The relationship between the levels of total sialic acid and complement proteins, C 3 and C 4, in the chemotherapy-untreated and –treated patients with acute lymphoblastic leukemia

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Abstract:

Blood samples were collected from chemotherapy-treated and -untreated patients with acute lymphoblastic leukemia (ALL). Samples were analyzed for the serum levels of total sialic acid (TSA), C3, and C4, as well as TSA levels in the white blood cells (WBCs) homogenate. In the group of untreated patients, TSA levels in the sera and WBCs were significantly increased as compared to normal subjects. A similar significant increase was observed in the levels of serum C3 and C4. In the group of treated patients, TSA levels in the sera and WBCs were significantly reduced, while insignificant decreases were observed in the serum levels of C3 and C4 as compared to the untreated patients. Correlation analysis showed weak relationships between TSA and complement levels in the sera of normal subjects, untreated, or treated patients. Thus, these results suggest that, despite the apparently similar behavior of the levels of TSA and the complement proteins before and after chemotherapy, TSA can not be used as an indicator to monitor the levels of complement proteins in response to chemotherapy.

Introduction :

Among the most widely distributed malignant diseases in Iraq, leukemia comes in the first place with the number of cases being doubled in the last decade of the 20th century (1). Finding suitable diagnostic and therapeutic tools for this disease is the scope of many investigations. At the level of diagnosis, the 9-carbon terminal sugar, sialic acid (SA), was found to be a reliable marker for the disease (2, 3, 4), as well as, for the status of the different components of the immune system during the disease course (5, 6, 7). At the level of treatment, chemotherapy is one of the most common protocols for leukemia treatment and it is also known to cause a state of immune suppression that involves different components of the immune system (8).

Complement activation (CA) is one of the defense mechanisms employed by the immune system against different tumorogenic diseases, including leukemia. Lymphocytes from certain forms of leukemia were found to be able to activate the complement system, but cells from other forms did not (9). Different complement receptors were also found to be expressed on the surfaces of various malignant cell lines, including leukemia cells (10, 11).

The relationship between CA and SA comes from the observations that nucleated cells can evade killing by homologous complement using several strategies, including cell surface SA (12). Moreover, certain cell types were shown to be more sensitive to complement -mediated cell lysis after

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removal of SA from their surfaces with neuraminidase (NA).This was shown to be true for normal human lymphocytes(13), red blood cells, rabbit lymph node cells(12), different carcinoma cell lines(10), bacterial cells(14), parasitic cells (15), and leukemic cells(16). Other studies, however, demonstrated no relationship between SA removal (17) and the membrane content of NA (9), from one side, and CA activation by acute and chronic leukemic cells, respectively, from the other side. To the limits of our knowledge, there was no investigation that has studied the relationship between CA and SA with reference to chemotherapy in leukemic patients.

In the present study, the attempts were made to draw direct relationships between serum and leukocyte contents of total sialic acid (TSA) and the serum levels of C3 and C4 in Iraqi patients with acute lymphoblastic leukemia (ALL). The attempts were extended to show how these relationships are affected in the chemotherapy-treated patients.

Materials and Methods:

Subjects. Two main groups of subjects were investigated. The first group included ALL patients who were referred to Baghdad Teaching Hospital to receive different programmes of chemotherapy. This group was divided into two subgroups; Chemotherapy-untreated and -treated ALL patients, each with 14 patients. The second group (n=14) included healthy individuals having no history of leukemia (Control group).

Estimation of TSA. Blood samples were collected from patients and control, and sera were separated according to Garvey *et al.* (18). Levels of TSA in the serum and WBCs homogenate were estimated using the colorimetric (Resorcinol reagent) method with absorbency read under optical density of 580nm (19).

Detection of C3 and C4: Serum levels of C3 and C4 were estimated using the Single Radial Immunodiffusion method (20).The method is based on measuring the diameter of the precipitation ring, and the complement 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). Correlation relationships were drawn using the SPSS programme. Results were expressed as mean ± standard error.

Results:

TSA in the serum. The level of TSA was significantly increased ($P<0.001$) in the sera of the chemotherapy-untreated ALL patients (177.52±3.69 mg/dl) as compared to the normal subjects (113.70± 0.92 mg/dl) (Fig.1). This level was significantly decreased ($P<0.001$) in the sera of chemotherapy-treated ALL patients (116.43 ± 13.59 mg/dl) as compared to the untreated patients, reaching values comparable to the normal ones (Fig.1).

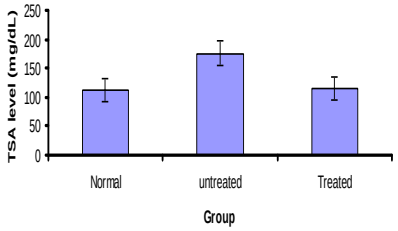

Figure 1. TSA levels in the sera of normal subjects and chemotherapy-treated and -untreated patients with ALL.

TSA in the white blood cells (WBCs) homogenate. The level of TSA was significantly increased (P<0.05) in the WBCs homogenate of the chemotherapy-untreated ALL patients (125.20 ± 8.06 mg/dl) as compared to normal subjects (79.91±1.71 mg/dl) (Fig.2). This level was significantly decreased (P<0.05) in the WBCs homogenate of chemotherapy-treated ALL patients (84.29 ± 10.51 mg/dl) as compared to the untreated patients, reaching values close to the normal ones (Fig.2).

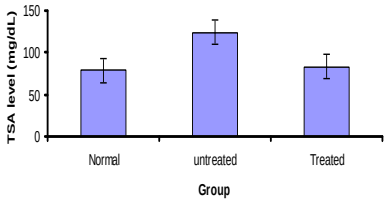

Figure 2. TSA levels in the white blood cell homogenates from normal subjects and chemotherapy-treated and -untreated patients with ALL.

C3 in the serum. The level of C3 was significantly increased (P<0.001) in the sera of the chemotherapy-untreated ALL patients (213.14±15.72 mg/dl) as compared to the normal subjects (130.15±11.35 mg/dl) (Fig.3). This level was insignificantly decreased (P<0.05) in the sera of chemotherapy-treated ALL patients (182.83±14.70 mg/dl) as compared to the untreated patients (Fig.3).

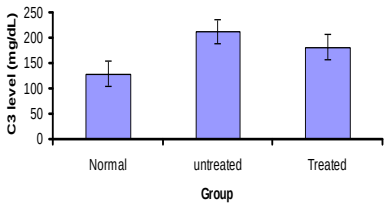

Figure 3. C3 levels in the sera of normal subjects and chemotherapy-treated and –untreated patients with ALL.

C4 in the serum. The level of C4 was significantly increased (P<0.05) in the sera of the chemotherapy-untreated ALL patients (50.78±4.78 mg/dl) as compared to the normal subjects (34.95±2.76 mg/dl) (Fig.4). This level was insignificantly decreased (P<0.05) in the sera of chemotherapy-treated ALL patients (45.87±5.32 mg/dl) as compared to the untreated patients (Fig.4).

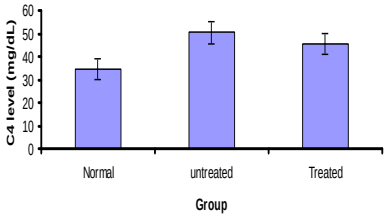

Figure 4. C4 levels in the sera of normal subjects and chemotherapy-treated and –untreated patients with ALL.

Correlation between serum levels of TSA, C3 and C4

TSA and C3: The results showed a weak correlation relationship between serum levels of TSA and C3 both in the normal subjects (r^2= 0.1079; Fig.5) as well as the chemotherapy-untreated (r^2= 0.2643; Fig.6) and -treated (r^2= 0.0976; Fig.7) ALL patients.

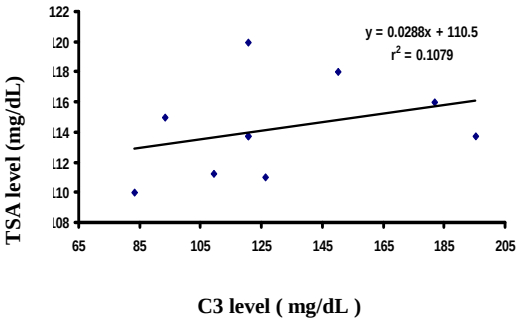

Figure 5: The correlation relationship between serum levels of TSA and C3 in the control group.

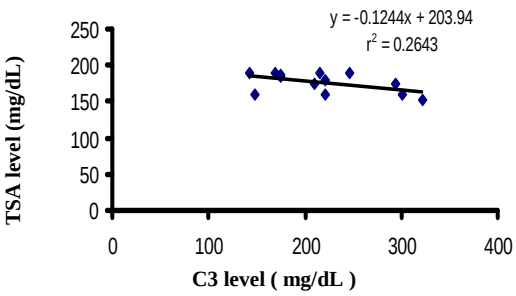

Figure 6: The correlation relationship between serum levels of TSA and C3 in the chemotherapy-untreated ALL patients.

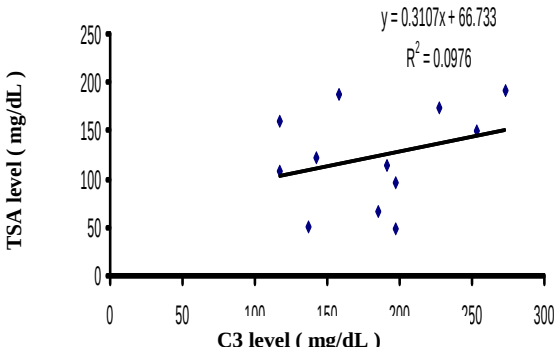

Figure 7: The correlation relationship between serum levels of TSA and C3 in the chemotherapy-treated ALL patients.

TSA and C4: The results showed a weak correlation relationship between serum levels of TSA and C4 both in the normal subjects (r^2= 0.0088; fig.8) as well as the chemotherapy-untreated (r^2= 0.015; fig.9) and -treated (r^2= 0.0124; fig.10) ALL patients.

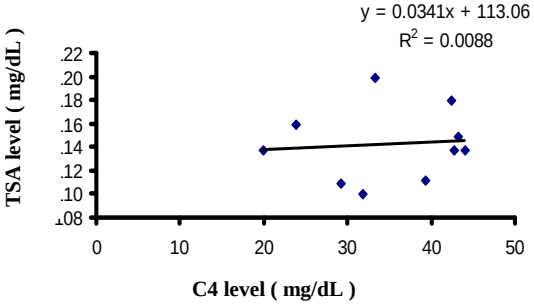

Figure 8: The correlation relationship between serum levels of TSA and C4 in the control group.

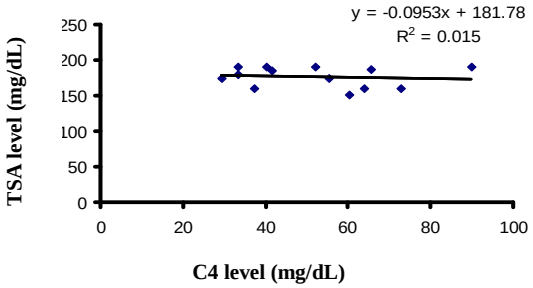

Figure 9: The correlation relationship between serum levels of TSA and C4 in the chemotherapy-untreated ALL patients.

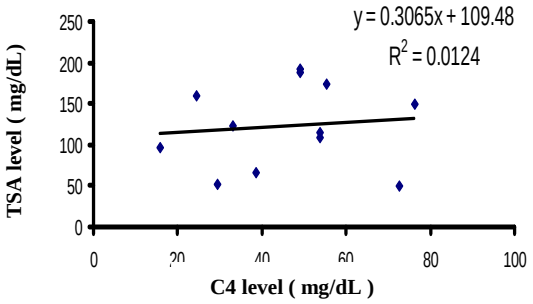

Figure 10: The correlation relationship between serum levels of TSA and C4 in the chemotherapy-treated ALL patients.

Discussion:

The present study showed a highly significant increase in the levels of serum TSA in the patients with ALL. These results are in agreement with those of other previous studies conducted with various types of tumors (21, 22). This increase could be attributed to the increased shedding of sialic acid from cancer cells, which might reflect an increase in the activity of NA on the membranes of these cells (23), or to the hyper-production of SA-rich acute phase proteins by the liver in response to an inflammatory reaction to the tumor (24). The increased levels of serum TSA were associated with similar increases in the TSA levels of the WBC homogenates in the same ALL patients. This increase can be explained in the ground of a compensation mechanism that adopted by the WBCs in which increased levels

of SA are produced to replace those molecules shed to the serum. There is a need , however, to investigate these changes in TSA levels along with the activities of both NA and sialyltransferase , the enzymes that mediate the processes of SA shedding and synthesis, respectively.

Other increases in the present study were observed in the levels of the complement proteins C3 and C4 in the sera of patients with ALL. Once again, these increases are not surprising due to the well expected activation of the host defense mechanisms, including complement, during malignant diseases (9, 10, 11, 13). These increases and the above mentioned increases in the levels of TSA were simultaneous in ALL patients. This observation agrees with the previous studies which showed that SA is one of the strategies used by different pathogenic cells, including tumor cells, to evade the killing activity of the complement system, especially the alternative pathway (12). Thus, removal of sialic acid from the plasma membranes of malignant cells, as reflected in the present study by the elevated levels of serum TSA, probably resulted in the exposure of these cells to the lysis activities by complement proteins and, thereby, increased levels of complement proteins necessary to attack the exposed cells.

In the patients treated with chemotherapy, significant decreases were uniformly observed in the levels of TSA in the serum and WBCs homogenate as well as insignificant decreases in the serum levels of C3 and C4 when compared with the untreated patients. The decrease in the serum TSA levels of ALL patients is consistent with the previous data showing that these levels attains the value of control and even reaches a lower value (25). The reason for that might be the damaging effects that chemotherapy drugs were found to have on the mitotic activity of the tumor cells (8) and, thereby, on the levels of TSA being produced and shed. Another possible explanation is a direct effect for chemotherapy drugs on the activity of NA, leading to a decreased shedding of TSA into serum, or sialyltransferase, leading to a reduced synthesis of SA by the tumor cell machinery. The associated decrease in the serum levels of complement proteins is probably a direct reflection of the immune suppressive function that chemotherapy drugs have.

In conclusion, the present study directly demonstrates uniform behaviors for the levels of TSA and complement proteins in the sera of patients with ALL. These behaviors took the forms of simultaneous elevations in the patients and then simultaneous decreases in the chemotherapy-treated patients. Yet, due to the weak correlation relationships between these levels, it is not possible to employ TSA levels as an indicator for the serum levels of C3 and C4 neither in the chemotherapy-untreated nor in the chemotherapy-treated patients with ALL.

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العلاقة بين مستويات حامض السياليك الكلي وبروتينات المتمم C3 و C4 في مرضى ابيضاض الدم اللمفاوي الحاد الخاضعين وغير الخاضعين للعلاج الكيميائي

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الخلاصة:

جمعت عينات الدم من مرضى ابيضاض الدم اللمفاوي الحاد (ALL) المعالجين وغير المعالجين بالعلاج الكيميائي. تم تحليل العينات بالنسبة لمستويات حامض السياليك الكلي (TSA) وبروتينات المتمم C3 و C4 في مصل الدم فضلاً عن مستويات TSA في مجانس خلايا الدم البيض . شهدت مجموعة المرضى غير المعالجين ارتفاعاً معنوياً في مستوىTSA في المصل ومجانس الخلايا البيض بالمقارنة مع عينة السيطرة . لوحظت زيادة معنوية مشابهة في مستويات C3 و C4 في المصل . شهدت مجموعة المرضى المعالجين انخفاضاً معنوياً في مستوى TSA في المصل ومجانس الخلايا البيض ، بينما لوحظ انخفاض غير معنوي في مستويات C3 و C4 في المصل لهذه المجموعة وذلك عند مقارنتها مع مجموعة المرضى غير المعالجين . أظهر تحليل معامل الارتباط عدم وجود ارتباط معنوي بين مستويات TSA وبروتينات المتمم في مصل الدم لكل من الاشخاص السليمين والمرضى غير المعالجين والمرضى المعالجين . لذلك تقترح هذه النتائج انه بالرغم من السلوك المتشابه ظاهرياً لمستويات TSA وبروتينات المتمم قبل وبعد العلاج الكيميائي الا ان TSA لا يمكن استخدامه كمؤشر لمراقبة مستويات بروتينات المتمم استجابةً للعلاج الكيميائي.