

Pervin VURAL<sup>1</sup>  
Mukaddes CANBAZ<sup>1</sup>  
Dilek SELÇUK<sup>2</sup>

## Total and Lipid-Bound Sialic Acid Levels in Actinic Keratosis and Basal Cell Carcinoma

Received: September 07, 1998

**Abstract:** Malignant cells have more sialic acid in their cell membrane than normal cells. Many studies have shown that mean values of total sialic acid (TSA) or lipid-bound sialic acid (LSA) were higher in patients with cancer than in normal subjects. Sialic acid levels correlate with stage of disease, tumor burden, degree of metastasis and recurrence of disease. The aim of the present study was to determine the plasma TSA, LSA, total protein (TP) and TSA/TP values in basal cell carcinoma (BCC) and evaluate if they are sensitive markers for malignancy.

Plasma TSA and LSA were measured according to the method described by Katopodis and co-workers and TP on an autoanalyser in 16 healthy controls, 13 pathologic controls (actinic keratosis: AK) and 12 patients with BCC.

Data analysis indicated a significant increase in TSA ( $P<0.0001$ ) and TSA/TP ( $P<0.0001$ ) values in the BCC group compared with the healthy controls. There was a significant increase in TSA ( $P<0.001$ ) and TSA/TP ( $P<0.0001$ ) values in AK (Pathologic controls) when compared with the healthy ones. When AK and BCC groups were compared, no differences between TSA, LSA, TP and TSA/TP values were observed. No significant difference was observed between the groups in terms of LSA values. The results indicate that TSA and TSA/TP along with other clinical and histopathological criteria may be valuable in establishing diagnosis of BCC.

**Key Words:** Total sialic acid (TSA), Lipid-bound sialic acid (LSA), Actinic keratosis (AK), Basal cell carcinoma (BCC).

Departments of <sup>1</sup>Biochemistry and Clinical Biochemistry, <sup>2</sup>Dermatology, Istanbul Medical Faculty, Istanbul University, 34390 Capa, Istanbul-Turkey

### Introduction

Sialic acid (SA), an important component of glycoproteins and glycolipids, is found in negatively charged surface polyanions on various cell membranes and plays an important role in the antigenic characterization of cells (1). Serum sialic acid levels have been used as laboratory markers in a variety of pathological conditions (2–7). Marked elevation of serum sialic acid concentrations (TSA and/or LSA) that correlate with the clinical activity of a disease have been documented in many malignancies (8–16). Increased sialic acid levels in malignant melanoma have been reported (8, 9). Plucinsky et al. have studied TSA, LSA and TSA/TP ratios in various cancers (including skin cancer, but it was not determined which type of cancer) and indicated that TSA and TSA/TP values are elevated in patients with cancer compared with healthy controls (17). Apart from their paper there are no reports on serum/plasma TSA

and LSA in skin cancer and also no data about precancerous disorders of the skin.

The role of sialic acid in the neoplastic process prompted us to investigate the TSA, LSA, total protein (TP) and TSA/TP values of plasma in BCC and AK. To the best of our knowledge, this is the first study of sialic analysis of the plasma of BCC and AK.

### Materials and Methods

A total of 25 patients, 13 with actinic keratosis (pathologic controls) and 12 with basal cell carcinoma (BCC group) followed up at the Dermatology Department of the Medical Faculty of Istanbul were included in the study. Actinic keratosis and basal cell carcinoma diagnosis was supported by routine histopathological evaluation of the biopsy material. The median age of patients with AK (10 women, 3 men) was 64 (range 47–80) and of

these with BCC (7 women, 5 men) was 61 (range 44–73). Duration of the disease was  $4.5 \pm 1.7$  years for AK, and  $5.5 \pm 1.2$  years for BCC patients. Smoking patients and those with hypertension, atherosclerosis, diabetes mellitus or any heart diseases were excluded from the study. 16 healthy nonsmoking adults (9 women, 7 men), 62 years old (range 45–74) were the control group (healthy controls). Patients were omnivores taking a free range diet. Venous blood samples from the study and control group were collected in heparinized tubes from the antecubital vein at 10AM after overnight fasting and centrifuged at 2000 rpm (10 minutes) to remove the plasma. The plasma samples were stored frozen ( $-20\text{ }^{\circ}\text{C}$ ) until used. Stability studies showed that the sialic acid values were constant under these conditions for up to 6 months and that freezing and thawing had no effect (18, 19).

LSA was measured according to the method described by Katopodis and co-workers with some modifications (18, 19). Forty-five  $\mu\text{L}$  serum was placed in screw-capped tubes with 150  $\mu\text{L}$  water. The tubes were vortexed for 10 seconds and immediately placed in ice. Three ml of cold ( $4\text{ }^{\circ}\text{C}$ ) chloroform/methanol (2:1, v/v) mixture was added to each tube for total lipid extraction, the tubes were capped and vortexed for 30 seconds. 0.5 ml cold water was added, the tubes were recapped and mixed for 30 seconds by repeated inversion. The tubes were centrifuged for 5 minutes at 2500 rpm at room temperature. One milliliter of the upper phase was transferred to another screw-capped tube. Fifty  $\mu\text{L}$  of phosphotungstic acid solution (1 g/ml) was added to each tube, the tubes were vortexed and allowed to sit at room temperature for 5 minutes. Then the tubes were centrifuged at room temperature for 5 minutes at 2500 rpm. After that the supernatant was decanted and the remaining pellets were redissolved in 1 ml  $37\text{ }^{\circ}\text{C}$  water by vigorously vortexing for 1 minute. For determination of TSA values, 20  $\mu\text{L}$  of plasma and 980  $\mu\text{L}$  of water were

pipetted into screw-capped tubes, the tubes were vortexed and placed in ice. Then to each tube for TSA and LSA was added 1 ml of resorcinol reagent (including 10 ml 2% (w/v) stock resorcinol in water, 9.75 ml water, 0.25 ml 0.1 M  $\text{CuSO}_4$ , brought to a final volume of 100 ml with concentrated HCl). Then each tube was capped, vortexed, and placed in  $100\text{ }^{\circ}\text{C}$  boiling water (15 minutes), followed by 10 minutes in an ice bath. Two milliliters of butylacetate/n-butanol (85: 15 v/v) was added to the reaction mixture, the tubes were vortexed and centrifuged at 2500 rpm for 10 minutes at room temperature. The supernatant was read spectrophotometrically at 580 nm. As a standard 5, 10, 20, 30  $\mu\text{g/ml}$  solutions of N-acetylneuraminic acid (NANA) (Sigma Chemical Co.), were used, prepared from 40  $\mu\text{g/ml}$  stock solution. For calculation of LSA values, the final experimental value was multiplied by 1.3 (a factor to correct for the volume in the extraction step). Coefficients of variation (CV%) for TSA and LSA measurements were 3.2% and 3.7%, respectively. Plasma total protein was measured on an autoanalyser. Data was given as mean  $\pm$  standard deviation (SD) and student's t-test for impaired data and simple correlation analysis were used for the statistical evaluation.

**Results**

Table 1 shows the comparison of TSA, LSA, TP and TSA/TP values from plasma samples of patients with AK, BCC and the healthy controls. Data analysis indicated significant differences in TSA, and TSA/TP values in BCC group when compared with the healthy controls ( $P < 0.0001$ ). Also there was a significant increase in TSA and TSA/TP values in the AK group in comparison with the healthy controls ( $P < 0.001$  and  $P < 0.0001$ , respectively). When the AK and BCC groups were compared, we did not observe any difference between the four parameters. No significant difference was observed

Group	TSA (mg/dl)	LSA (mg/dl)	TP (g/dl)	TSA/TP (mg/g)
Controls (16)	$59.70 \pm 7.22$	$15.12 \pm 1.80$	$7.29 \pm 0.51$	$8.14 \pm 0.91$
AK (13)	$70.87 \pm 8.63$ $P < 0.001^*$	$15.07 \pm 3.45$ NS*	$7.38 \pm 0.54$ NS*	$9.73 \pm 1.16$ $P < 0.0001^*$
BCC (12)	$75.24 \pm 10.91$ $P < 0.0001^a$ NS <sup>+</sup>	$17.68 \pm 5.02$ NS <sup>a</sup> NS <sup>+</sup>	$7.54 \pm 0.41$ NS <sup>a</sup> NS <sup>+</sup>	$9.93 \pm 1.16$ $P < 0.0001^a$ NS <sup>+</sup>

Table 1. Plasma total sialic acid (TSA), lipid-bound sialic acid (LSA), total protein (TP) and TSA/TP values of patients with actinic karatosis (AK), basal cell carcinoma (BCC) and control group (Mean  $\pm$  SD and p values).

\* When AK and Control groups were compared <sup>a</sup>When BCC and control groups were compared, <sup>+</sup>When BCC and AK groups were compared, NS: no significance,  $P > 0.05$ .

in LSA levels between the healthy controls and AK, the healthy controls and BCC, or the AK and BCC groups. No significant difference was observed in TP concentrations in the control, AK or BCC groups. There was no correlation between duration of disease and TSA, LSA levels in both BCC and AK groups. A positive correlation

was found between TSA and LSA concentrations in BCC and AK patients ( $r=0.8018$ ,  $p<0.01$  and  $r=0.8974$ ,  $P<0.01$ ) and the controls ( $r=0.7737$ ,  $P<0.02$ ) (Figure 1). Normal TSA and LSA values detected in our healthy controls were similar to the ones reported in the literature (14, 17).

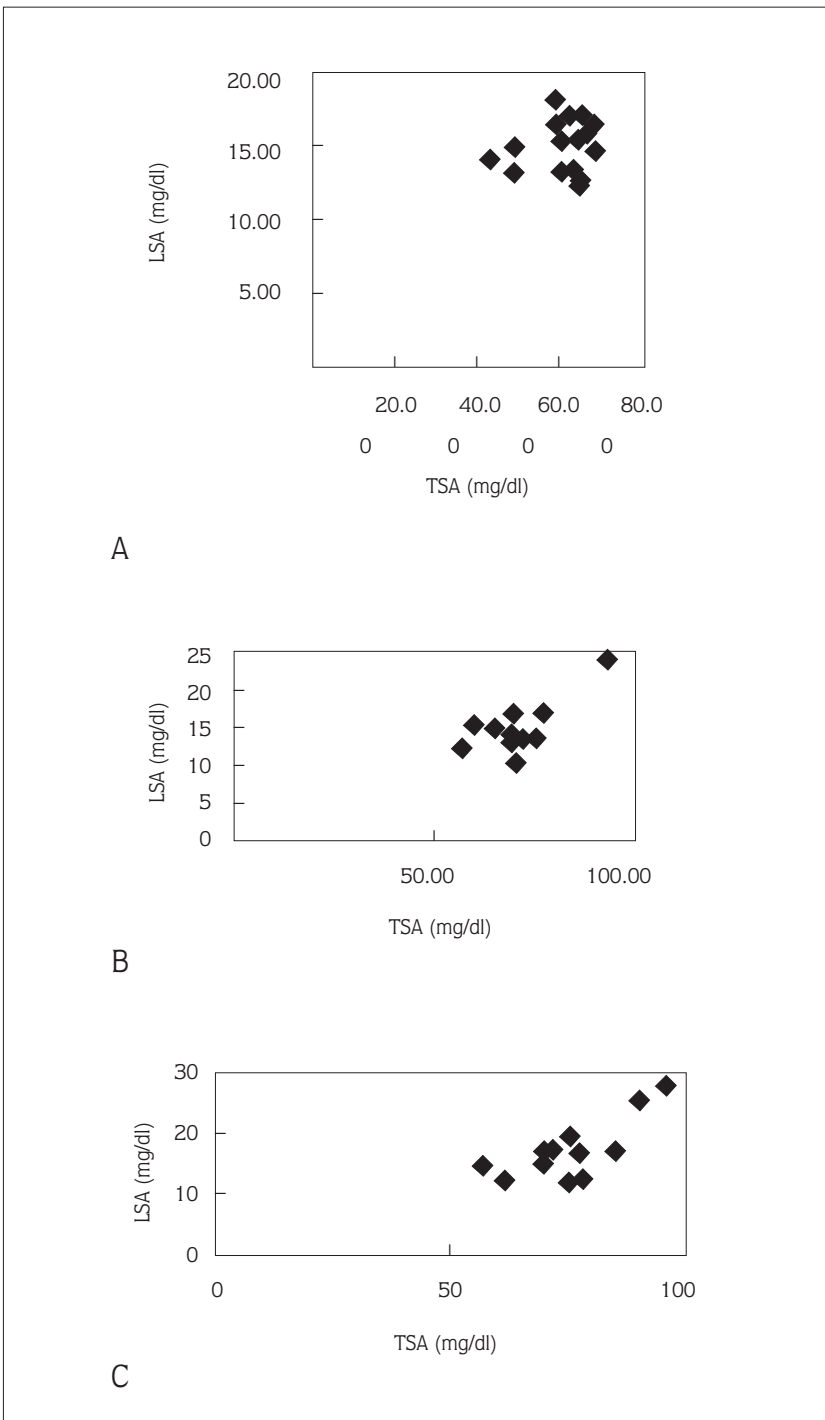


Figure 1. The correlation between plasma TSA and LSA in control (A,  $r=0.7737$ ,  $p<0.02$ ), actinic keratosis (B,  $r=0.8974$ ,  $P<0.01$ ) and basal cell carcinoma (C,  $r=0.8018$ ,  $P<0.01$ ) patients.

## Discussion

Neoplastic cells, surface glycoproteins and glycolipids have different carbohydrate compositions. As a result of increased turnover, secretion, and/or shedding, these glycoproteins and glycolipids can be released into the sera (5, 7). The major constituent of glycoproteins and glycolipid is sialic acid, that usually occurs as a terminal component at the nonreducing end of their carbohydrate chains (1). Malignant cells have been reported to have more sialic acid in their cell membrane than normal cells (5–7). Moreover, elevated serum levels of TSA and/or LSA have been observed in many malignancies (5–8, 18). Serum TSA levels are significantly increased in malignant melanoma (8, 9) and these levels are directly related to tumor burden (8) and disease recurrence (9). A significant correlation has been demonstrated between TSA levels, activity and tumor stage of breast cancer (12), and between TSA, LSA values and extent of metastases in colorectal cancer (11). Plucinsky et al. have studied TSA, LSA and TSA/TP values in various cancers and indicated that TSA and TSA/TP are elevated in patients with cancer compared with healthy subjects (17). However, elevated TSA levels have been found in many circumstances apart/from malignancy, such as myocardial infarction (20) and some autoimmune disorders (21–23).

In the present study plasma TSA levels in the BCC group were significantly increased in comparison with the healthy controls. There was no difference in TSA values in BCC when compared with the pathologic controls (AK). TSA concentrations in the pathologic controls were also increased compared with healthy subjects. LSA levels were essentially comparable in all three groups. A statistically significant increase was observed between

BCC and the healthy controls, and between the pathologic controls (AK) and healthy ones with respect to TSA/TP values. No significant difference of TSA/TP values was observed when BCC patients were compared with the AK group. All these findings show that in BCC there are elevated TSA levels as in cancer in general. TSA/TP values follow the same pattern as plasma TSA concentrations. The TSA increment is probably related to increased turnover of malignant cells (5–7). Significant TSA and TSA/TP elevations were seen also in the pathologic controls (AK), indicating the lack of specificity of these markers. The explanation of the fact that there is no significant difference between BCC and AK (there is a nonsignificant increase in TSA in BCC) is probably the slow clinical progression of BCC. The relatively small number of patients enrolled into the study, is probably another explanation of the above mentioned finding. Some studies report that LSA is a better indicator of malignancy in prostate and bladder (15) and breast (14) cancer. We found nonaltered concentrations of plasma LSA levels, which suggest that LSA cannot be used as an indicator of malignancy in BCC.

The results of this investigation indicate that TSA and TSA/TP are sensitive markers for detecting BCC. However, although TSA and TSA/TP are sensitive, they lack specificity since we also found increased levels in the pathologic controls (AK). TSA and TSA/TP values along with other clinical and biochemical criteria may be valuable in establishing diagnosis of BCC.

*Correspondence author:*

*Pervin VURAL*

*Belediye Cad. Hakan Apt. No: 4/3*

*34840 Avclar-Istanbul*

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