

# Elevated Levels of Sialic Acid and Lipid-Associated Sialic Acid in Plasma of Rheumatoid Arthritis Patients

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## Abstract

The objective of this study is to evaluate plasma levels of total Sialic acid TSA and Lipid-associated Sialic acid LSA as a marker of Rheumatoid Arthritis AR. Plasma Sialic acid is known as a parameter of inflammation. In the present study, in order to explore the potential role of sialic acid in arthritis rheumatoid, plasma sialic acid levels, plasma LSA and total protein in patients with arthritis rheumatoid were measured. A total 40 patients were compared with 40 healthy control subjects. Plasma TSA, LSA and TP level were determined spectrophotometrically in plasma samples. Plasma Sialic acid levels were significantly increased in RA ( $88.48 \pm 14.15$  mg/dl,  $P < 0.05$ ) and LSA level were significantly increased in RA ( $26.37 \pm 2.25$  mg/dl,  $p < 0.05$ ). By contrast there wasn't any significant elevation of TP in RA patients, compared with healthy control (TSA,  $53.31 \pm 7.58$  mg/dl,  $P < 0.05$ ), (LSA,  $18.46 \pm 1.45$  mg/dl) and (TP,  $7.39 \pm 0.34$  g/dl).

Key word: Sialic acid, Lipid-associated Sialic acid.

## Introduction

Sialic acid (N-acetylneuraminic acid NANA) are acetylated derivatives of neuraminic acid. They are attached to non reducing residues of the carbohydrate chains of glycoproteins and glycolipids. The suggested biological functions of sialic acid are as following: (a) stabilizing the conformation of glycoproteins and cellular membranes; (b) assisting in cell to cell recognition and interaction; (c) contributing to membrane transport; (d) affecting the function of membrane receptors by providing binding sites for ligand; (e) influencing the function stability and survival of blood glycoproteins; and (f) regulating the permeability of the basement membrane of glomeruli [1]. Sialic acid concentration varies physiologically with age, but its level may also be influenced by such a condition as inflammation [2]. Neoplastic tumors or inborn genetic disorder which cause abnormal sialic acid metabolism [3]. Sialic acid and its derivatives were used in the treatments of several diseases, including neuropathic and inflammatory diseases as well as certain tumors [4]. Lipid-associated sialic acid (LSA) is a useful adjunct in the management of a variety of malignancies [5]. Elevation in blood LSA level was reported in patients with mammary (63%), gastroenteric (65%), pulmonary (79%) and ovarian (94%) neoplasms as well as those with leukemia (91%), lymphoma (87%), melanoma (84%), and Hodgkin disease (91%). [6 – 11]. Sialic acid level do not appear to be a good marker for discriminating malignant from non malignant disease of the Lung [12]. Higher levels of total sialic acid were found in women with metabolic gestation syndrome [13] and during periodontal disease [14]. ESR is the most commonly performed laboratory test for inflammation in rheumatic disorder reactive protein, transferrin, ceruloplasmin, albumin,  $\alpha$ 1-antitrypsin were similarly used [15, 16]. The present study was undertaken to evaluate the utility of TSA and LSA as an index to measure the disease activity and inflammation in Rheumatoid Arthritis (RA). TSA, LSA and

Total Protein TP were studied in patients with Rheumatoid Arthritis .These were compared with normal healthy controls.

## Materials and Methods

### Chemicals:

Standard solution for sialic acid 500micro gram/ml concentration was prepared by dissolving 50 mg 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(5,10,15,20,25,30 micro gram/ml)for calibration curve measurement.

### Patiens:

Plasma for the measurement of TSA , LSA and TP levels was obtained from 40 patients with rheumatoid arthritis of subjects followed at the central lab in Sulaimania for the period from Dec. 2007 to Apr.2008.The diagnosis of this case was specified by consultants.

### Plasma preparation:

Venous blood was collected from fasting patients in the early morning. The samples were taken in tubes containing anticoagulant, their plasma were separated and used immediately ,otherwise , plasma samples were stored at [-15]until analysis.

### Biochemical methods:

#### 1-Total sialic acid

##### A-Principle

The principle of this method depends on the formation of chromogen from the addition of resorcinol reagent in to the test tube. The chromogen formed was extracted by butyl acetate/methanol reagent and measured at 580 nm[17].

##### B-Reagents

Reagent I: Resorcinol stock: made up by dissolving 2 gm of resorcinol in 100 ml of deionized water. This reagent is stable for many months in the refrigerator.

Reagent II: Resorcinol HCL: 10 ml of the stock solution is added to the mixture of 80 ml of concentrated HCL and 0.25 ml of 0.1M CuSO<sub>4</sub>. The volume is then made up to 100ml with deionized water. This should be prepared at least four hours before use and stable for two weeks in the refrigerator.

Reagent III: butyl acetate/methanol: 85ml of butyl acetate is added to 15ml of methanol.

### C-Procedure:

The reaction was performed in 180×150mm Pyrex test tube labeled as test, standard and blank, into which the following reagents were pipettes as follows:

| Reagent     | Test<br>μl | Standard<br>μl | Blank μl |
|-------------|------------|----------------|----------|
| sample      | 20         | -              | -        |
| D.W         | 980        | 980            | 980      |
| standard    | -          | 20             | -        |
| Reagent II  | 1000       | 1000           | 1000     |
| Reagent III | 2 000      | 2000           | 2000     |

The tubes were capped with glass bulbs and heated for 15 min in a boiling water bath (100C) and then cooled in tap water bath or ice for 10 min. Vortexes and centrifuged for 10min at 3000 rpm, the extracted chromophore was read at 580nm.

**LSA determination:**

LSA was measured according to the method described by Katopodis and his co-worker [18, 19] Fifty µl plasma aliquots were placed in screw-capped tubes, 3ml of cold of (4C)chloroform/methanol(2:1v/v)mixture was added to each tube for total lipid extraction, the tubes were then capped and vortexes for 30 seconds, 0.5ml of cold water was added to each tube and the tubes were centrifuged for 5 min. at 2500 rpm at room temp.

The upper phase (aqueous Layer containing LSA) was transferred to another screw-capped tube and 50micro ml of phosphotungstic acid (1g/ml) was added to each tube. The tubes were vortexes again and allowed to stand at room temp. for 5min. Then the tubes were centrifuged at room temp for 5min. at 2500rpm. After that, the supernatants were decanted and the remaining pellets were redissolved in 1ml of water at 37°C by vigorously vortexing them for 1min and sialic acid content was determined as mentioned for TSA.

**TP determination:**

Colorimetric method was described by Gornall et al [20, 21]. The peptide bonds of protein react with Cu<sup>2+</sup> in alkaline solution to form a colored complex whose absorbance is proportional to the concentration of the total protein in the specimen, and was measured at 550 nm. The biuret reagent contains sodium potassium tartrate to complex cupric ions and maintain their solubility in alkaline solution.

**Reagents:**

**Reagent I:**

Sodium hydroxide 370 mmol/L + Na-K Tartrate 10 mmol/L + Potassium iodide 3 mmol/L + Copper sulfate 3 mmol/L  
Standard solution –Bovine Albumin 6 gm/dl.

**Procedure:**

Let stand reagent and specimens were left at room temperature

| Pipette into test tube | Blank µl | Standard µl | Test µl |
|------------------------|----------|-------------|---------|
| Reagent                | 1000     | 1000        | 1000    |
| standard               |          | 20          |         |
| specimen               |          |             | 20      |
| D.W                    | 20       |             |         |

The above components in the tubes were mixed, left to stand for 10 minutes at room temperature.

Record absorbance's at 550nm against reagent blank.

**Calculation**

Result = Abs(Assay) ÷ Abs(standard) × standard conc.

## Results

The levels of plasma sialic acid TSA indicate significant statistical differences in the Rheumatoid arthritis patients group compared to the control group ( $P < 0.05$ ). There is also a significant statistical difference ( $P < 0.05$ ) with the respect to the levels of plasma-associated sialic acid between the control group and rheumatoid arthritis patients group (table-1)(fig-3,fig-4). Figs (1 and 2) show the percent of TSA, LSA and TP in rheumatoid arthritis and control group. TP not changed in this rheumatoid Fig-5. Table (1) also shows the Mean $\pm$  SD, variance, P-value, Confidence interval, selectivity and sensitivity for all tests (TSA, LSA and TP).

The correlation coefficient equals -0.27, indicating a relatively weak relationship between the variables TSA and LSA (Fig -6-a). The correlation coefficient equals 0.25, indicating a relatively weak relationship between the TSA and TP (Fig-6-b), where the correlation coefficient equals 0.10, indicating a relatively weak relationship between the variables LSA and TP (Fig-6-c). The present study shows that the magnitude of the selectivity varies between (60% for TP) for patients with RA, while equals 82.5% for TSA and 85% for LSA for patients with RA.

## Discussion

Several studies were performed about the determination of a new marker for the diagnosis of rheumatoid arthritis. The present data confirms the previous evidence that TSA and LSA are significantly higher in patients with rheumatoid arthritis (RA). RA which is along-term disease that causes inflammation of joints and surrounding tissues. It can also affect other organs. Most glycoproteins contain mannose, galactose, fucose, sialic acid and in some instances glucose as the carbohydrate moiety. Spiro, in 1959 established that liver is the major organ involved in the normal synthesis of glycoproteins [22]. All the TSA and LSA were significantly elevated in the plasma of patients with (RA), TP from patients of RA which showed minimal but statistically insignificant elevation. TSA and LSA may, thus be able to differentiate an inflammatory arthritis from a dehydrative, non-inflammatory arthritis. Our results are supported with previously published work [23].

Previous studies were centered largely around the demonstration of increased levels of carbohydrates of the carbohydrate-protein complex and carbohydrate-lipid complex in plasma of RA. More over, elevated plasma or serum levels of TSA and / or LSA were observed in many cases (23 - 25). There was a positive correlation of ESR with glycoproteins studied, the best correlation being with hexosamine followed by sialic acid and other hexose in that order [26]. The elevation of plasma TSA and LSA in RA patients is of note and we believe our data add to the literature showing changes in TSA and LSA status in RA. The mechanisms are unclear and we can only speculate as to the reason. In conclusion, the increase of plasma TSA and LSA levels is associated positively with the presence of inflammation and appears to be a consequence of the disease itself, and could be suggested as one of the newly discovered marker for RA.

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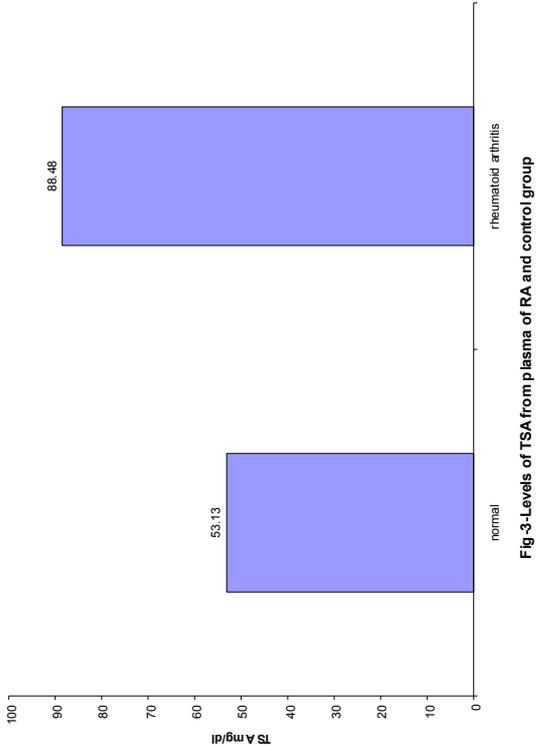


Fig-3-Levels of TSA from plasma of RA and control group

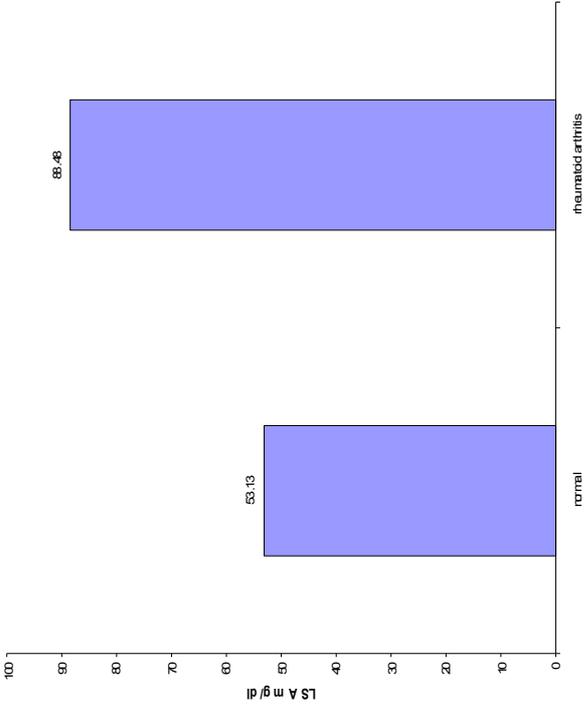


Fig-4-Levels of LSA from plasma of RA and control group

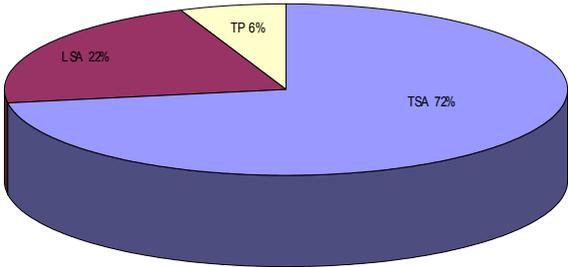


Fig-2-Percent o TSA, LSA and TP from plasma of RA patients

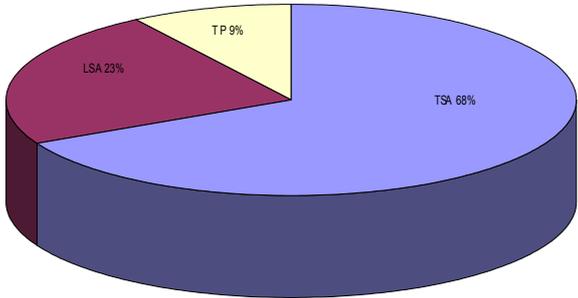


Fig-1-Percent of TSA, LSA and TP from plasma of control group

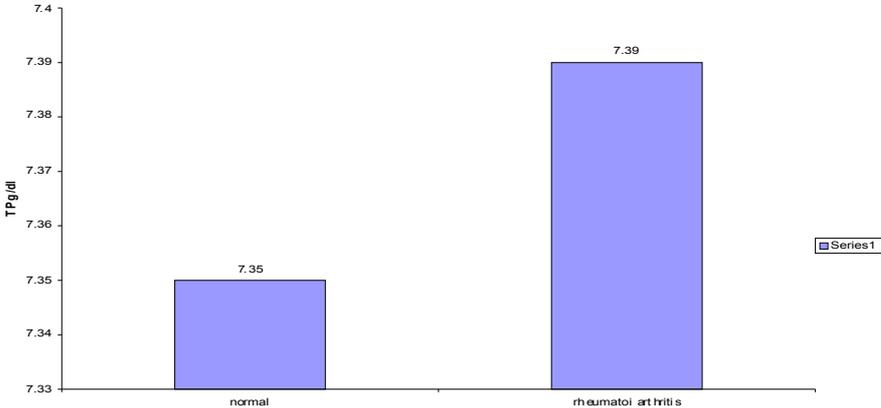


Fig-5-Levels of TP from plasma of RA pateints and control group

Fig 6-c:-Coorrelation between LSA and TP

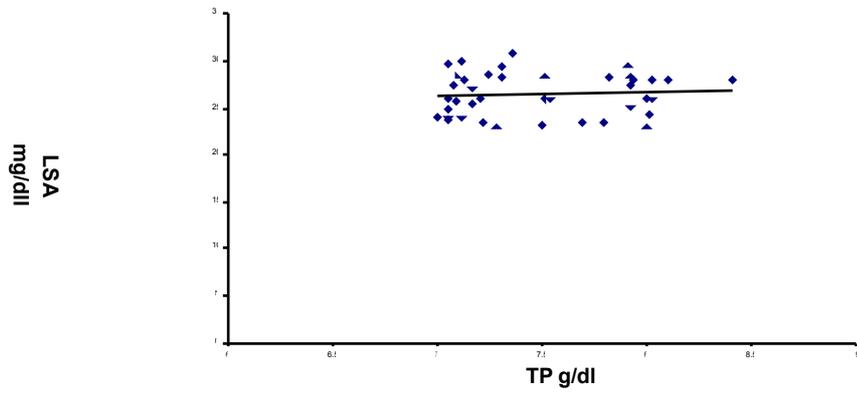


Fig-6-b-correlation between TSA and TP

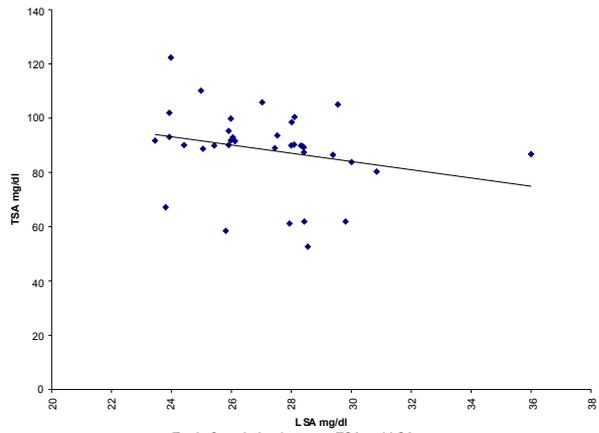
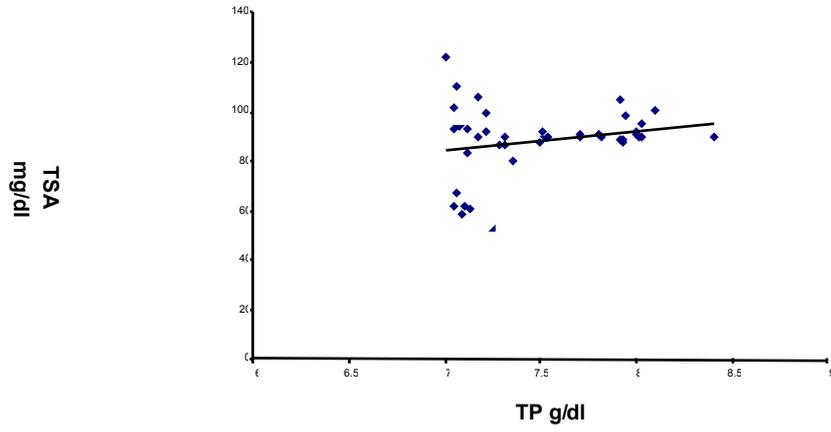


Fig 6:-Correlation between TSA and LSA

**Table (1):** Data obtained revealing different statistical parameters

| Parameter | Disease                         | Range<br>Mg/dl        | Mean+ <sub>-</sub> SD<br>mg/dl  | Variance | Confidence<br>-interval | P-<br>value | selectivity | Sensitivity |
|-----------|---------------------------------|-----------------------|---------------------------------|----------|-------------------------|-------------|-------------|-------------|
| TSA       | Rheumatoid<br>Arthritis<br>N=40 | 52.65-<br>122.33      | 88.48+ <sub>-</sub> 14.15       | 200.34   | %95                     | P<0.05      | %82.5       | %10         |
|           | Normal<br>N=40                  | 35.48-<br>69.41       | 53.31+ <sub>-</sub> 7.58        | 57.48    | %95                     |             |             |             |
| LSA       | Rheumatoid<br>Arthritis<br>N=40 | 23.02-<br>30.85       | 26.37+ <sub>-</sub> 2.25        | 5.08     | %95                     | P<0.05      | %85         | %12.5       |
|           | Normal<br>N=40                  | 15.33-<br>21.96       | 18.46+ <sub>-</sub> 1.45        | 2.11     | %95                     |             |             |             |
| TP        | Rheumatoid<br>Arthritis<br>N=40 | 7.01-<br>8.02<br>g/dl | 7.39+ <sub>-</sub> 0.34<br>g/dl | 0.11     | %95                     | P<0.05      | %60         | %22.5       |
|           | Normal<br>N=40                  | 7.04-<br>8.0<br>g/dl  | 7.35+ <sub>-</sub> 0.33<br>g/dl | 0.11     | %95                     |             |             |             |