# Serum total sialic acid levels as an indicator for the humoral immune status in the chemotherapy-treated and untreated patients with acute lymphoblastic leukemia

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## Abstract

Forty patients with acute lymphoblastic leukemia (ALL) were tested for the serum levels of total sialic acid (TSA) and the immunoglobulins IgG, IgM, and IgA before and after treatment with six different chemotherapy protocols. While significantly increased ( $P<0.001$) as compared to the healthy individuals group, serum TSA levels in ALL patients were significantly decreased ( $P<0.001$) in response to all chemotherapy protocols as compared to ALL untreated patients. A linear correlation relationship( $r^2 = 0.936$) was found between TSA levels and the period of chemotherapy treatment. Serum levels of IgG, IgM, and IgA showed significant increases ( $P<0.001$) in ALL patients. These levels were 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 ($r^2 = 0.909$) was found between serum IgA levels and the period of chemotherapy treatment. The results of this study support the role of TSA as an indicator for the disease and the humoral immune status in the untreated ALL patients and suggest such a role for TSA in the chemotherapy treated ALL patients as well.

## Introduction

Leukemia occupies the first place among the most common malignant diseases in Iraq. The number of leukemia patients doubled in the last decade of the twentieth century (1). Intensive efforts have been made to make progress both in diagnosis and treatment of the disease. Sialic acid(SA), the terminal component of the carbohydrate chains located in the outermost position of plasma membranes, is of increasing interest in this regard. Elevated levels of serum total SA(TSA) were reported in the majority of patients with various malignant tumors (2), including leukemias

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(3).Thus, serum levels of TSA have been reported to be efficient as an early diagnostic tool for leukemia (4).At the level of treatment, hemotherapy s among several methods for cancer treatment. Chemical drugs were first introduced as single agents, such as adrenocorticosteroids, methotrexate, mercaptopurine...etc. Combination chemotherapy was then proved to be more effective in treating different kinds of cancer (5). The humoral immune response, the arm of the immune system which is highly activated during malignancy, is severely inhibited fter xposure o ifferent cancer treatments, including chemotherapy(6).

The present study was designed to elucidate ore bout he ole f A s a possible iological arker hat ould be employed to monitor the humoral immune tatus oth efore nd fter treatment ith hemotherapy n patients with acute lymphocytic leukemia(ALL).

## Materials and Methods

***Patients.*** The present study included 40 patients 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) (Table 1).

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

| Group | Number | Chemotherapy protocol | Period of treatment |
|---|---|---|---|
| 1 | 5 | Vincristine,Prednisolone, Adramycin | 3 Weeks |
| 2 | 5 | Vincristine,Prednisolone, Adramycin | 5 Weeks |
| 3 | 5 | Vincristine,Prednisolone, 6-mercaptopurin | 7 Weeks |
| 4 | 5 | Vincristine,Prednisolone, 6-mercaptopurin | 11 Weeks |
| 5 | 5 | Vincristine, Prednisolone, Adramycin, Methotrexate | 15 Weeks |
| 6 | 5 | Vincristine, Prednisolone, Adramycin, Methotrexate | 25 Weeks |
| Patients | 10 | Untreated | - |
| Control | 10 | Healthy | - |

***Estimation of TSA.*** Blood samples were collected from patients and control, and sera were separated according to Garvey *et al.*(7). Levels of TSA were estimated using the colorimetric ( Resorcinol eagent) method with absorbency read under optical density of 580nm (8).

***Detection of Immunoglobulins***: Serum levels of IgG,IgM and IgA were estimated using the Single Radial Immunodiffusion method (9).The method is based on measuring the diameter of the precipitation ring and the immunoglobulin level was obtained from the table accompanying the test kit provided by Biomeghreb (Tunisia).

***Statistical analysis.*** Data were analyzed using the Analysis of Variance (ANOVA) test. The level of significance was estimated using the least significant difference (LSD test). Results were expressed as mean ± standard error.

## Results

1. ***TSA levels in ALL patients.*** A significant ncrease ( $P< 0.001$)was observed in the serum levels of TSA in ALL patients 56.23±8.13mg/dL) as compared to the control group of healthy individuals 113.44±1.06mg/dL)(Figure 1).

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

A. ***Vincristine,Prednisolone, Adramycin treatment protocol.*** Serum TSA levels in ALL patients treated with this protocol significantly decreased ( $P< 0.001$) after 3 weeks (161.65 ± 2.33mg/dL) and 5 weeks (133.15 ±1.94mg/dL) of treatment as compared to untreated ALL patients ( 256.23 ±8.13mg/dL) (Figure 1).

B. ***Vincristine,Prednisolone, 6-mercaptopurin treatment protocol.*** Serum TSA levels in ALL patients treated

with this protocol significantly decreased ( P< 0.001) after 7 weeks (125.22 ±2.63mg/dL) and 11weeks (107.25 ±4.75mg/dL) of treatment as compared to untreated ALL patients ( 256.23 ±8.13mg/dL)(Figure 2).

C. ***Vincristine, Prednisolone, Adramycin, Methotrexate treatment protocol.***

Serum TSA levels in ALL patients treated with this protocol significantly decreased ( P<0.001) after 15 weeks (106.20±7.38mg/dL) and 25 weeks (70.68±3.22mg/dL) of treatment as compared to untreated ALL patients ( 256.23±8.13mg/dL)(Figure 3).

Overall, a linear correlation relationship with $r^2$ value of 0.936 could be drawn between the levels of TSA and the period f reatment (Figure 4).

3. ***Immunoglobulin levels in ALL patients.*** Serum level of IgG (2035.11±29.89mg/dL), IgM (278.60±21.23mg/dL), and IgA (400.20±14.42mg/dL)showed significant increases( P<0.001) when compared to their respective control values (1510±83.24, 193.25±31.84, and 277 ±42.05mg/dL; respectively)(Fig.1).

4. ***Immunoglobulin levels in chemotherapy-treated ALL patients.***

A. ***Vincristine,Prednisolone, Adramycin treatment protocol.*** After 3 weeks of treatment with this protocol, significant decreases(P<0.001) were observed in the levels of IgG( 1120.55 ±188.01mg/dL), IgM( 161 ± 30.79 mg/dL), and IgA(194.11±25.41mg/dL) as compared to their respective values in the untreated ALL patients (2035.11 ±29.89, 278.60±21.83, and 400.20± 14.42mg/dL; respectively ) (Fig.1).

After 5 weeks of treatment, significant decreases( P<0.001) were observed in the levels of IgG( 1160.20± 210.71 mg /dL), IgM( 138±33.679 mg/dL), and IgA(182.15±23.53mg/dL) as compared to their respective values in the untreated ALL patients( 2035.11±29.89, 278.60± 21.83, and 400.20±14.42mg/dL; respectively)(Fig.1).

B. ***Vincristine,Prednisolone, 6-mercaptopurin treatment protocol.***

After 7weeks of treatment, significant decreases( P<0.001) were observed in the levels of IgG( 892±197.62mg/dL), IgM(125±22.13mg/dL), and IgA(168± 22.22mg/dL) as compared to their respective values in the untreated ALL patients (Fig.2).

After 11weeks of treatment, significant decreases( P<0.001) were observed in the levels of IgG( 896±93.09mg/dL), IgM(114.20±16.63mg/dL), and IgA (150.75±22.58mg/dL) as compared to their respective values in the untreated ALL patients (Fig.2).

C. ***Vincristine, Prednisolone, Adramycin, Methotrexate treatment protocol.***

After 15 weeks of treatment, significant decreases( P<0.001) were observed in the levels of IgG( 760.40±94.57mg/dL), IgM(105±13.03mg/dL), and IgA(126±18.86mg/dL) as compared to their respective values in the untreated ALL patients (Fig.3).

After 25 weeks of treatment, significant decreases( P<0.001) were observed in the levels of IgG( 912±179.37mg/dL), IgM(108±11.57mg/dL), and IgA(114 ± 16.61mg/dL) as compared to their respective values in the untreated ALL patients (Fig.3).

A linear correlation relationship with $r^2$ value of 0.909 could only be found between he evels f IgA and the period of chemotherapy treatment( Fig.5).

## Discussion

The significant increase in serum TSA of ALL patients in the present study is consistent ith hat was demonstrated y revious tudies in various types of cancers 10,11).In advanced cancer with metastasis, the level of serum A as hown o increase significantly ue o he act that certain cancer markers which are glycoproteins and glycolipids are shed from cancer cells into serum (12) .The increase in SA shedding may primarily reflect an increase n he ctivity o sialidases on membranes of cancer cells. Another explanation omes rom the observation that SA-rich acute phase proteins are produced by the liver as a result to an inflammatory reaction to the tumor(13) .

The present study has also revealed increases in the levels of the immunoglobulins IgG,IgA,and IgM in the sera of ALL patients. These results are in agreement with the results of previous investigations (14,15). This increase seems to have a relationship with the elevated levels of SA in the sera of these patients. First, Sialic acids are known to be important determinants for various cellular activities including immunogenicity(16). Thus, it has been proposed that the altered carbohydrate composition f he alignant ell surface may contribute to aberrant cellular antigenicity (17).Second, sialic acids have the ability to act as biological masks by preventing ligands , including immunoglobulins from recognizing receptors (18).Thus, shedding of SA molecules from surfaces of malignant cells, as proposed above, might expose those receptors eading o he nduction f the humoral immune response and increase of antibody production. In addition, the presence of such markers as SA in certain umor ypes as postulated to act as a target for the binding of natural antibodies nd or attempts of active immunization (19). However, since antibodies are glycoproteins in nature, the elevation in their levels can be directly explained by the fact that the levels of serum glycoproteins are elevated in cancer patients(19).

The present study has also demonstrated that the levels of serum SA 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 SA levels in the chemotherapy-treated patients attains the value of untreated control and even reaches a lower value(6). Previous studies as hown hat drugs ,like methotrexate and 6-mercaptopurine have damaging ffects n he ynthesis of DNA,RNA, and pyrimidines (5).The usage f hese ighly cytotoxic chemical drugs has probably lowered the levels of SA through its negative effect on the mitotic activity of tumor cells. This will normally affect the amount of SA produced nd hed y the cell. In patients with different kinds of malignant tumors, it was reported that TSA value might have utility in detecting the disease and following patients on chemotherapy treatment(2). The strong correlation between SA levels and the period of chemotherapy treatment

in the present study supports a role for TSA as a marker to the patient's response to the treatment in ALL.

Our results have also revealed that the levels of immunoglobulins (IgG, IgM ,and IgA) significantly decreased in response to the gradually increased doses of chemotherapy. his esult s in accordance with the previously reported inhibitory effects of chemotherapy on the immune system. It has been shown that patients under

high doses of chemotherapy are more susceptible to infectious diseases than those under maintenance treatment stage(20), which reflects severe inhibition to the immune system.Ried et al. have reported that the decrease in the levels of immunoglobulins starts one month after the beginning of the remission induction stage as a result to treatment with vincristine and pridnisolone(21).

Other investigators have demonstrated that the chemotherapy or radiotherapy, or both, significantly reduce the number of plasma cells, B-cells ,and T-cells in the periphery as a result to their effects on the interleukins, especially IL-2 (22).

Our results suggest a special and strong correlation between IgA and the period of treatment.

In conclusion, the elevated levels of serum SA and immunoglobulins of the patients ith LL upports he ole f SA as biological marker for both the disease and the humoral immune status of untreated atients. ost importantly, the decreased levels of serum SA and immunoglobulins in our chemotherapy-treated patients shows the reliability of SA levels in monitoring patients on treatment.

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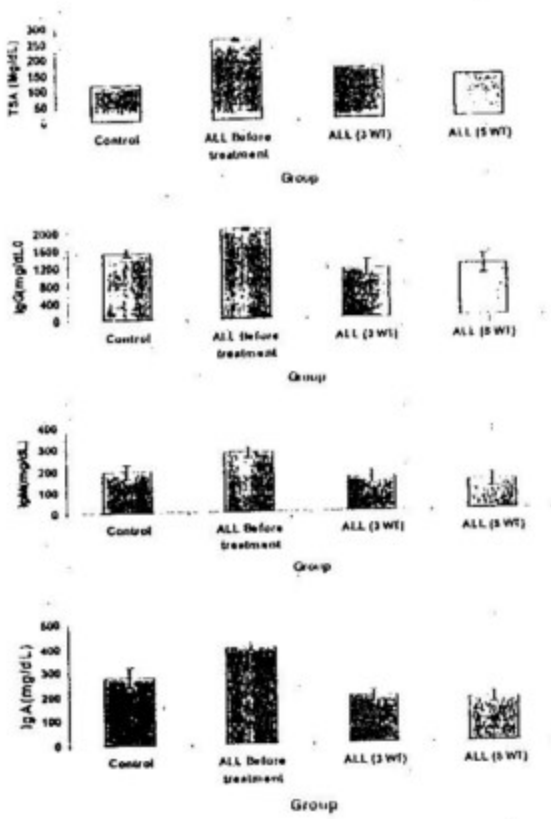


**Figure 1 : The effect of vincristine, prednisolone, and adriamycin for 3 and 5 weeks on serum levels of TSA,IgG,IgM, and IgA in ALL patients.**
**WT= weeks of treatment**

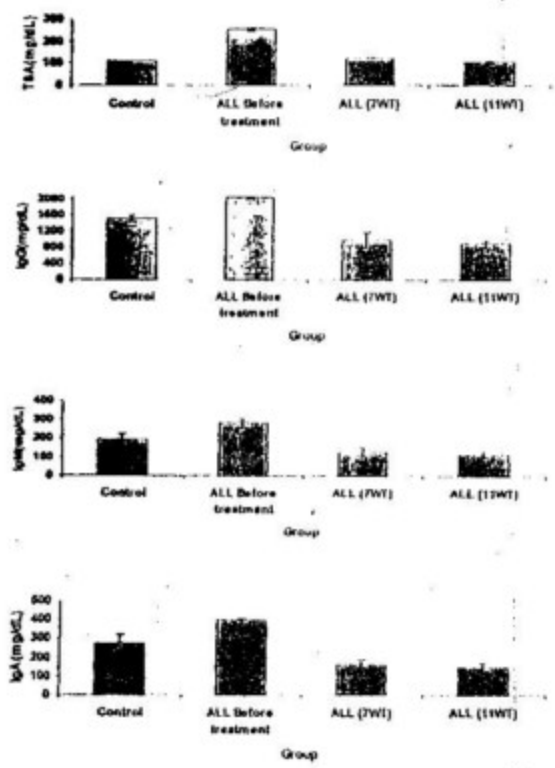


**Figure 2 : The effect of vincristine,vredinisolone, and 6 mercaptopurin for7 and 11weeks on serum levels of TSA,IgG,IgM, and IgA in ALL patients.**
**WT= weeks of treatment**

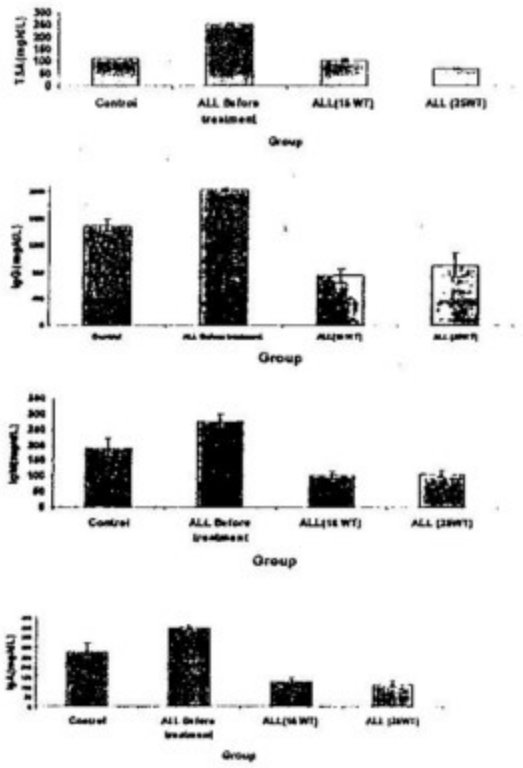


Figure 3: The effect of vincristine, prednisolone, adramycin, and methotrexate for7 and 11 weeks on serum levels of TSA,IgG,IgM, and IgA in ALL patients. WT= weeks of treatment

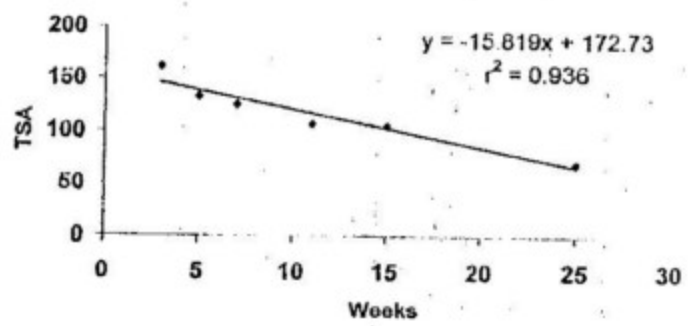


Figure 4: The linear correlation between the serum levels of TSA and the period of chemotherapy in ALL patients.

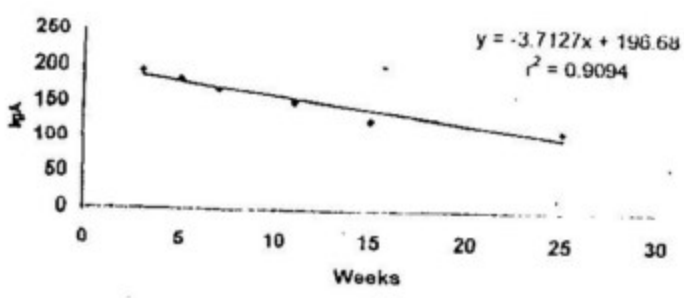


Figure5: The linear correlation between the serum levels of IgA and the period of chemotherapy in ALL patients.

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# مستويات حامض السياليك الكلي في المصل كمؤثر للحالة المناعية الخلطية في مرضى ابيضاض الدم اللمفاوي الحاد المعالجين وغير المعالجين كيمياوياً

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الخلاصة

تم اختبار ٤٠ مريضا بابيضاض الدم اللمفاوي الحاد بالنسبة لمستويات حمض السياليك الكلي TSA والكلوبيولينات المناعية IgA,IgM,IgG في مصل الدم قبل وبعد العلاج باستخدام ستة بروتوكولات مختلفة للعلاج الكيماوي. ازدادت مستويات TSA بشكل معنوي ( P<0.001) في مصل الدم لمرضى ابيضاض الدم اللمفاوي الحاد مقارنة بالأشخاص السليمين، بينما انخفضت مستوياته بشكل معنوي ( P<0.001 ) استجابة لجميع بروتوكولات العلاج الكيماوي مقارنة بالمرضى غير المعالجين. لقد وجدت علاقة ارتباط خطي ( $r^2$= 0.936 ) بين مستويات TSA وفترة العلاج الكيماوي . أظهرت مستويات IgA,IgM,IgG في مصل الدم لمرضى ALL زيادات معنوية ( P<0.001 ) مقارنة بالأشخاص السليمين. لقد انخفضت هذه المستويات بشكل معنوي ( P<0.001 ) بعد العلاج باستخدام كل من البروتوكولات الستة للعلاج الكيماوي مقارنة بمرضى ALL غير المعالجين. لقد وجدت علاقة ارتباط خطي ( $r^2$ =0.909) بين مستويات IgA في مصل الدم وفترة العلاج الكيماوي. تدعم نتائج الدراسة الحالية وجود دور لمستوى حمض السياليك الكلي في مصل الدم كمؤشر لتقصي كل من المرض والحالة المناعية الخلطية لمرضى ALL غير المعالجين، وتقترح وجود مثل هذا الدورلحامض السياليك الكلي في مرضى ALL المعالجين كيماويا كذلك.