EVALUATION OF TOTAL SERUM SIALIC ACID AND LIPID ASSOCIATED SIALIC ACID IN BRAIN TUMOR PATIENTS

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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 patients with different types of brain tumors before and after surgical removal of brain tumor.

Methods: TSA and LSA levels have been estimated in serum of patients with benign brain tumors (n=28) and malignant brain tumors (n=31) in addition to healthy controls (n=34).Also the TSA and LSA measured in the patients (7) days after surgical removal of the tumor.

Results: Data analysis reflects a significant increase ($p<0.05$) in the TSA and LSA in the malignant tumor patients as compared with healthy controls. The results revealed a significant decrease ($p<0.05$) in the TSA but not LSA (7) days after operation.

Conclusions: It has concluded that there is a correlation between the type of tumor and the concentration of TSA and LASA. The surgical removal of the tumor leads to decrease in TSA and LASA in the serum.

Keywords: Brain tumor, Cancer, Sialic acid, Lipid associated sialic acid

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Introduction

N-acetylnuraminic acid (sialic acid) is 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). 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. Sialic acids are the predominant carbohydrate of these compounds[1]. The relevance of sialic acids to the tumor cell is apparent from the increased sialylation and sialytransferase activity observed in many cancer cells[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[3-6].

Sialic acid containing glycosphingolipids could be a microglial activator[7] and it modulates cell-cell and cell-matrix interactions[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[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[10-11]. The objective of this work is to estimate (TSA) and (LSA) as a possible useful diagnostic and prognostic parameter in patients with different types of brain tumor.

Patients & methods

Patients

Fifty nine patients with different brain tumors referred to Al-Kadhimiya Teaching Hospital for surgical interventions. The patients with benign tumors were 28 patients (17 patients with meningioma, 7 patients with schwanoma, and 4 patients with dorsal neurofibroma). Patients with malignant tumors were 31 cases (16 patients with glioma, 6 patients with glioblastoma, 4 patients with medullary blastoma, 5 patients with brain metastases from breast and lung carcinoma, astrocytoma). The tumors in these

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patients were removed by surgery. 34 healthy peoples were taken as control.

Venous blood samples were collected before initiating the operation and one week after operation. Sera were separated and kept at (-20ºC) until analysis.

Assay

TSA was measured using Svennerholm (1957)[12] method as modified by Miettinen and Takka-Luukkainen (1959)[13]. In brief, 20µL of serum was diluted into 500µL in a test tube with distilled water .five hundred micro liter of resorcinol reagent (0.2 grams of resorcinol was dissolved in 10 ml 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 and 1ml of butyl acetate-n-butanol (85:15 v/v) mixture was added at room temperature, vortex and centrifuge for 5 minutes at 2500rpm. Read absorbance of the extracted blue color at 580nm.

LASA is measured according to procedure of Katopodis and Stock[14] in which 50µL of serum 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 & discussion

The results of the TSA and LASA of the patients with benign and malignant brain tumor in addition to healthy controls are presented in Table 1 & 2.

Table 1: The results of TSA in patients with benign and malignant brain tumors & healthy controls

TSA	Mean±SD mg/dl	No. of patients		Sensitivity
		≥89.35*	<89.35	
Controls	61.83±13.76	0	34	0
Benign:				
Before surgery	72.32±16.79	6	22	21.43
After surgery	63.05±11.93	1	27	3.75
Malignant:				
Before surgery	81.22±20.73	13	18	41.94
After surgery	68.28±14.39	5	27	16.13

Table 2: The results of LSA in patients with benign and malignant brain tumors and healthy controls

LSA	Mean±SD mg/dl	No. of patients		Sensitivity
		≥36.04*	<36.04	
Controls	21.54±7.25	0	34	0
Benign:				
Before surgery	29.14±8.03	11	17	39.29
After surgery	23.58±5.81	3	25	10.71
Malignant:				
Before surgery	34.57±8.57	17	14	54.84
After surgery	25.36±5.5	8	23	34.78

The results revealed that there was a significant increase ($p<0.05$) in the TSA and LASA level in the patients with malignant brain tumor as compared with healthy control (Table 3).

Table 3: P values between the compared groups (control, patients with benign and malignant brain tumors)

Groups	P value	
	TSA	LSA
Control Vs benign *	0.185	0.788
Control Vs Malignant *	0.025	0.0009
Benign* Vs Malignant *	0.038	0.057
Benign* Vs Benign **	0.071	0.658
Malignant* Vs Malignant**	0.006	0.949

While no significant variation ($p<0.05$) was indicated in TSA and LASA between benign brain tumor and control. Several types of tumors have been reported to have elevated 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 explanation for the higher serum sialic acids content in brain cancer patient could not be excluded .Such increase not only in the concentration of serum 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 with agreement with these suggestions.

The insignificant increase in TSA level in patients with benign tumors as compared with healthy controls was also agreed with other observations[28]. Serum sialic acids were found to be increase in patients with metastatic diseases when compared with patients having only localized involvement[29].

Considering their pretreatment levels as base line, elevation in levels of TSA was found in patients with benign and malignant brain tumors. While there is no significant changes in LASA levels before and after surgery. These results may be explained by the fact that the cause of increase TSA is mainly due to increase in the acute phase reactant protein as an inflammatory reaction. After treatment by drugs and surgery, the anti-inflammatory drugs which mainly hydrocortisone and its derivatives that decreases the inflammatory reaction and hence decrease the acute phase reactant proteins in the blood. Also the removal of all tumor tissue in benign brain tumor patients and subtotal removal of malignant tumors leads to decrease the level of TSA.

References

1. Yarema, K.J., Goon, S., and Bertozzi, C.R.: Metabolic selection of glycosylation defects in human cells. Nat Biotechnol, 2001; 19(6): 553-8.
2. Scauer, R.: Sialic acids and their role as biological markers. Trends Biochem Sci, 1985; 10: 357.
3. Narayanan, S.: Sialic acid as a tumor marker. Ann Clin Lab Sci, 1994; 24(4): 376.
4. Prabhudas, S.: Evaluation of serum sialic acid, heat stable alkaline phosphatase and fucose as markers of breast cancer. Anticancer Res, 1990; 10: 107.
5. Ginsbourg, M., Musset, M., and Misset, J.L.: Relationship between profilerative activity of bone Marrow micrometastasis and plasma levels of a new cancer marker lipid associated sialic acid in human breast cancer. Biomed Pharmacother, 1986; 40: 388-95.
6. Yanisey, C., Yenice, S., Guner, G., and Guner, S.: Evaluation of tumor-associated antigens and total sialic acid levels in effusions of malignant and benign origin. Biomed Soc Trans, 1996; 24(2): 301.
7. Painbeni, T., Gamelin, E., Cailleux, A., Le-Bouil, A., Boisdron-Celle, M., and Daver, A.: Plasma sialic acid as marker of the effect of the treatment on metastatic colorectal cancer. Eur J Cancer, 1997; 33(13): 2216-20.
8. El-Abbadi, M., Seyfried, T.N., Yates, A.J., Orosz, C., and Lee, M.C.: Ganglioside composition and histology of a spontaneous metastatic brain tumor in the VM mouse. Br J Cancer, 2001; 85(2): 285-92.
9. Lagana, A., Martinez, B., and Marin, D.: Evaluation of lipid-bound sialic acid (LSA) as a tumor marker. Int J Biol Marker, 1995; 10(3): 174-9.
10. Bhuvarahamurthy, V., Balagubramanian, N., Vijayakumar, S., and Govindasamy, S.: Effect of radiation on serum glycoproteins and glycosidases in patients with cervical carcinoma. Int J Gynaecol Obstet, 1995; 48(1): 49-54.
11. Kokoglu, E., Suer, S., Ozyart, E., Syahhan, A., and Sonmz, H.: Plasma fibronectin and sialic acid levels in various types of human brain tumors. Cancer Biochem. Biophys, 1995; 15(1): 35-40.
12. Svennerholm, L.: Quantitative estimation of sialic acids II. A colorimetric resorcinol-hydrochloric acid method. Biochemica Biophysica Acta, 1957; 24: 604-11.
13. Miettinen, T., and Takka-Luukkainen, L.T.: Use of butyl acetate in determination of sialic acid. Acta Chem Scand, 1959; 13(4): 856-7.
14. Katopodis, N., and Stock, C.: Improved method to determine lipid bound sialic acid in plasma or serum. Res Common Chem Path Pharm, 1980; 30(1): 171-80.
15. Harvey, H.A., Lipton, A., and White, D.: Glycoprotein and human cancer. Cancer, 1981; 47: 324.
16. Horgan, E.I.: Total and lipid associated sialic acids in sera from patients with cancer. Clin Chem Acta, 1982; 118: 327.
17. Ann, M., Schwart, Z., and Nonda, K.: Serum lipid- bound sialic acids as a marker in breast cancer. Cancer, 1982; 50: 1815.
18. Schauer, R.: Sialic acids and their role as biological markers. Trends Biochem Sci, 1985; 10: 357.
19. Emmelot, P.: Biochemical properties of normal and neoplastic cell surfaces. A review. Eur J Cancer, 1973; 9: 319.
20. Hogan, R., Yan, A., Fennelly, S.J., Jones, J., Canteveil, B., and Duffy, M.J.: Serum sialic acid and CEA concentration in human breast cancer. Br J Cancer, 1980; 41: 587.
21. Yue, K., Bian, M., Zhu, D., Liu, W., and Siu, S.: Serum lipid associated sialic acid (LSA) in diagnosis and monitoring ovarian cancer. Chung Kuo I Hsueh Ko Hsueh Yuan Hsueh Pao, 1995; 17(2): 128-32.
22. Crook, M.A., Couchman, S., and Tutt, P.: Serum sialic acid in patients with multiple myeloma. Br J Biomed Sci, 1996; 53(3): 185-6.
23. Senra-Varela, A.: Evaluation of lipid associated sialic acid as a tumor marker. Int J Biol Mark, 1995; 10(3): 174.
24. Gaaninger, U., and Deutsch, E.: Plasma sialyltransferase levels as a parameter in the diagnosis and follow-up of gastrointestinal tumors. Cancer Res, 1982; 40: 1300.
25. Griffits, J., and Reynolds, S.: Plasma sialyltransferase total and isoenzyme activity in the diagnosis of cancer of the colon. Clin Biochem, 1982; 15: 46.
26. Stefenelli, N., Klotz, H., Engel, A., and Bauer, P.: Serum sialic acid in malignant tumors, bacterial infections and chronic liver diseases. J Cancer Res Clin Oncol, 1985; 109: 55.
27. Turner G.A., Skillen, A.W., and Buamah, P.: Relation between raised concentrations of fucose ,sialic acid, and choosing suitable serum glycoprotein markers. J Clin Pathol, 1985; 38: 558.
28. Moss, A.J., Bissada, N.K., Boyd, C.M., and Hunter, W.C.: Significance of protein- bound neuraminic acid levels in patients with prostatic and bladder carcinoma. Urology, 1979; 8(2): 182.
29. Macbeth, R., and Bekasi, J.: Plasma glycoproteins in various disease states including carcinoma. Cancer Res, 1962; 22: 1170.