# The Influence of Body Weight on the Serum Sialic Acid Levels in Lymphoma Patients

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## Abstract

The a prospective study of the effect of body weight of 70 patients with lymphoma on the levels of serum sialic acid forms, total sialic acid, bound sialic acid, free sialic acid and lipid sialic acid were measured. The results were compared with those obtained from control groups which consisted of 50 person of matched age and sex.

**Objective**: To determine the influence of body weight on the serum sialic acid forms.

**Methods:** The patients were categorized into four groups according to the certain statistical methods. The first group comprised 24 patients who have weight 5-20Kg range. The second group comprised 13 patients who have weight 21-40Kg range. The third group comprised 12 patients who have weight 41-60Kg range. The fourth group comprised 21 patients who have weight >60Kg.

**Results:** Significant elevations (P<0.005) of total sialic acid, bound sialic acid, free sialic acid, and lipid associated sialic acid (TSA, BSA, FSA, and LSA respectively) levels were seen in all groups of lymphoma patients when compared with those of the healthy subjects. Total serum protein was decreased significantly (P<0.005) and TSA/TP, BSA/TP, FSA/TP, and LSA/TP ratios raised significantly (P<0.005) in the cancer patients in comparison with those of the healthy individuals.

**Conclusion:** Sialic acid is not specific marker for detecting lymphoma patients.

**Keywords:** Body weight, lymphoma patients, serum sialic acid

## الخلاصة

في دراسة مستقبلية لتأثير وزن المريض لسبعين مريض من مرضى السرطانات اللمفاوية على مستويات الاشكال المختلفة لحامض السياليك ,حامض السياليك الكلي وحامض السياليك المرتبط والحر والمترافق مع الدهن .النتائج تم مقارنتها مع مجموعة السيطرة والتي كانت مطابقة لمجموعة المرضى من حيث العمر والجنس.

هذه الدراسة تهدف إلى تقويم تأثير الوزن لمرضى السرطانات اللمفاوية على مستويات الإشكال المختلفة لحامض السياليك.

تم تقسيم المرضى إلى أربع مجاميع تضمنت المجموعة الأولى 24 مريضا من لهم معدل وزن بين5-20 كغم. المجموعة الثانية تألفت من 13 مريضا من الذين لهم معدل وزن بين 21-40 كغم. المجموعة الثالثة تضمنت 12 مريضا من الذين لهم معدل وزن بين 41-60 كغم. المجموعة الرابعة تألفت من 21 مريضا من الذين لهم معدل وزن اكثر من 60 كغم.

دلت النتائج على زيادة معنوية في تركيز حامض السياليك الكلي و المرتبط و المترافق مع الدهن و الحر في مجاميع المرضى عند مقارنتها مع مثيلاتها لدى الأشخاص الأصحاء.كما بينت الدراسة ارتباطا خطيا سالبا بين حامض السياليك المترافق مع الدهن إلى تركيز البروتين الكلي و الوزن.

حامض السياليك لا يمكن اعتباره كدالة للكشف عن مرضى السرطانات اللمفاوية.

## Introduction

Sialic acid has been hypothesized to play an antiatherogenic role in lipoprotein metabolism through the electrostatic inhibition of lipoprotein interaction with chondroitin -6-sulphate-rich arterial proteoglycans (APG) [9]. Many investigators have been searching for a specific, reliable and easily identifiable marker for cancer. No such single marker has yet been found. However, several different biological substances are elevated in cancer substances including antigens, biological metabolites ectopic hormones and isoenzymes [10].

Alteration in cell surface and glycoproteins are common during carcinogenesis and may play a key role in determining the metastatic behavior of tumor cells [11- 14].

The structural diversity of sialic acid is reflected in the Varity of its biological functions [4, 15-17].Due to its size and the hydrophilic character, along with its negative charge this sugar can have a simple physiochemical effect on its environment.

The current project was aimed to evaluate the effect of body weight on the levels of serum sialic acid in lymphoma patients.

## Materials and methods

Serum sialic acid forms levels were obtained from 70 lymphoma patients. Hodgkin's and Non-Hodgkin's patients. An accurate diagnosis of these patterns was made by specialists in oncologist and in close co-operation with pathologist depending on detection of molecular markers in peripherals blood and lymph node. The majority of patients were under treatment. Duration of such patients who receive chemotherapy ranged between less than one year for 42 patients and the others 18 patients who under treatment more than year. Ten patients who did not take any treatment. In addition to 50 matched healthy individuals. The study carried out at the department of Biochemistry, college of medicine, university of Kufa.

**Specimen collection**

Disposable syringes and needles were used for blood collection. Blood samples were obtained from patients and the control group by vein puncture. Samples were allowed to clot at 37°C, and then centrifuged at 3000Xg for 10 min. Sera were removed and stored at -20°C until analysis.

**Chemicals and apparatus.**

All laboratory chemicals and reagents were of Analar grade (Fluka, BDH, and Merck companies). Sialic acid was obtained from Sigma Company USA.

Total sialic acid assay was performed using colorimetric method according to Doumas and Peters [18]. The concentration of total sialic acid was calculated from the standard curve constructed in the range 10-80 mmole/l of pure N-acetyl neuraminic acid. The reagents used for the quantitative assay were: (a) 0.04M periodic acid; (b) 0.6g of resorcinol in a solution containing 60ml of 28% HCl, 40ml of distilled water, and 25µmole of $CuSo_4.2H_2O$; (c) 95% tert-butyl alcohol. The reagents were prepared fresh daily from stock 0.4M periodic acid and 6% resorcinol solution; the stock reagents were stable to storage for several weeks at -20C° in the dark. All assay reagents were stored at 0C° except tert-butyl alcohol which was maintained at room temperature. Bound sialic acid was estimated using the protocol of determination of total sialic acid except that the oxidation step is carried out at 37C° rather than 0C°. Free sialic acid

was determined from the difference of level indicated with the oxidation at 0C° and that obtained by oxidation at 37C°. Lipid associated sialic acid was measured by the procedure of Katopid and Stock [19]. The reagents used for the quantitative assay were: (a) chloroform: methanol (2:1 v/v) at 4C°; (b) phosphotungestic acid 1g/ml.Total proteins in serum were evaluated by Biuret method [20]. Albumin concentration was estimated according to the method of (Bromocresol green) [18].

**Statistical analysis.**

The results were illustrated as mean ± S.D and analyzed statistically. The differences between the results of lymphoma patients and healthy individuals were assessed by Student's *t* test. Significant variation was considered when P value was less than 0.05. The analysis of variance (ANOVA) was used for the assessment of the effect of the weight on the cancerous patients.

## Results

To evaluate the effect of body weight on the levels of the serum biomarkers in a total 70 lymphoma patients. There were 24 females and 46 males. Their ages ranged from 3-71year. the enrolled patients were categorized into four groups. The first contained 24 patients who have weight <20Kg. The second comprised 13 patients who have weight range between 21-40Kg.

The third consisted 12 patients who have weight range between 41-60Kg. The fourth contained 21 patients who have weight >60Kg.The data were analyzed statistically by the student *t* test. TSA, BSA, FSA, and LSA levels were found to be elevated significantly (P<0.005) in all patients groups when compared with healthy subjects (Table1).

Table 1. Effect of weight on serum sialic acid concentration in lymphoma patients and healthy subjects

| Subject | Healthy | Patients with | | | |
|---|---|---|---|---|---|
| | N =50 | Group I | Group II | Group III | Group IV |
| | | N =24 | N =13 | N =12 | N =21 |
| TSA mmole/l Mean± S.D P value | 1.35±0.14 | 1.96±0.6 P<0.005 | 2.28±0.54 P<0.005 | 2.09±0.53 P<0.005 | 2.32±0.79 P<0.005 |
| BSA mmole/l Mean± S.D P value | 1.14±0.16 | 1.58±0.63 P<0.005 | 1.76±0.43 P<0.005 | 1.64±0.67 P<0.005 | 1.84±0.76 P<0.005 |
| FSA mmole/l Mean± S.D P value | 0.22±0.11 | 0.38±0.17 P<0.005 | 0.51±0.22 P<0.005 | 0.51±0.3 P<0.005 | 0.48±0.22 P<0.005 |
| LSA mmole/l Mean± S.D P value | 0.46±0.19 | 1.30±0.6 P<0.005 | 1.3±0.58 P<0.005 | 1.18±0.58 P<0.005 | 1.12±0.54 P<0.005 |

Group I; Group II; Group II; Group IV Lymphoma patients have weight range between < 20; 21- 40 ; 41- 60 >60 Kg.

TSA, BSA, FSA, and LSA normalized to total serum proteins (TSA/TP, BSA/TP, FSA/TP, and LSA/TP respectively) were evaluated in cancer patients and healthy individuals. However, these ratios were found to be to be increased significantly (P<0.005) in the cancerous patients when compared with the healthy subjects (Table 2).

Table 2. Effect of weight on serum sialic acid concentration normalized to total serum proteins in lymphoma patients and healthy subjects

| Subject | Healthy | Patients with | | | |
|---|---|---|---|---|---|
| | N =50 | Group I | Group II | Group III | Group IV |
| | | N =24 | N =13 | N =12 | N =21 |
| TSA/TP mmole/g<br>Mean ± S.D<br>P value | 0.19±0.02 | 0.39±0.21<br>P<0.005 | 0.39±0.17<br>P<0.005 | 0.38±0.17<br>P<0.005 | 0.38±0.14<br>P<0.005 |
| BSA/TP mmole/g<br>Mean ± S.D<br>P value | 0.15±0.03 | 0.30±0.17<br>P<0.005 | 0.31±0.15<br>P<0.005 | 0.32±0.18<br>P<0.005 | 0.32±0.13<br>P<0.005 |
| FSA/TP mmole/g<br>Mean ± S.D<br>P value | 0.03±0.02 | 0.07±0.03<br>P<0.005 | 0.07±0.05<br>P<0.005 | 0.07±0.05<br>P<0.005 | 0.07±0.04<br>P<0.005 |
| LSA/TP mmole/g<br>Mean ± S.D<br>P value | 0.10±0.03 | 0.21±0.12<br>P<0.005 | 0.20±0.1<br>P<0.005 | 0.20±0.12<br>P<0.005 | 0.15±0.02<br>P<0.005 |

Group I; Group II; Group II; Group IV Lymphoma patients have weight range between < 20; 21- 40 ; 41- 60 ; >60Kg.

Total serum proteins, albumin contents, globulin, and albumin /globulin magnitudes were measured in all lymphoma patients and healthy individuals. The values were analyzed by student *t* test. As shown in (Table 3) there are significant P<0.005 decreases in serum total proteins, and albumin contents, whereas globulin content increased significantly P<0.005, P<0.09, and P<0.04 for group I, III, and IV respectively, but non-significant for group II when compared with healthy subject, albumin/globulin as shown raised P<0.007, P<0.095, P<0.05, P< 0.01 significantly for group I, II, III, and IV respectively when compared with healthy subjects.

Table 3. Effect of weight on serum total protein concentration in lymphoma patients and healthy subjects

| Subject | Healthy | Patients with | | | |
|---|---|---|---|---|---|
| | N =50 | Group I | Group II | Group III | Group IV |
| | | N =24 | N =13 | N =12 | N =21 |
| TP g/dl<br>Mean± S.D<br>P value | 7.5±0.75 | 5.9±0.95<br>P<0.005 | 6.1±1.3<br>P<0.005 | 5.71±0.88<br>P<0.005 | 6.04±1.1<br>P<0.005 |
| Albumin g/dl<br>Mean± S.D<br>P value | 5.5±0.63 | 2.9±1.5<br>P<0.005 | 3.8±0.73<br>P<0.005 | 3.1±1.1<br>P<0.005 | 3.35±1.1<br>P<0.005 |
| Globulin g/d<br>Mean± S.D<br>P value | 1.9±0.9 | 3.02±1.5<br>P<0.005 | 2.3±1.1<br>N.S | 2.6±1.2<br>P<0.09 | 2.6±0.9<br>P<0.04 |
| Albumin/Globulin Mean± S.D<br>P value | 4.01±4.9 | 1.63±2.02<br>P<0.007 | 2.12±1.6<br>P<0.095 | 1.91±0.66<br>P<0.059 | 1.64±0.92<br>P<0.01 |

Group I; Group II; Group II; Group IV Lymphoma patients have weight range between < 20; 21- 40 ; 41- 60 ; >60Kg.

Further evaluation of the effect of the weight of lymphoma patients was carried out upon sub-grouping of cancerous patients in four groups. The analysis of variance (ANOVA) is used for the statistical analysis of the results. The major differences were found to be between I with II groups (Table 4). Such differences were significant for four parameters (TSA, FSA, Albumin, and Globulin), and I with IV such differences were significant for four

parameters (TSA, BSA, FSA, LSA/TP). In general, the order of significant variation was found to be; group I with II, I with IV > group II with III, group II with IV > group I with III, II with IV.

Table 4. Results of analysis of variance (ANOVA) for effect of weight in the serum sialic acid concentration in lymphoma patient.

| Subjects | Patients | | | | | |
|---|---|---|---|---|---|---|
| | I with II | I with III | I with IV | II with III | II with IV | III with IV |
| TSA mmole/l | P<0.065 | N.S | P<0.01 | N.S | N.S | N.S |
| BSA mmole/l | N.S | N.S | P<0.08 | N.S | N.S | N.S |
| FSA mmole/l | P<0.04 | P<0.05 | P<0.09 | N.S | N.S | N.S |
| LSA mmole/l | N.S | N.S | N.S | P<0.06 | P<0.01 | P<0.06 |
| TP g/dl | N.S | N.S | N.S | N.S | N.S | N.S |
| Albumin g/dl | P<0.009 | N.S | N.S | P<0.08 | N.S | N.S |
| Globulin g/dl | P<0.07 | N.S | N.S | N.S | N.S | N.S |
| Alb/Glob ratio | N.S | N.S | N.S | N.S | N.S | N.S |
| TSA/T mmole/g | N.S | N.S | N.S | N.S | N.S | N.S |
| BSA/TP mmole/g | N.S | N.S | N.S | N.S | N.S | N.S |
| FSA/TP mmole/g | N.S | N.S | N.S | N.S | N.S | N.S |
| LSA/TP mmole/g | N.S | N.S | P<0.01 | N.S | P<0.06 | N.S |

N.S: non significant.

Table 5. Results of univariate analysis of sialic acid contents, sialic acid normalized to total proteins, albumin and globulin in sera and weight in lymphoma patients

| Subjects | r | P value |
|---|---|---|
| TSA | 0.12 | N.S |
| BSA | 0.085 | N.S |
| FSA | 0.12 | N.S |
| LSA | 0.1353 | N.S |
| TP | 0.014 | N.S |
| Albumin | 0.12 | N.S |
| Globulin | 0.13 | N.S |
| TSA/TP | 0.055 | N.S |
| BSA/TP | 0.0071 | N.S |
| FSA/TP | 0.054 | N.S |
| LSA/TP | 0.23 | P< 0.05 |

## Discussion

In the body, sialic acid, synthesized from glucosamines, turns into a derivative form cytidine mono phosphate(CMP). The product reacts with oligosaccharides and polysaccharides and then acts as a sialic acid source, with sialyltransferase being the enzyme which transfers the sialic acid from the CMP derivative to the terminal glucose chain of the glycoprotein [21, 22].

The changes in the biosynthesis and post-translational glycosylation processing of the acute-phase glycoprotein in the liver is likely to cause considerable raises in sialic acid [23-25]. On the other hand, elevations of sialic acid may be related to the intensified cell metabolism and increased serum sialiyltransferase activity expressed by the tumor cells [26]. Then causes excess amount of sialic acid penetration in to plasma.

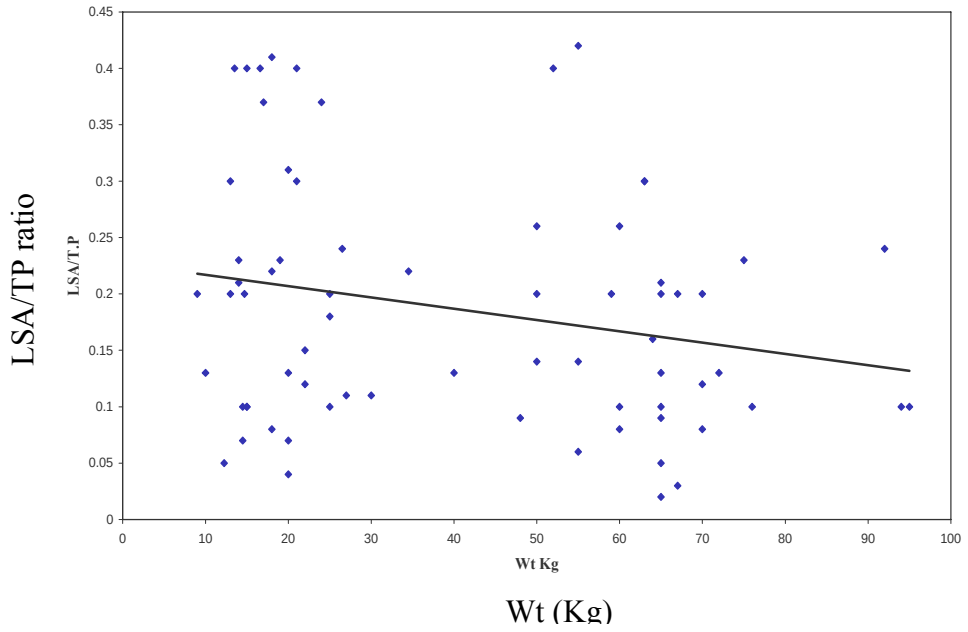


Fig 1. Correlation of lipid associated sialic acid normalized total protein (LSA/TP) levels and weight in lymphoma patients.

In this study of body weight influence indicate significant elevation in sialic acid forms in cancerous tissues compared with those normal tissues. The explanation is that Sialic acid found on the cell surface is responsible for the negative charge of the cell due to its ionized carboxyl group[27]. An increase of sialic acid content in the membrane causes more binding of the positively charged such as $Ca^{+2}$ to the membrane, consequently leading to structure destruction by aggregate formation. This finding is considered to be an effective factor in the formation of cancer by some investigators.

According to current results we could conclude a panel of sialic acid estimation as a routine assessment of lymphoma patients. Further biomarkers are needed to determine the prognostic elevation of lymphoma.

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