Total Sialic Acid Concentrations as a Supporting Marker of Malignancy in Acute Lymphoblastic Leukemia

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Abstract

To follow up the response of acute lymphoblastic leukemic (ALL) patients to chemotherapy treatment and for prognosis, diagnosis, the total Sialic acid (TSA) levels was studied as a tumor marker. The study included 40 patients (male = 22, female =18) with (ALL) were tested for the serum and leukocytes homogenate concentrations of total sialic acid (TSA) before and after treatment with six different chemotherapy protocols. While significantly increased ($P< 0.001$) as compared to the healthy individuals group, serum and leukocytes homogenate TSA concentrations dropped significantly ($P<0.001$) after treatment with each of the six chemotherapy treatment protocols, as compared to ALL untreated patients. A linear correlation relationship ($r^2 - 0.9514$; $r^2 = 0.9451$) was found between TSA concentrations and the period of chemotherapy treatment in serum and leukocytes homogenate. A linear correlation relationship ($r^2 = 0.9965$) could be found between percentage of serum total sialic acid and percentage of leukocytes homogenate total sialic acid. The results of this study strongly support the role of TSA as a marker for the disease and suggest that it could be used for progression and prognosis of ALL patients, in addition we strongly suggest that there was a positive relationship between TSA level in serum and leukocytes.

Introduction

Leukemia occupies the first place among the most common ten malignant diseases in Iraq. The number of leukemia patients doubled in the last decade of the twentieth century (1). Intensive efforts are being made to make progress both in diagnosis and treatment of the disease. Although several biological markers can be used to monitor

cancer, predict the therapeutic response and prognosis of cancer, and in some certain situations even diagnosis cancer are currently used routinely, which can be measured in serum, plasma, or other body fluid and their concentration becomes changed in the presence of cancer, all these markers have some limitations in their clinical efficiency (2). Sialic acid (N-acetylneuraminic acid; NANA (SA)) is composed of alkylated derivatives of the neuraminic acid, which are present in various mucoprotiens and as the carbohydrate component of cell membrane glycolipids. The carbohydrate moiety characterizes the cohesive, adhesive and antigenic properties by its effect on cell-to-cell contacts. These properties often change substantially after malignant transformation of cell. In addition, development of malignancy could affect the serum and tissue sialic acid levels as well (3). Elevated levels of serum total SA (TSA) were reported in the majority of patients with various malignant tumors (4), including cancer of oral cavity (5), endometrial cancer (6), lymphomas (7) and leukemias (8).The incidence and mortality rate of cancer is still unacceptably high and only a few studies aiming at investigating simultaneous employing of both serum and leukocytes levels of sialic acid in diagnosis and prognosis of cancer patients have been reported. Therefore, the current study aimed to elucidate more about the role of SA as a possible biological marker and to investigate association of alteration in serum and leukocytes levels of sialic acid with diagnosis and treatment monitoring of acute lymphoblastic leukemia (ALL).

Materials and Methods

Patients. The present study included 40 patients (male = 22, female =18) who were referred to Baghdad Educational Hospital as ALL patients. According to the chemotherapy protocol employed and the period of treatment, patients were divided into six groups in addition to the group of chemotherapy-untreated ALL patients and the healthy individuals group (control) (male = 4, female = 6) (Table 1).

Estimation of TSA: Blood samples were collected from patients and control, and divided into two parts, sera were separated and the other part was putted into glass tube with heparin as anticoagulant; then, they were centrifuged at 2000 rpm for 15 minutes. The Buffy coat was separated and homogenized. Concentrations of TSA were estimated for the serum and leukocyte homogenate by using the colorimetric

(Resorcinol reagent) method with absorbency read under optical density of 580nm (9).

Statistical analysis: Data were analyzed using the Analysis of Variance (ANOVA) test. The level of significance was shown using the least significant difference (LSD) test. Correlations were analyzed by calculating Pearson's product- moment correlation coefficient for normally distributed variables. Values are given as mean ± standard error .A P- value of less than 0.05 was considered statistically significant.

Results

sialic acid levels in both serum and leukocyte homogenate did not show any statistically significant difference ($p< 0.05$) between male and female in all groups (patients with ALL and healthy individuals).

1. **TSA concentrations in untreated ALL patients (male = 2, female =8)**: A significant increase ($P< 0.001$)was observed in the serum and leukocytes homogenate concentrations of TSA in ALL patients (256.23±8.13 ; 188.72±3.24 mg/dL; respectively) as compared to the control group of healthy individuals (113.44±1.06 ; 72.66±2.53mg/dL; respectively)the resulted have shown highly elevation in TSA percentage value in untreated patients (225.87% ;259.73% ; respectively)as compared to control group(100.00% ;100.00% ; respectively) (Table 2).

2. **TSA concentrations in chemotherapy-treated ALL patients.**

A. Vincristine+Prednisolone+Adramycin treatment protocol (male = 6, female =4). Serum and leukocytes homogenate TSA concentrations in ALL patients treated with this protocol significantly decreased ($P< 0.001$) after 3 weeks (161.65±2.33 ; 126.00±1.69 mg/dL; respectively) and 5 weeks (133.15±1.94 ; 100.14±2.93mg/dL; respectively) of treatment as compared to untreated ALL patients (256.23±8.13;188.72±3.24 mg/dL; respectively) (Table 2).While the resulted TSA percentage was highly decreased (142.49%;173.41%; respectively) after 3 weeks and (117.37%;138.19%; respectively) after 5 weeks of treatment as compared to untreated ALL patients (225.87%;259.73%; respectively) (Table 2).

B. Vincristine+Prednisolone+6-mercaptopurine treatment protocol (male = 6, female =4). Serum and leukocytes homogenate

TSA concentrations in ALL patients treated with this protocol significantly decreased (P< 0.001) after 7 weeks (125.22±2.63;85.83±3.05mg/dL; respectively) and 11weeks (107.25±4.75;74.66±2.75mg/dL; respectively) of treatment as compared to untreated ALL patients (256.23+8.13;188.72+3.24mg/dL; respectively) (Table 2). While the resulted TSA percentage was highly decreased (110.38%; 118,12%; respectively) after 7 weeks and (94.54%;102.66%; respectively) after 11 weeks of treatment as compared to untreated ALL patients (225.87%;259.73%; respectively) (Table 2).

C. Vincristine+ Prednisolone + Adramycin +Methotrexate treatment protocol (male = 8, female =2).

Serum and leukocytes homogenate TSA concentrations in ALL patients treated with this protocol significantly decreased (P<0.001) after 15 weeks (106.20±7.38; 71.43±1.80mg/dL; respectively) and 25 weeks (70.68±3.22; 38.30±5.17mg/dL; respectively) of treatment as compared to untreated ALL patients (256.23±8.13; 188.72±3.24 mg/dL; respectively) (Table 2). While the resulted TSA percentage was highly decreased (93.61%; 98.30%; respectively) after 15 weeks and (62.30%; 52.71%; respectively) after 25 weeks of treatment as compared to untreated ALL patients (225.87%; 259.73%; respectively) (Table 2).

Overall, a linear correlation relationship with r^2 value of (0.9514; 0.9451) could be drawn between the concentrations of TSA in (serum and leukocytes homogenate; respectively) and the period of treatment (Fig.1 and Fig.2) besides a linear correlation relationship (r^2 =0.9965) could be found between the percentage of TSA in serum and percentage of TSA in leukocytes homogenate (Fig.3).

Discussion

Glycosylation has been demonstrated to play a critical role during malignant transformation (10, 11).The present study found that ALL patients had significantly higher levels of leukocytes and serum sialic acid as compared to the control. The increase of sialic acid in cells may be due to enhanced activity of enzymes involved in sialic acid synthesis and / or transfer. Some reports have indicated a 3-5 times increased sialytransferase activity in various virally transformed cells as compared to the corresponding normal cells (12). The concentration

of serum TSA in current study might be increased through changes in the biosynthesis and post translational glycosylation processing of acute – protein phase glycoprotein in the liver (13) or the phenomenon may be related to the intensified cell metabolism and increased serum sialytrans-ferase activity expressed by the tumor cell (14). However, malignancy with simultaneous infection increased the TSA concentrations significantly more than infection alone. An elevation in serum TSA has been reported in the majority of children with leukemias (8) and in adults with acute myeloid leukemia (AML), chronic myeloid leukemia (CML) (15), chronic lymphoblastic leukemia (CLL) (16), acute lymphoblastic leukemia (ALL) (4) and lymphomas (7) .Elevated TSA values have also been reported in the majority (36 % - 90 %) of AML, CML and ALL patients (17). The influence of sialic acid on oncogenicity of tumor cells may be based on i) a negative charge determining constituent on the cell surface, resulting in the cell of contact inhibition, ii) an antigen – masking agent and iii) a component of the cell surface involved in the adherence of tumor cells to the mesothelial membrane prior to their dissemination to form metastases (18).

The present study has also demonstrated that levels of cellular and serum sialic acid significantly decreased in the chemotherapy – treated ALL patients. The decrease was evident in all the six groups of patients in a chemotherapy– time dependent manner. This result is in agreement with previous data showing that the serum SA levels in the chemotherapy – treated patients attains the value of healthy individuals and even reaches a lower value (19). The usage of chemotherapy drugs has probably lowered the level of sialic acid through its negative effect on the mitotic activity of tumor cells, which have damaging effects on biosynthesis of DNA and cell reproduction (20). In growing cells, the rate of carbohydrate synthesis is significantly higher compared with non growing cells (21) and about 70% of total sialic acid is generally found on the cell surface (22) so the decrease of serum sialic acid in ALL patients after chemotherapy may be associated with this decrease of cellular content, specially when we found a special and strong correlation between leukocytes SA and serum SA as well as the correlation relation ship between TSA levels and the period of chemotherapy treatment .

Thus, the present findings suggest that SA changes in tumor cells and serum could be an important step during tumor growth /

malignancy as well as tumor regression after chemotherapy treatment and they support the view of multilevel / multistep effects of the drugs in cancer chemotherapy, but infection must be taken into account when interpreting increased SA values.

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Table (1): Groups of ALL patients according to chemotherapy protocol and period of treatment with the group of untreated patients and control group

Group	Chemotherapy protocol	Period of treatment (week)
1	Vincristine,Prednisolone, Adramycin	3
2	Vincristine,Prednisolone, Adramycin	5
3	Vincristine,Prednisolone, 6-mercaptopurine	7
4	Vincristine,Prednisolone, 6-mercaptopurine	11
5	Vincristine, Prednisolone, Adramycin, Methotrexate	15
6	Vincristine, Prednisolone, Adramycin, Methotrexate	25
Patients	Untreated	-
Control	Healthy	-

Table 2: The effect of the chemotherapy protocol on total sialic acid in serum and leukocytes homogenate of ALL patients.

Groups	Protocol of chemotherapy	Serum TSA mg/dl	Percentage of Serum TSA (%)	Cell TSA mg/dl	Percentage of Cell TSA (%)
Control Male = 4 Female = 6	-	111.44 ± 1.06	100	72.66 ± 2.53	100
ALL patients Untreated Male = 2 Female = 8	-	256.23 ± 8.13 A	225.87	188.72 ± 3.24 A	259.73
ALL patients Treated Male = 5 Female = 0 *	Vincristine , Prednisolone Adramycin	161.65 ± 2.23 B	142.49	126.00 ± 1.69 B	173.41
ALL patients Treated Male = 1 Female = 4 **	Vincristine , Prednisolone Adramycin	131.15 ± 1.91 B	117.37	100.41 ± 2.93 B	138.19
ALL patients Treated Male = 2 Female = 3 +	Vincristine, Prednisolone 6-Mercaptopurine	125.22 ± 2.36 B	110.38	85.83 ± 2.05 B	118.12
ALL patients Treated Male = 4 Female = 1 ++	Vincristine, Prednisolone 6-Mercaptopurine	107.25 ± 4.75 B	94.54	74.66 ± 2.75 B	102.66
ALL patients Treated Male = 5 Female = 0 *+	Vincristine , Prednisolone Adramycin , Methotrexate	106.20 ± 7.38 B	93.61	71.43 ± 1.80 B	98.30
ALL patients Treated Male = 3 Female = 2 *++	Vincristine , Prednisolone Adramycin , Methotrexate	70.68 ± 3.22 B	62.30	38.30 ± 5.17 B	52.71

A Significantly increased at (P<0.001) as compared to control. * Patients treated for 3 weeks. + Patients treated for 7 weeks. *+ Patients treated for 15 weeks.
B Significantly decreased at (P<0.001) as compared to untreated. ** Patients treated for 5 weeks, ++ Patients treated for 11 weeks. *++ Patients treated for 25 weeks.

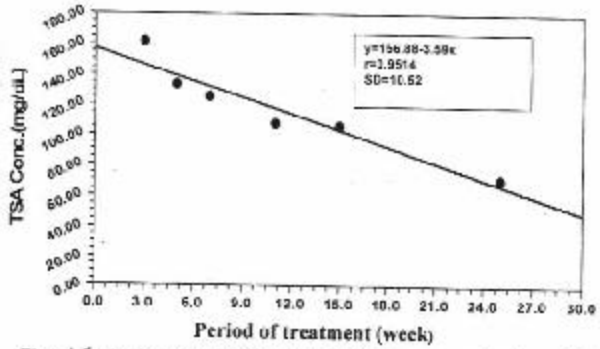

Fig. (1) The linear correlation between the serum concentrations of TSA and the Period of chemotherapy in ALL patients

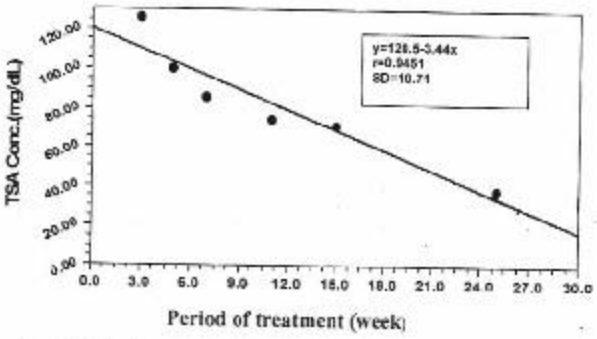

Fig. (2) The linear correlation between leukocytes homogenate concentrations of TSA and the Period of chemotherapy in ALL patients

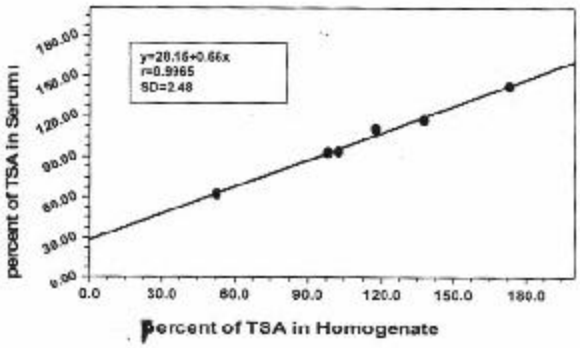

Fig. (3) The linear correlation between percent of serum and leukocytes Homogenate Concentrations of TSA in ALL patients

تراكيز حمض السياليك الكلي علامة داعمة لتشخيص الورم في ابيضاض الدم اللمفاوي الحاد

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الخلاصة

تم اعتماد حمض السياليك الكلي في كل من المصل وكريات الدم البيض لغرض متابعة استجابة مرضى ابيضاض الدم اللمفاوي الحاد للعلاج الكيميائي والتشخيص المبكر للورم. تم قياس تركيز حمض السياليك لـ 40 مريضا (22 ذكرا و 18 أنثى) خاضعين وغير خاضعين للعلاج الكيميائي بالاعتماد على ست أنظمة علاجية، حيث لوحظ حصول ارتفاع معنوي ($P< 0.001$) لكل من تراكيز حمض السياليك الكلي لمصل وكريات الدم البيض في مرضى ابيضاض الدم اللمفاوي الحاد غير الخاضعين للعلاج الكيميائي مقارنة مع الاشخاص السليمين بينما أدى العلاج الكيميائي إلى حصول انخفاض معنوي ($P< 0.001$) في تركيز حمض السياليك لكل من المصل وكريات الدم البيض عند مقارنتهم مع المرضى غير الخاضعين للعلاج الكيميائي.

بينت الدراسة الحالية إلى وجود علاقة ترابط قوية بين تركيز الحمض والمدة الزمنية للعلاج الكيميائي لكل من المصل والخلايا حيث كانت ($r^2 = 51$, $r^2 = 0.9514$ 0.94) على التوالي فضلا عن إلى وجود علاقة ترابط قوية($r^2 = 0.9965$) بين النسبة المئوية لتركيز الحمض في المصل والنسبة المئوية لتركيزه في الخلايا مما يدل على أن زيادة مستوى الحمض في الخلايا تؤدي إلى زيادة مستواه في المصل ومن هذا تشير الدراسة الحالية إلى اعتماد حمض السياليك الكلي في كل من المصل والخلايا كعلامة لتقدم المرض واستجابة مرضى ابيضاض الدم اللمفاوي الحاد للعلاج الكيميائي.