TOTAL AND LIPID-BOUND SIALIC ACID IN THE SERUM AND CEREBROSPINAL FLUID OF PATIENTS WITH BRAIN TUMORS

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SUMMARY

Total sialic acid (=TSA) and lipid-bound sialic acid (=LBSA) were determined in serum and cerebrospinal fluid (=CSF) from 40 patients with various neurosurgical disorders. Of these 27 cases who had brain tumors have been compared with 13 non-neoplastic neurosurgical cases and 18 normal volunteers.

TSA levels were significantly increased in both the serum and CSF of glioma patients, and the serum TSA values were indistinguishable from the benign tumor groups. Conversely the LBSA levels particularly in the CSF, were gradually elevated, patients with brain tumor in line with to their grading of tumor malignancy ($p<0.05$).

This data and other studies suggest that measurement of TSA and LBSA, as a malignant tumor marker, particularly in the CSF, should prove to be useful for diagnosis and follow-up assessment of patients undergoing treatment for malignant brain tumors.

KEY WORDS :

Intracranial tumors, Sialic acid.

INTRODUCTION

Glycoproteins and gangliosides of the cell membrane are known to undergo significant alterations during the malignant transformation. One of the characteristics of this transformation is the production of glyco-compounds with a high content of N-acetylneuraminic acid (=sialic acid) (8).

Warren et al. (14) as well as others have observed that only traces of tumor characteristic sialo-focusyl glycopeptides are found in the serum of healthy subjects while it is found in high concentrations in malignant transformed cells.

Other tumor markers such as carcinoembryonic antigen (=CEA) expected to help the differentiation of malignant tumors from benign ones, proved to be of little value (2). CEA had a high specificity only in the case of CNS metastases.

Increased levels of glycolipid-bound sialic acid in the serum of mice and humans with mammary carcinoma and also of serum glycoproteins in the cancer suggest that measurement of total serum and/or lipid-bound sialic acid might be useful markers of malignancy (1, 9, 10).

Despite the extensive studies of serum sialic acid in relation to many types of tumors, information as regards sialic acid levels in the serum and particularly in the CSF of patients with brain tumors is scanty (5).

We have examined the clinical significance of sialoglycoproteins in both serum and CSF by measuring the concentration of N-acetylneuraminic acid, as total and lipidbound sialic acid (TSA and LBSA), in patients with intracranial tumor compared to other non-tumoral neurosurgical disorders or normal volunteers.

METHODS AND MATERIALS

Both TSA and LBSA levels were assayed in 40 patients with intracranial tumor or a non-tumoral neurosurgical disorder and in 18 normal volunteers.

Patients and tumors examined

The age of patients with benign tumors was between 4 and 65, the average age being 41.8. The patients with malignant tumors were between 8 and 70 years of age with an average of 42.6. All the tumors were removed surgically and graded according to the WHO classification (15) as: Grade 0 (8 cases), Grade 1-2

(6 cases), Grade 3 (7 cases), Grade 4 (4 cases). Histopathological documentation is given in Table I.

TABLE I. Histopathological documentation of intracranial tumors and diagnosis in non-tumoral control group.

Patients	Gradede (WHO)	(n)		
Brain tumors				
Astrocytma G1-2	1-2	(3)		
Astrocytoma G3	3	(5)	(10	
Astrocytoma G4	4	(2)		
Ependymoma	1-2	(2)	(3)	
Ependymoma	3	(1)		
Optic glioma	1-2	(1)		
Meningioma	0	(4)		
Medulloblastoma	4	(1)		
Schwannoma	0	(2)		
Anaplastic meningioma	3	(1)		
Epidermoid tumor	0	(1)		
Dermoid tumor (infected)	0	(1)		
Monstrocellular sarcoma	4	(1)		
Metastatic tumor (primary site lung)	4	(2)		(27)
Controls				
Lomber HNP		(7)		
ACM Aneurysm		(1)		
Benign intracranial hypertension		(1)		
Cervical HNP		(1)		
Epilepsy (Amgdalectomy performed)		(1)		(11)
Cerebral abscess				
Temporal intracerebral abscess		(2)		(02)
		Total		**(40)**
Normal volunteers		(18)		
		Over-all Total		**(58)**

All brain tumor patients underwent glucocorticoid treatment (12-16) mg/day). Glucocorticoid administered to non-neoplastic neurosurgical patients was assessed as another control group. CSF was obtained via ventricular tap pre-or peroperatively in the tumor groups. Samples were subjected to centrifugation at 3000 rpm/5min., and the supernatant was assayed immediately or stored at -20°C until used.

Sialic Acid Assay

Total sialic acid was determined by using perchloric acid, paradimethyl-amino benzaldehyde and hydrochloride acid depending on the assay of sialic acid by colorimetric method (4). Lipid-bound sialic acid was measured with some modifications according to a recent technique for serum described by Katapodis et al. (6). Briefly, it depends basically on the assay of LBSA coloration by resorcinol after extraction with chloroform/methanol in cold conditions.

The presence of inflammation caused by bacterial or viral infection was ruled out in non-tumoral controls as well as the tumor group by determining the leucocyte count (cut-off=8000 cells/ml) and the concentration of C-reactive protein (CRP, cut-off=1.0 mg/dl).

Statistical Methods

The distribution within different diagnostic groups was investigated for deviations from the norm. The various tumor groups were compared using the student's t-test (11).

RESULTS

Serum TSA and LBSA levels

TABLE II shows the mean values and standart deviations of the sialic acid concentration determined in various tumors and in normal volunteers. With the exception of brain abscesses there was a significant difference in the sialic acid serum concentrations in the patients with various tumors compared with the normal volunteers, as far as LBSA levels were concerned. Comparison with the average sialic acid concentrations (both TSA and LBSA) in benign tumors with the normal, yielded similar results but were not considered statistically significant.

Both the mean TSA and LBSA levels were significantly elevated in the malignant tumor group ($p<0.005$ and $p<0.01$ respectively). In glioma patients the serum LBSA levels were significantly elevated compared to the serum TSA levels ($p<0.02$).

CSF Total and Lipid-bound sialic acid levels

Total and lipid-bound sialic acid levels in the CSF of patients with brain tumor, and non-tumoral neurosurgical disorders as controls are summarized in TABLE III.

TABLE II. Serum Total and Lipid-bound Sialic Acid in Brain tumors and controls (mg/100ml serum)

Paients	(n)	Age (range)	TSA + s.d. (n)	LBSA + s.d. (n)
Brain Tumors	(25)	39.5 (4-65)	92.63* p 0.005 +25.17	48.28* p 0.001 +30.59
Benign tumors (WHO:GO)	(6)	45.0 (17-65)	95.15 NS +22.29	36.73 NS +22.94
(WHO:GO-2)	(10)	44.8 (4-65)	95.88 NS +30.02	45.08* p 0.05 +31.90
Glioma (WHO:G1-4)	(9)	39.8 (4-64)	91.71 NS +29.02	54.09* p 0.02 +32.94
Malignant tumors (WHO:G3-4)	(9)	38.3 (8-64)	89.65* p 0.005 +14.00	52.56* p 0.01 +27.37
Cerebral abscess & Infected dermoid Tm.	(3)	18.7 (14-25)	90.73 NS +31.42	26.84 NS +33.56
Glucocorticoid administered non-neoplastic neurosurgical patients	(6)	27.0 (17-39)	53.65 NS +10.59	26.84 NS +11.90
Control Epilepsy	(1)	39	45.99 (1)	16.64 (1)
Normal volunteers	(18)		72.83 +10.35	18.32 +01.68

TSA=Total sialic acid
LBSA=Lipid-bound sialic acid
NS=Nonsignificant compared with normal (Student's t test)
* Statistically significant

TABLE III. CSF Total and lipid-bound sialic acid in Brain Tumors and non-tumoral neurological disorders. (mg/100ml CSF)

Patients	(n)	Age (range)	TSA + s.d. (n)	LBSA + s.d. (n)
Brain Tumors	(11)	34.8 (4-70)	2.50* p 0.005 +1.52	0.52* p 0.05 +0.23
Benign tumors (WHO:GO)	(5)	30.8 (4-57)	1.4 NS +0.51	0.50 NS +0.19
Glioma (WHO:G1-4)	(8)	35.4 (4-70)	2.75* p 0.01 +1.13	0.62* p 0.01 +0.20
Malignant tumors (WHO:G3-4)	(6)	38.2 (8-70)	3.37* p 0.05 +1.52	0.54* p 0.05 +0.26
Glucocorticoid administered non-neoplastic neurosurgical patients	(6)	42.0 (30-40)	0.98 NS +0.67	0.48 NS +0.22
Non-tumoral disorders	(10)	40.5 (33-56)	1.20 (1) +1.22	0.32 (9) +0.12
Lumber HNP				
Cervical HNP				
ACM Aneurysm				
BIH			4.77 (1)	0.26 (1)

TSA=Total sialic acid
LBSA=Lipid-bound sialic acid
HNP=Herniated nucleus pulposus
BIH=Benign intracranial hypertension
NS=Not-significant compared with the non-tumoral group (Student's t test)
* Statistically significant.

The average CSF total sialic acid (TSA) value was 1.20 and LBSA value was 0.32 in the non-neoplastic group. Mean TSA and LBSA values were significantly elevated in malignant tumors and gliomas ($P<0.05$ and $p<0.01$) respectively.

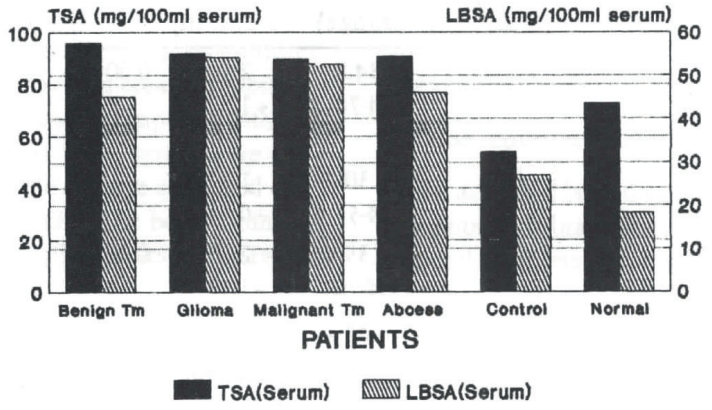
Over-all calculation of the cumulative frequency of benign and malignant tumors showed 90.0 % of the malignant tumors were above the cut-off level while 29.6 % of the benign tumors were below the cut-off level. (Cut-off levels for TSA were 83.0; 2.4 mg/100 ml and for LBSA were 20.0; 0.4 mg/100ml serum and CSF respectively) From these data, the sensitivity was calculated to be 93.0 %. In all these patients an infectious inflammatory process was excluded by normal leucocyte count and immunoglobulins were also found within the normal range. A patient in the non-neoplastic group with a high level of TSA diagnosed as benign intracranial hypertension was excluded from the study.

DISCUSSION

Both the sialic acid containing glycoproteins and the gangliosides of the cell membrane are known to undergo significant alteration during malignant transformation (3, 13). Determination of total sialic acid in the serum of in the CSF reflects the combined levels of these glycoconjugates (5, 9, 12). The mechanism of elevated sialic acid in the CSF has been suggested to be a selective cleavage of surface glycoproteins that may result in the "shedding" of glycopeptides which eventually find their way into the blood circulation and the CSF (5).

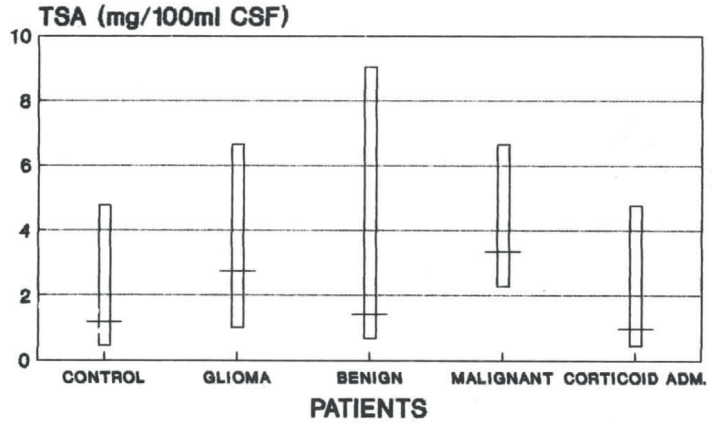
Tumors described as benign histologically showed a significantly lower sialic acid concentration than many of those which were described as malignant (7). There was correlation between the size of the tumor and sialic acid concentration. Similarly, intraventricular location of the neoplasm and obstructive hydrocephalus presented by the tumoral mass were not

SIALIC ACID
TSA&LBSA(Serum)



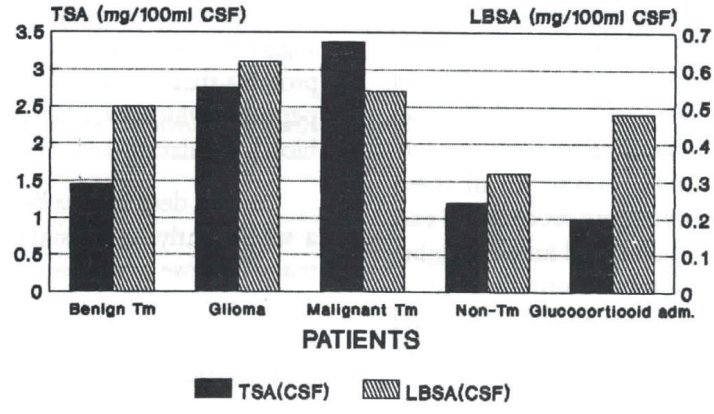
TSA=Total sialic acid
LBSA=Lipid-bound sialic acid

SIALIC ACID
TSA(CSF)



TSA=Total sialic acid
CSF=Cerebro-spinal fluid

SIALIC ACID
TSA&LBSA (CSF)



TSA=Total sialic acid
LBSA=Lipid-bound sialic acid

found to be significant effective factors in increasing the sialic acid concentration even in the CSF. This must be attributed to the varying amounts of glycocompounds on tumor cell membranes.

There are reports stating that the glycoprotein sialic acid in the CSF of the adult is approximately 0.7 mg/100 ml, and that significant elevations of CSF glycoproteins occur in patients with brain tumors (5). Although glycoproteins in CSF are derivied mainly from blood, glycoproteins originating from the brain may also exist.. As for the permeability of the blood-brain or blood-CSF barriers to glycoconjugates it is known to increase in CNS diseases, including neoplasia. Our TSA data on gliomas are in agreement with the increased serum and CSF total sialic acid in patients with glioma reported elsewhere (5, 9). On the other hand, all our patients with glioma and malignant tumors had increased LBSA concentrations, even thought their TSA levels particularly in the glioma group, were indistinguishable from the controls and the normal volunteers.

The discrepancies between TSA and LBSA levels in the CSF of patients with brain tumors could be explained by the fact that in malignancy the metabolism of both glycoproteins and gangliosides can be aberant.

We have found that both TSA and LBSA assays in the serum and the CSF are valuable particularly in glioma and malignant tumor patients with a relatively higher specificity rate observed compared with the benign brain tumor group (95.3 % vs 81.8 %).

Comparison of sensitivity of TSA ve LBSA levels in the brain tumors showed slight priority of LBSA over TSA with values of 97.2 % to 88.9 % respectively.

High levels of sialic acid may indicate that tumor resistance against previously effective chemotherapy is increased particularly during the follow-up assessment of patients with malignant brain tumor.

The measurement of sialic acid concentration in serum and/or CSF can be performed easily and quickly, and it is valuable in determining the tumor malignancy especially in patients undergoing treatment for gliomas and other malignant brain tumors.

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