Serum Total Sialic Acid and Lipid Bound Sialic Acid Levels Among Patients with Different Sites of Oral Cavity Cancer

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Abstract

Serum total sialic acid (TSA) and lipid bound sialic acid (LSA) levels were determined by spectrophotometric methods in 23 patients with oral cavity cancer(OCC). Of them 5 patients have carcinoma of the anterior 2/3 of the tongue with an average age of {60±8.0 years} and 2 males patients with carcinoma of the inner surface of the cheek with an average age {68±8.0 years} and 8 patients had carcinoma of the floor of the mouth with an average {62±9.0 years} and 8 patients with carcinoma of the hard palate with an average age {58±8.0 years}. They were compared with 32 healthy controls. Significant increase ($P<0.001$) in serum TSA and LSA levels in overall patients with OCC as compared to that of normal healthy controls and more elevated levels of serum TSA and TSA in patients with carcinoma of the anterior 2/3 of the tongue.

Introduction

Sialic acid (N-acetylneuraminic acid) is composed of alkylated derivatives of the neuraminic acid (2-Keto-5-amino-3, 5-dideoxy-D-nonulosonic acid) is widely distributed in nature and in mammals as a terminal components at the nonreducing end of carbohydrate chain of glycoproteins and glycolipids (1). Selected glycoproteins and glycolipids may prove to be tumour marker (2), since cell surface and membrane components play a prominent role in neoplastic behavior (3,4). The unique structural features of this molecule, which includes a

negative charge owing to a carboxyl group, enable it to play an important role in cellular functions, such as cell-to-cell recognition or cell-to-matrix interactions and transformation to malignancy (5). Sialic acid residues on the cell surface may also be involved in masking cell surface antigens and may serve as receptors for virus particles, some hormones and antibodies (6) However, they can also act as critical components of ligands recognized by a variety of animal, plant and microbial proteins termed sialic acid binding lectins (7). The surface properties of tumor cell differ from their normal counterparts, owing in part to altered sialoglyco-conjugates that are expressed on the plasma membrane (8). Sialic acid concentrations vary physiologically with age, but their level may also influenced by such conditions as inflammation, neoplastic tumor or inborn genetic disorder, which cause abnormal sialic acid metabolism (9).

Marked elevation of serum sialic acid as reflected by serum total N-acetyl-neuraminic acid (NANA) concentrations are not specific for one type of cancer, as elevations have been reported in patients with lymphoma, malignant melanoma, cancers of the lung, prostate, bladder and gastrointestinal system (10,11). Increased levels of sialic acid in cancer patients can be explained by spontaneous release (shedding) of aberrant sialic acid rich glycoproteins and glycolipids (12,13). In contrast ,a clear correlation of changes in sialic acid concentrations and malignancy has not emerged, some reports show a decrease and not an increase in sialic acid in association with malignancy (14).

Evaluation of sialic acid changes might contribute to diagnosis of cancer patients and to monitoring the tumor progression, detection of early recurrence and response to treatment (15).

The important role of sialic acid in neoplastic process prompted us to investigate total sialic acid (TSA) and lipid –bound sialic acid (LSA) values in serum of cancer patients in the oral cavity (OC) and compared with normal group and to our best knowledge this is the first study of sialic acid analysis in the serum of patients with squamous carcinoma of the (OC) in Iraq.

Materials and Methods

Chemicals: standard solution for sialic acid 500µg/ml concentrations was prepared by dissolving 50mg of standard N-acetylneuraminic acid in 100ml of distilled water, and on the day of

determination, the stock solution was diluted with phosphate buffer saline at pH 7.4 to give the following standard solutions (5.0.10.0,15.0,20.0,25.0,
30.0 μg/ml) for calibration curve measurement

Patients: Sera for the measurement of TSA and LSA levels were obtained from five groups of subjects followed up at the E.N.T and Maxillofacial Departments in the Medical City (Surgical Specialities Hospital) for the period from March 2001 to December 2004.The cases were either refered from primary district healthy center, dental clinics or visiting E.N.T and Maxillofacial Departments.

The patients with cancer were divided into the following groups according to the American Joint on Cancer Tumor staging scheme, based on TNM (T, tumor size; N, node invasion; M, metastasis) (16).

Group (1): consisted of 5 patients with carcinoma of the anterior two thirds (2/3) part of the tongue; included 4 males and 1 female with an average age (60±8.0) years.

Group (2): consisted of 2 male patients with carcinoma of the inner surface of the cheek with an average age (68±6.0) years.

Group (3): consisted of 8 patients with carcinoma of the mouth floor (with different degree of invasions); included 7 males and 1 female with an average age (62±9.0) years.

Group (4): consisted of 8 patients with carcinoma of the hard palate included 6 males and 2 females with an average age (58±8.0) years.

Group (5): This group comprised of 32 healthy blood donors as control subjects with the male to female ratio being 1.9 and age range (35-70) without receiving any medications.

The diagnoses of these tumors were carried out by E.N.T, maxillofacial surgeons.Moreover the disease had to be measured in two dimensions by a computed tomographic scan (CTS). Biopsy were taken and submitted for histopathological examination for the type of tumor and degree of differentiation. since most of the studies patients are in third and fourth stage.

Serum preparation:

Venous blood samples were collected between 8-9 AM considering the Circadian rhythm, then kept at room temperature for 10 minutes for cloting; centrifuged at 3000 rpm for 10 minutes, then the serum was separated and store at (-20C^0) until analysis.

Measurement of serum TSA by resorcinol reagent:

Serum TSA values determination was performed as previously described (17). Briefly, 20 µl of serum was diluted to 500 µl in to screw-capped tubes with distilled water; the tubes were vortexes and placed in ice. To each tube, for TSA test, 1ml of resorcinol reagent (including 10ml 2%(w/v) stock resorcinol in water, 9.75 ml water, 0.25 ml 0.1M $CuSO_4$, brought to a final volume of 100 ml with concentrated HCl).Each tube was capped, vortexes, and placed in 100C° boiling water (15minutes), then cooled for 10 minutes in an ice bath. One ml of butylacetate/n-butanol (85:15 v/v) was added to the reaction mixture, and the tubes was vortexes and centrifuged at 2500 rpm for 10 minutes at room temperature. The absorbance of the blue color supernatant was recorded at 580 nm.

Measurement of serum LSA: LSA was measured according to the method described by Katopodis and his co-workers (18,19). Fifty-µl serum aliquots were placed in screw-capped tubes, 3ml of cold (4C°) chloroform/methanol (2:1;v/v) mixture was added to each tube for total lipid extraction, the tubes were then capped and vortexes for 30 seconds, 0.5 ml of cold water was added to each tube and the tubes were centrifuged for 5 minutes at 2500 rpm at room temperature. The upper phase (aqueous layer containing LSA) was transferred to another screw-capped tube and 50 µl of phosphotungstic acid (1g/ml) was added to each tube .The tubes were vortexes again and allowed to stand at room temperature for 5 minutes. Then the tubes were centrifuged at room temperature for 5 minutes at 2500 rpm. After that the supernatants were decanted and the remaining pellets were redissolved in 1ml of water at 37C° by vigorously vortexing them for 1 minute and sialic acid content was determined as mentioned for TSA.

Statistical Methods

The data were expressed as mean values±standard deviation. Statistical significants in the values were evaluated by the student's t test. Both TSA and LSA sensitivities were calculated as the percentage of patients having values above the cut-off level 2SD(standard deviation).

Results

Patient's characteristics:

To investigate whether the changes in serum TSA and LSA levels of patients are conversely related to the location of oral cavity cancer (OCC), separate calculations were made for each group of patients. (Table 1), shows the values of serum TSA level for patients with (OCC) and for healthy controls. Data analysis revealed significant differences in serum TSA level in different patient groups in comparison with the healthy controls. The overall serum TSA level in 23 patients was 83.65±10.61 mg/dl, while the corresponding level in 32 healthy control was 65.20±5.44 mg/dl, this increase of 28% was statistically significant ($p<0.001$). The mean value of serum TSA level in patients with carcinoma of the anterior two thirds (2/3) part of the tongue was 86.40±12.82 mg/dl; which represent 33% increase in the TSA level which was statistically significant ($p<0.001$). The mean TSA level for the patients with carcinoma of inner surface of the cheek was found to be 25% higher than that of the healthy control ($p<0.001$). On the other hand the level of serum TSA content for the patients with carcinoma of the mouth floor was 26% higher than that of normal serum content ($p<0.001$). In 30% of patients with hard palate carcinoma, serum TSA was statistically significant ($p<0.001$).

Fig (1); shows the levels of serum TSA in health controls and in patients with (OCC).A significant elevated levels of TSA were detected in patients with OCC compared to the levels of the healthy control. Particularly, 4 of 5 cases of anterior 2/3 of the tongue, all of patients with inner surface of cheek, 6 of 8 cases of carcinoma of the mouth and 5 of 8 cases of hard palate carcinoma have elevated levels above the cut-off level for the healthy controls plus 2SD (standard deviation) (76.08 mg/dl).

The present study shows that the magnitude of the sensitivity is varied between 63% for patients with hard palate carcinoma to 100% for the patients with inner surface of the cheek, while the values reach to 75% and 80% for the patients with carcinoma of floor of the mouth and anterior 2/3 of the tongue respectively (fig 2), this increase in sensitivity might contributed to the disease stage.

Table (2); shows the comparison of serum LSA values of patients with (OCC) and the healthy controls, the mean serum LSA level in 32 healthy controls was found to be 21.88±1.09 mg/dl, while that for serum LSA in all cancer patients was 27.57±4.19 mg/dl, this represent

a 35% increase which is statistically significant (P<0.001). The mean value of serum LSA level for patients with carcinoma of the anterior 2/3 of the tongue was 27.27±3.53 mg/dl, this increase (32%) was statistically significant (P<0.001). The mean serum LSA level for patients with carcinoma of inner surface of the cheek was 27% higher than that for the healthy controls.

The most pronounced changes were found in the level of serum LSA in patients with carcinoma of floor of mouth 41% and 39% in serum patients with hard palate carcinoma.

Fig (3); shows the distribution of the serum LSA levels in the healthy control and in the cancer patients according to the sites of disease. Nevertheless, only 4 of 5 cases of anterior 2/3 of the tongue, all cases with inner surface of cheek, 5 of 8 cases of carcinoma of the floor of the mouth and 5 of 8 cases of hard palate carcinoma have elevated levels above the cut-off level for the healthy controls plus 2SD (24.06 mg/dl).

The extent of the increased sensitivity varied between 63% for both patients with carcinoma of the floor of the mouth and hard palate to 100% for the patients with inner surface of the cheek, while the values reach to 80% for the patients with carcinoma of anterior 2/3 of the tongue (fig 4).

Discussion

The surface glycoproteins and glycolipids of tumor cells have different carbohydrate compositions, which may contribute to the aberrant cell-cell recognition, cell adhesion, antigenicity and invasiveness of malignant cells. As a result of increased turnover, secretion, and/ or shedding, these glycoproteins and glycolipids can be released into the sera (20) .The major constituent of glycoproteins and glycolipids is sialic acid, that usually occurs as a terminal component at the non-reducing end of their carbohydrate chains (21) acid induces an electronegative charge (22) because it is a relatively strong acid (pKa=2.6), which completely ionized at physiological pH (23). This ionization plays a major role because the distribution of cell surface dense anionic sites is correlated in vitro with tumor cell aggregation (24) .The quantity of glycoconjugates on membranes of neoplastic cells is higher than that on membranes of normal cells. This surface sialylation correlates positively with the metstatic potential of cultured murine tumor cell lines (25) .It has been hypothesized that tumor cells

use their heavily sialylised surface as a mask to avoid recognition by the immune surveillance system and thus facilitate metastatic spreading (22).

Previous studies were centered largely around the demonstration of increased levels of carbohydrates of the carbohydrate-protein complexes in sera of cancer patients.In the present study serum TSA and LSA levels in the OCC groups were significantly increased in comparison with the healthy controls. Morever, elevated serum levels of TSA and /or LSA have been observed in many malignancy (26,27), and these levels are directly related to tumor burden [28] and disease recurrence (29).

A significant correlation has been demonstrated between TSA levels, activity and tumor stage of breast cancer (30), and between TSA and LSA values and extent of metastases in colorectal cancer (31). An increased output acute phase protein could explain the increase of TSA concentration in patients with malignant diseases from the liver as a non-specific secondary reaction and by an intensified output of tumor cells with high contents of sialic acid. This latter explanation is supported by the fact that sialyltransferase levels increased simultaneously with those of sialic acid (22).

In conclusion, the increased serum TSA and LSA levels are associated positively with presence of malignant tumor and appear to be a consequence of the disease itself, and could be suggested as one of the newly discovered tumor marker in the early diagnosis, progression and follow up for the patients with OCC. In addition it's an easily predictable way for the help of the physicans and biochemists in their researches.

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Table (1): TSA levels in the serum of normal control and of patients with oral cavity cancer

Disease	Mean± SD (mg/dl)	Range (mg/dl)	P.value
Anterior 2/3 of the tongue cancer n=5	86.40± 12.82	71.24-108.22	P<0.001
Inner Surface of the cheek cancer n=2	81.75± 3.50	78.24-85.25	P<0.001
Mouth floor cancer n=8	81.27± 8.50	70.09-97.09	P<0.001
Hard palate cancer n=8	84.80± 10.97	69.42-103.11	P<0.001
Total cases n=23	83.65± 10.61	69.42-108.22	P<0.001
Normal n=32	65.20± 5.44	55.69-73.12	-

Table (2): LSA levels in the serum of normal control and of patients with oral cavity cancer

Disease	Mean± SD (mg/dl)	Range (mg/dl)	P.value
Anterior 2/3 of the tongue cancer n=5	27.27± 3.53	20.23-29.24	P<0.001
Inner Surface of the cheek cancer n=2	26.24± 1.00	25.24-27.25	P<0.05
Mouth floor cancer n=8	26.92± 4.97	20.11-31.24	P<0.001
Hard palate cancer n=8	26.87± 3.71	20.01-32.11	P<0.001
Total cases n=23	27.57± 4.19	20.01-32.11	<0.001
Normal n=32	21.88± 1.09	16.21-23.96	-

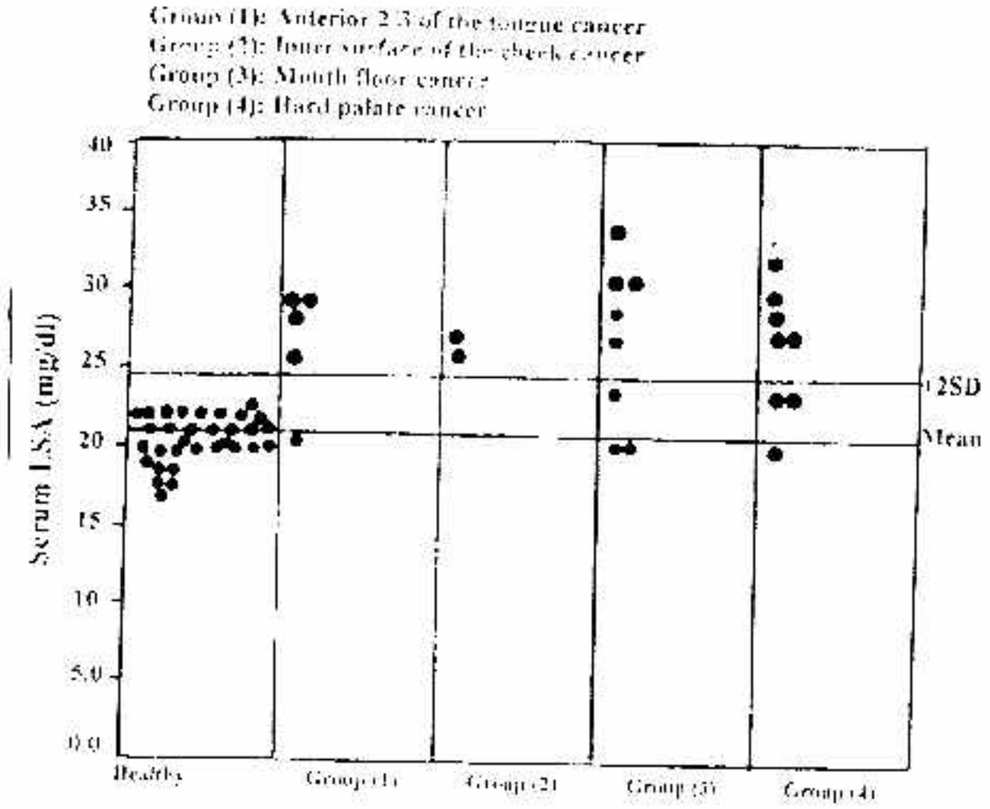

Fig (3): Levels of LSA in serum from healthy donors and cancer patients

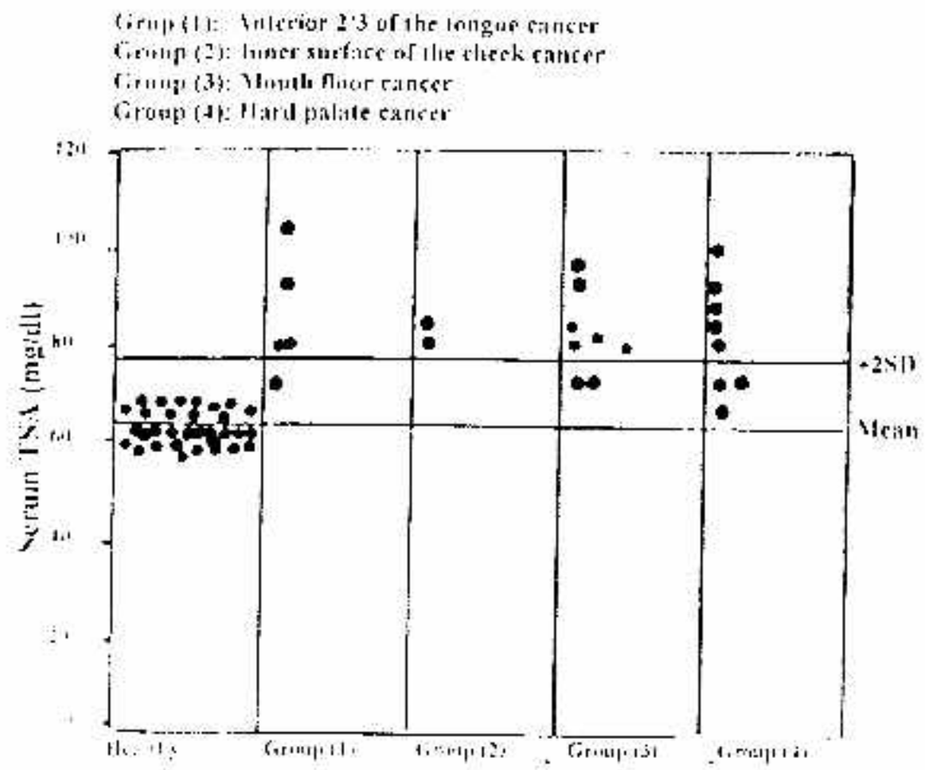

Fig (1): Levels of TSA in serum from healthy donors and cancer patients

Fig (4): Sensitivity of LSA in cancer patients

Fig (2): Sensitivity of TSA in cancer patients

مستويات حامض السايليك والدهن المرتبط بحامض السايليك عند مصل مرضى سرطان تجويف الفم ذو المواقع المختلفة

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الخلاصة

تم تعين مستويات حامض السايليك الكلى والدهن المرتبط بحامض السايليك في المصل بالطريقة الطيفية قى 23 مريضا يعانون من سرطان تجويف الفم ، منهم خمسة مرضى مصابين بسرطان الجزء الامامي لثلثي اللسان بمعدل عمر(8.0±60) سنوات واثنان من الذكور مصابين بسرطان الجزء الداخلي لسطح الخد بمعدل عمر(8.0±68) سنوات وثمانية مرضى مصابين بسرطان قاع تجويف الفم بمعدل عمر(9.0±62) سنوات وثمانية مرضى مصابين بسرطان الجزء الصلب من اللهاة بمعدل عمر(8.0±58) سنوات ومقارنة النتائج مع 32 شخصا سليما.

اظهرت النتائج وجود زيادة دالة ($P<0.001$) في مستويات حامض السايليك الكلي والدهن المرتبط بحامض السايليك في مصل مرضى سرطان تجويف الفم مقارنة مع مجموعة التحكم السوية مع وجود نسبة مرتفعة من حامض السايليك الكلى والدهن المرتبط بحامض السايليك عند مرضى سرطان الجزء الامامى لثلثى اللسان.