estimate total Sialic acid (TSA) and (LSA) as a possible useful diagnostic parameter in the serum and tumor tissue in patients with breast tumor.

Patients & Method:

Twenty-eight female patients with breast tumors referred to Al-Yarmouk hospital for surgical interventions and Twenty-eight (female) healthy women were taken as control of the same average age.

Venous blood samples were collected in the day of operation before initiating the operation. Sera were separated and kept at (-20°C) until analysis.

Tumor tissue was taken from the lesion at the day of surgery, which immediately transferred, for mincing and homogenization. An equal volume of sodium phosphate buffer (pH=6.86) is added to the minced tissue, and then cold centrifugation (at 4°C) was performed at 4000 rpm for 30 minutes.

The centrifugal supernatants were aspirated and stored frozen at (-20°C) in polyethylene tube until assayed.

Assay:

This assay carried in private sector. TSA was measured using Svennerholm (1957)\textsuperscript{[12]} method as modified by Miettinen and Takka-Luukkainen (1959)\textsuperscript{[13]} . In brief, 20µL of serum or supernatant was diluted into 500µL in a test tube with distilled water \textsuperscript{.0.5 ml} of resorcinol reagent (0.2 grams of resorcinol was dissolved in 10ml of distilled water and added to 80ml of concentrated HCl containing 0.25ml of 0.1M copper sulfate and completed to 100ml with distilled water) were added. The test tube was heated for 15 minutes at 100°C in boiling
water bath. After heating the tubes were cooled in running water, 1ml of butyl acetate-n- butanol (85:15 v/v) mixture at room temperature, vortex and centrifuge for 5 minutes at 2500rpm. Read absorbance of the extracted blue color at 580nm.

LSA is measured according to procedure of Katopodis and Stock [14] in which 50µL of serum or supernatant were extracted with 3ml of chloroform: methanol (2:1 v/v) at 4°C. The lipid extract was partitioned with 0.5 ml of cold distilled water, and the aqueous layer containing LSA was precipitated with 50µL of phosphotungstic acid (1g/ml). After centrifugation, the supernatant was aspirated, and the precipitate was resuspended in 1ml of distilled water and sialic acid content was determined as mentioned for TSA.

Results:
The results of the TSA and LSA of the patients with breast tumor (28) in addition to healthy controls (34) are presented in Table I. Which revealed that there is a significant increase (p<0.05) in the TSA and LSA level in the serum and tissue of patients with malignant breast tumor as compared with healthy control Fig. (1)&(2).

<table>
<thead>
<tr>
<th></th>
<th>MALIGNANT (n=28)</th>
<th>CONTROL (n=34)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Serum</td>
<td>Tissue</td>
</tr>
<tr>
<td>TSA</td>
<td>73.9±6.3</td>
<td>48.1±3.9</td>
</tr>
<tr>
<td>t.test</td>
<td>128, P&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>LSA</td>
<td>26.5±2.1</td>
<td>16.6±1.5</td>
</tr>
<tr>
<td></td>
<td>St.=15.52 P&lt;0.0002</td>
<td></td>
</tr>
</tbody>
</table>

Figure (1): Results of the TSA of the patients with malignant breast tumor and healthy controls.

Table I: Serum and Tissue TSA, LSA for the patients and controls
Discussion:

Different types of tumors have been reported to elevate serum contents of sialic acids including human tumors of breast, lung, stomach, and lymphoma \[15-17\].

Sialic acids present as components of surface glycoconjugate and of soluble glycoconjugate in the animal cells and tissue, appear to be involved in the regulation of cell surface functions and thus in malignant transformations \[18-19\]. Several studies have shown that neoplastic transformation leads to elevated serum sialic acids concentration. Elevated TSA or its other forms have been reported in sera of patients with different malignant diseases \[20-23\]. Other explanations for the higher tissue sialic acids content in brain cancer patients could not be excluded. Such increase was noticed not only in the concentration of serum and tissue glycoprotein and glycolipids, but also in the degree of sialylation of these substances. In fact, an elevation in the activity of serum sialyltransferase in patients with different types of cancer has been demonstrated \[24-25\]. Some authors have suggested that increased serum sialic acid in patients with cancer reflects an inflammation reaction to the tumor, leading to an elevated output of the acute phase reactant proteins from liver \[26-27\]. Hence our results are in agreement with these suggestions.

Serum sialic acids were found to be increase in patients with metastatic diseases when compared with patients having only localized involvement \[29\].

Conclusion:

The changes in serum and tissue sialic acids may be used as supporting tools in the identification of malignancy properties. More investigations are required to obtain a complete explanation of the causes of the increase in sialic acids in the tissue and serum of patients; and the follow up studies are recommended.

References:

Breast tumor; cancer; sialic acid; lipid-associated sialic acid.

Jameel Haz'a Al-Bayati et al


* General Surgeon, Emergency Unit, Al-Yarmouk Teaching Hospital, Baghdad, Iraq.
** Assistant Professor Al-Mustansryia Medical College, Consultant surgeon Al-Yarmouk Teaching Hospital
*** Consultant Surgeon Senior lecturer Al-Mustansryia Medical college