place where the populations sampled are living[12] supports the conjecture of a relation between ACP_1 and metabolism. It is possible that in A and BA subjects the pattern of flavo-enzyme activities may allow a full response to stimuli (environmental and/or genetic) aimed to maximize body mass.

It is interesting to note that considering the whole sample of obese children, no significant difference is observed in the distribution of ACP_1 phenotypes with respect to the general population. On the other hand, when the sample of obese children is subdivided according to severity of disease a highly significant pattern of association with the ACP_1 phenotype emerges. The data suggest that genetically determined variability of ACP_1 activity influences the degree of obesity, but only when departure from 'normality' has already been triggered.

The pattern of association described here in obese children is in line with that observed in newborns[4], and encourages[5] further investigations on the role of ACP_1 in obesity and especially in the predisposition to extreme body mass deviations.

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Plasma lipid-bound sialic acid alterations in neoplastic diseases

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Summary. Plasma lipid-bound sialic acid (LSA) was assayed in normal volunteers, patients with non-malignant diseases, and a variety of cancer patients. Mean plasma LSA in 50 normal volunteers, 16 patients with non-malignant diseases, 54 breast cancer, 17 lung cancer, 15 colon cancer, 7 ovarian cancer, 5 prostate cancer, 4 leukemia, 4 gastrointestinal, 3 thyroid cancer, 3 pancreas cancer and 2 adrenal cancer patients were 17.7, 23.2, 58, 85, 56.7, 46.2, 56.7, 53.3, 31.1, 33.2 and119.5 mg/dl, respectively. None of the normal volunteers had elevated plasma LSA values. Plasma LSA level was not significantly different in male and female volunteers. Two patients with rheumatic arthritis had LSA values slightly elevated over the mean + 2 SD for the normal volunteers. Two out of 114 different cancer patients had plasma LSA levels within normal range exhibiting 98.2% sensitivity of the assay. Plasma LSA, which is relatively simple to assay, may be used as a tumor marker in wide variety of neoplastic diseases.
Key words Lipid-bound sialic acid; cancer patients; tumor marker; non-malignant diseases.

The surface of cancer cells differs in many respects from normal cells[1]. Neoplastic transformations of a variety of cell types are associated with changes in the composition of membrance glycoproteins[2,3], a major structural component of the cell surface. One such change is in the level of sialic acid on the cell surface[4,5]. Sialic acid levels are higher in cancer patients than normal controls[6-9]. Studies have indicated that assay of lipid-bound sialic acid (LSA) may be more useful and discriminating than the assay of total sialic acid[10,11]. Dnistrian and Schwartz evaluated[12] the LSA and carcino-embryonic antigen (CEA) in cancer patients and concluded that LSA was increased in more patients with leukemias, lymphomas, Hodgkin's disease and melanomas in comparison to the CEA. The purpose of the present investigations is to extend our knowledge of LSA in a wide variety of neoplastic patients.

Materials and methods

Blood samples were obtained in citrate tubes from normal volunteers (age 25–35 years), patients with non-malignant diseases (2 sickle cell anemia, 1 hepatitis and 13 rheumatoid arthritis), and a variety of cancer patients

receiving services at Hubbard Hospital of Meharry Medical College. Plasma was collected by centrifugation and used directly or stored frozen at −80 °C. Frozen plasma samples were allowed to thaw at room temperature prior to the assay. N-acetylneuraminic acid was purchased from Sigma Chemical Co., St. Louis, MO. All other chemicals were bought from MCB Manufacturing Chemists Inc., Cincinnati, OH.

LSA ASSAY. LSA was assayed in plasma samples in triplicate by using the method described by Dnistrian et al.[11]. A cold mixture of plasma sample (45 µl) and water (150 µl) was extracted with 3 ml of chloroform-methanol (2:1 v/v) and the extract was partitioned with 500 µl of cold water. One ml of upper layer was transferred to another tube and 50 µl of phosphotungstic acid (1 g/ml) was added to the tube. The precipitate thus obtained was separated by centrifugation and resuspended in 1 ml of water and used for LSA determination. LSA was assayed by the resorcinol method. The extracted blue color was read at 580 nm spectrophotometrically and the sialic acid level was calculated from a standard curve obtained from N-acetylneuraminic acid.

Five random samples were assayed for plasma LSA for five consecutive days. All the values were within a range of 1 mg/dl indicating a good precision of this assay.

Results and discussion

LSA levels in normal volunteers, patients with non-malignant diseases, and various cancer patients is given in figure 1. Mean LSA level in 50 normal volunteers was 17.7. (range 6.3–30.0) mg/dl. All of the 50 volunteers were healthy adults (age 25–35 years). The mean LSA levels for the 28 male and 22 female volunteers were 18.5 (range 8.5–30.0) mg/dl and 16.9 (range 6.6–27.4) mg/dl, respectively. The LSA values were not significantly different from each other (Student's t-test was used for statistical analysis) between male and female volunteers. The mean LSA level in 16 patients with non-malignant diseases was 23.2 mg/dl. Mean LSA levels in 54 breast cancer, 17 lung cancer, 15 colon cancer, 7 ovarian cancer, 5 prostate cancer, 4 leukemia, 4 gastro-intestinal cancer, 3 thyroid cancer, 3 pancreas cancer and 2 adrenal cancer patients were 58, 85, 53, 56.7, 86.2, 56.7, 53.3, 31.1, 33.2 and 119.5 mg/dl, respectively. None of the normal volunteers had elevated plasma LSA levels. In addition, plasma LSA was not elevated in 2 sickle cell anemia, 1 hepatitis and 11 rheumatoid arthritis patients. However, 2 patients with rheumatoid arthritis had LSA values slightly elevated over the mean +2 SD for the normal volunteers. The data indicated 97% specificity of this test. However, two out of 114 cancer patients had values within normal range exhibiting 98.2% sensitivity of the

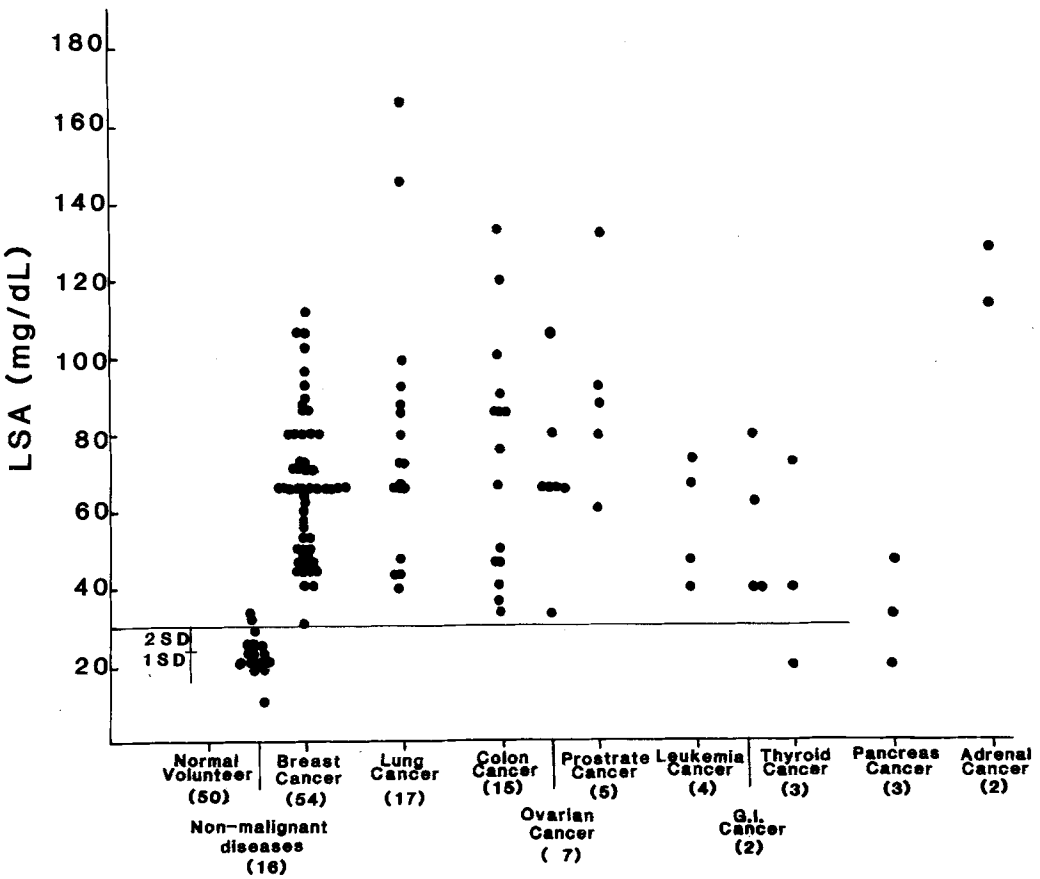

Figure 1. Plasma LSA in normal volunteers, patients with non-malignant diseases and variety of cancer patients. The data were analyzed statistically using Student's two-tailed t-test. Values for cancer patients were significantly ($p < 0.05$) higher than normal volunteers and non-malignant patients.

Plasma LSA levels in cancer patients

Type of cancer	Early stage		Advanced metastatic stage	
	Number of patients	Mean LSA level mg/dl	Number of patients	Mean LSA level mg/dl
Breast cancer	40	46.3	14	66.4
Lung cancer	4	41.8	13	108.9
Colon cancer	6	42.3	9	96.2
Ovarian cancer	1	33.4	6	82.3
Prostate cancer	–	–	5	86.2

assay. Plasma LSA level in patients with early stage (with lower tumor burden) and advanced metastatic stage (with higher tumor burden) is given in the table. It was generally observed that cancer patients with metastatic diseases had relatively higher LSA values.

Serial determination of LSA was made in 3 colon, 1 prostate and 1 ovarian cancer patients. The data is shown in figure 2. Two colon cancer patients, who died with the metastatic disease had significantly elevated LSA values. The prostate cancer patient also died with metastatic disease and had increased plasma LSA levels. The ovarian cancer patient plasma LSA was measured after surgery. As can be seen from figure 2, there was a gradual decrease in plasma LSA level.

The results from this study indicated that plasma LSA level is elevated in all types of cancer patient studied. Dnistrian et al. observed[11] plasma LSA levels <20 mg/dl in normal subjects but elevated levels in patients with benign diseases (13%), primary breast cancer (47%), and recurrent metastatic breast cancer (62%). LSA levels were decreased in patients who were responding to chemotherapy. Elevated LSA levels were observed with progression of the diseases. The LSA level was increased in more patients with leukemias, lymphomas, Hodgkin's disease, and melanomas[12]. A study by Munjal et al. reported[13] highest LSA values in lung cancer and lowest in breast cancer patients. A correlation was observed between total sialic acid and lipid-bound sialic acid[14]. In this study, highest LSA levels (119.5 mg/dl) were observed in adrenal cancer patients. However, LSA level was measured in only 2 adrenal cancer patients. Patients with lung cancer and prostate cancer also exhibited higher elevations in LSA level, which is in agreement with the data of Munjal et al.[13]. But in our experience, the lowest elevation in LSA level was not observed with breast cancer. Our data exhibited a 97% specificity with 98.2% sensitivity for LSA as a tumor marker, which is in agreement with results from other laboratories[12,15]. In contrast, a relatively low sensitivity of 40% and 44% was reported by Erbil et al.[16] and Kakari et al.[17], respectively. The difference in sensitivity may be due to different sample collection and analytical techniques, and/or patient selections.

The use of the assay of plasma sialyltransferase and total sialic acid as a tumor marker has been investigated in our laboratory[9,18,19]. The assay of plasma LSA in the same patients and normal volunteers exhibited better specificity and sensitivity than plasma sialyltransferase and total

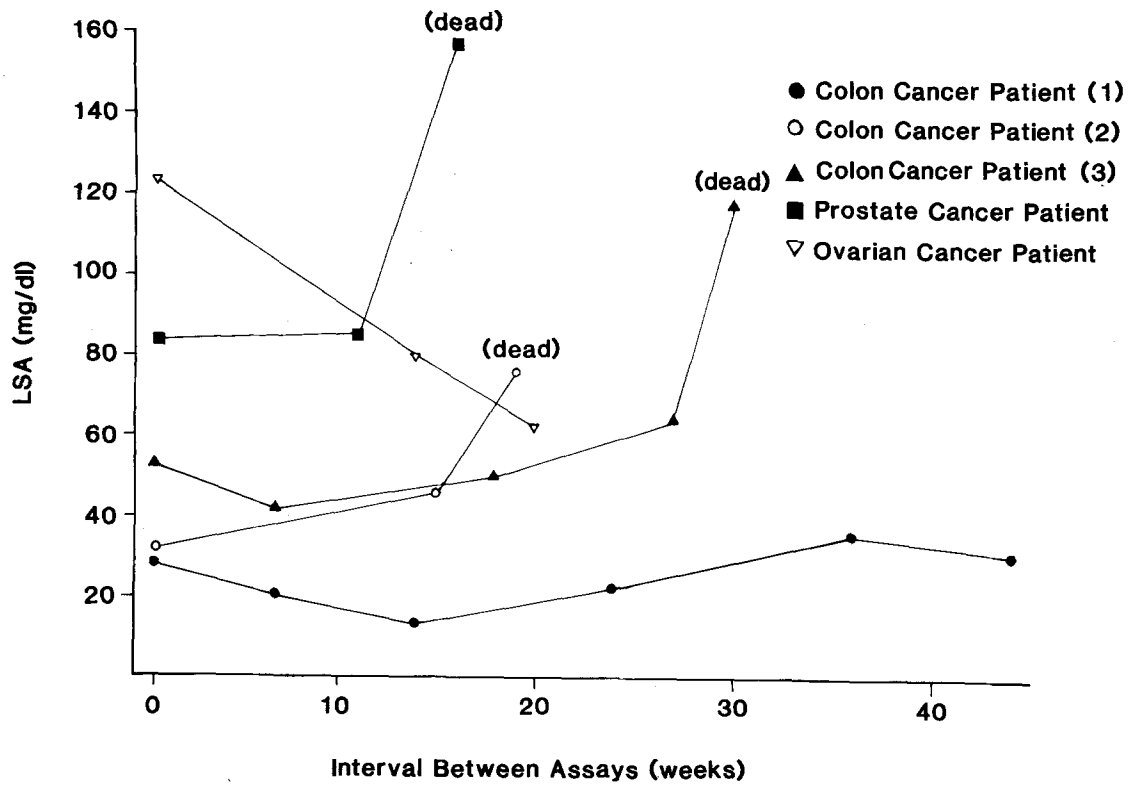

Figure 2. Serial change in plasma LSA in 3 colon, 1 prostate, and 1 ovarian cancer patients.

sialic acid determinations[18,19]. Since assay of plasma LSA is relatively simple and has better specificity and sensitivity in patients and normal individuals studied, it could be useful as a prognostic determinant in a variety of neoplastic conditions.

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Deficiency of kallikrein-like enzyme activities in cerebral tissue of patients with Alzheimer's disease

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Summary. We examined the changes in the intracerebral activities, at the time of postmortem autopsy, in patients with Alzheimer's disease. When compared with the control group, the activity of kallikrein-like enzyme was significantly decreased, while prolyl endopeptidase activity increased, in the patients group. Aprotinin inhibited 50% of the activity of the former enzyme at 2×10^{-7} M. Taken together with the results of a multivariate study, the above findings may indicate that intracerebral kallikrein deficiency plays an important role in the pathogenesis of Alzheimer's disease.
Key words. Alzheimer's disease; brain; proteases; kallikrein; prolyl endopeptidase.

Proteases not only govern protein metabolism in general, but are also concerned with turnover of the structural proteins of cells, and are thus closely related to cell functions. Because of this, many investigators have tried to connect age-related changes in cell functions with protease changes. Recently, several reports were published which suggested the accumulation of abnormal protein in the brains of patients with Alzheimer's disease[1-4]. Also, as more direct evidence, injection of a protease inhibitor into animal brains was shown to induce the formation of lysosome-associated granular aggregates (dense bodies) which closely resembled the ceroid-lipofuscin that accumulates in certain disease states and during aging[5]. These reports prompted us to study the protease changes in the brain of patients with Alzheimer's disease.

Materials and methods

We selected 1 case of typical Alzheimer's disease (60-year-old female), 6 cases of senile dementia of the Alzheimer type (5 females, 84.8 ± 3.5 years; 1 male, 91 years) and 6 control subjects (3 males, 88.7 ± 5.7 years; 3 females, 72.3 ± 1.3 years). Histologically, brains from the 7 cases with dementia had senile plaques and neurofibrillary tangles in various amounts, but no vascular lesions detectable by routine examination. Control subjects had neither a clinical record of dementia nor pathologically significant lesions in the brain. The cerebral tissues had been stored at the time of autopsy in a deep freeze at −70 °C. In all cases the occipital lobe of the brain was used for enzymatic examination. The brain homogenates were prepared in phosphate-buffered saline (PBS, pH 7.2) by using a tissue homogenizer, Ultraturrax, at the maximum speed for 1 min. The homogenate was centrifuged (3000 g for 20 min), and the supernatant fluid was withdrawn for the measurement of enzymatic activities. The diagnosis was made according to pathological and clinical findings.

Determination of enzyme activities. The substrates, enzymes and their sources were as follows (see table 1 for abbreviations): Glu · NA, Arg · NA, Pro · NA, Gly-Arg · NA, Lys-Ala · NA, and Gly-Pro · NA from