ROLE OF TOTAL AND LIPID -BOUND SIALIC ACID IN DISCRIMINATIG ACTIVITES OF RHEUMTOID ARTHRIRIS PATIENTS

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Abstract:

Background: It is known that total serum sialic acid (TSA) and lipid bound sialic acid (LSA) levels may be altered by different types of disease included Rheumatoid Arthritis (RA) and inflammatory disease, therefore the evaluation of these compounds in serum patients with RA may elucidate the relationship between its levels and disease activity.

Aim: This study was designed to evaluate the clinical application of serum total sialic acid (TSA) and lipid bound sialic acid (LSA) levels in patients with RA, considering the disease activity and compare the levels with a normal group.

Method: The study was carried out on ninety-seven healthy and sick adults. Fifty-four (37 female, 17 male) patients with RA disease. Of them 29 patients (21female, 8 male) had low disease activity [age range 32-65 years] and twenty-five patients (16 female, 9 male) had high disease activity [age range 22-52 years]. They were compared with 43 healthy persons [age range 19-64 years]. Colorimetric methods were used for determination TSA and LSA in serum samples.

Results: Total sialic acid and lipid bound sialic acid levels in serum patients with RA show significantly increased when compared to normal group, and more increased in patients with high activity disease.

Conclusions: Based on our results, serum TSA and LSA level would be used as a good marker for discriminating between activities of RA disease.

Key words: Rheumatoid Arthritis, sialic acid, and lipid bound sialic acid.

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Introduction

Sialic acid (SA), a class of important ketoses that contain nine-carbon atom, is an acetylated derivative of neuraminic acid (2-keto-5-amino-3, 5-dideoxy-d-nonulosonic acid)[1]. Sialic acid is widely distributed in nature as non-reducing termini of glycoprotein and glycolipids. About 70% of the total sialic acid (TSA) of eukaryotic cell is found on the cell surface and the remainder is distributed primarily in the endoplasmic reticulum, mitochondria and lysosomes[2].

Because of their acidic nature, SA impart a negative charge to the cell surface and are important in cell-to-cell or cell-to-matrix interactions.SA residues on the cell surface may also be involved in masking cell surface antigens and may serve as receptors for lectins, virus particles, some hormones and antibodies[3].However, they can also act as critical components of ligands recognized by a varity of animal, plant and microbial proteins termed sialic acid binding lectins[4].

In human, SA is present in Alpha-1-acid glycoprotein(AAG), haptoglobin, ceruloplasmin and transferrin , which are acute phase rectants[5,6]. Sialic acid levels vary physiologically with age, but their levels may also be influenced by such conditions as inflammation, neoplastic tumor or in born genetic disorders, which cause abnormal sialic acid metabolism[7].

Marked elevation of serum (TSA and LSA) that correlate with the clinical activity of a disease have been documented in many pathological states, included cardiovascular disease, different types of cancer and inflammatory reaction, where the

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underlying pathology is either of tissue destruction, tissue proliferation, depolymerization or inflammation[8,9]. The role of total sialic acid (TSA) includes a small amount of free sialic acid as well as glycoprotein and glycolipid-bound sialic acid (LSA) in the various pathological processes promoted us to investigate and find some typical differences between the content of TSA and LSA values in sera patient with Rheumatoid Arthritis and compared with normal group.

Materials and Methods

Chemicals: Standard solution for sialic acid of 500µg/ml concentration was prepared by dissolving 50 mg of standard N-acetylneuraminic acid in 100 ml 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 (5.0, 10.0, 15.0, 20.0, 25.0, 30.0 µg/ml) for calibration curve measurement.

Subjects: Sera samples of the measurement of TSA and LSA were obtained from five groups of subjects who were attending Rheumatology Outpatient Clinic Division in Baghdad Teaching Hospital- Medical City from April to August 2004.

The patients with RA were divided in to two groups after evaluation of their disease activity by the Tentative EULAR Criteria[10]. [Number of tender joints: maximum 28; number of swollen joints; maximum 28; pain score: zero (no pain) to 10 (very sever pain), erythrocyte sedimentation and morning stiffness of at least 30 min duration].

Group (1) consisted of 43 adult healthy blood donors as control subjects. Mean age was 44.3±4.2 (range: 19-64) with the male to female ratio being 0.72.

Group (2) and (3) consisted of patients (8 male, 21 female) were enrolled with less than 14 swollen joints and one hour morning stiffness were considered to have low activity of the disease.

Group (4) and (5) consisted of 25 patients (9 male, 16 female) had been diagnosed with a higher number of swollen joints and more prolonged morning stiffness, were considered to have a high degree of inflammation. Characteristics of the above groups were presented in table 1 and 2.

Table 1: Characteristics of the Rheumatoid Arthritis Groups (2) and (3) low activity

Character	Group (2)	Group (3)
Sex	Male	Female
No. of patients	8.0	21.0
Age range (yrs)	32-65	19-16
Mean age (yrs)	55.30±1.90	48.8±2.30
Duration of disease (yrs)	6.90±1.10	5.90±1.20
Morning stiffness (h)	0.70±0.20	1.10±0.30
No. of tender joints	15.20±3.0	16.20±2.30
No. of swollen joints	6.10±1.20	8.90±2.0
ESR (mm/h)	36.0±8.0	34.0±2.0
Positive rheumatoid factor	5/8	17/21

Table 2: Characteristics of the Rheumatoid Arthritis Groups (4) and (5) high activity

Character	Group (4)	Group (5)
Sex	**Male**	**Female**
No. of patients	9.0	16.0
Age range (yrs)	22-52	19-58
Mean age (yrs)	45.6±7.9	43.8±4.8
Duration of disease (yrs)	5.2±1.2	6.6±2.3
Morning stiffness (h)	3.4±1.1	3.3±0.3
No. of tender joints	18.9±2.3	24.0±0.8
No. of swollen joints	15.1±3.5	14.2±2.5
ESR (mm/h)	65.0±5.0	78.0±5.0
Positive rheumatoid factor	9/9	

Serum preparation:

Venous blood samples were collected by utilizing disposable needle and plastic syringes from each patient and control, and then allowed at room temperature for 10 minutes for clotting; sera were separated by centrifugation at 3000 rpm (10 min) and stored at (-20C^{o}) until tested for TSA and LSA levels.

Measurement of serum TSA by resorcinol reagent:

Serum TSA values determination was performed as previously described [(11)]. Briefly, 20 µl of serum was diluted to 500 µl in to screw-capped tubes with distilled water; the tubes were vortexes and placed in ice.

To each tube for TSA test, 1ml of resorcinol reagent (including 10ml 2%(w/v) stock resorcinol in water, 9.75 ml water, 0.25 ml 0.1M $CuSO_4$, brought to a final volume of 100 ml with concentrated HCL). Then each tube was capped, vortexed, and placed in100C^{o} boiling water (15minutes), then cooled for 10 minutes in an ice bath. One ml of butylacetate/n-butanol (85:15 v/v) was added to the reaction mixture, the tubes were vortexes and centrifuged at 2500 rpm for 10 minutes at room temperature. The absorbance of the blue color supernatant was recorded at 580 nm.

Measurement of serum LSA:

LSA was measured according to the method described by Katopodis and co-workers[12,13]. Fifty-µl serum was placed in screw-capped tubes, 3ml of cold (4^{o}C) chloroform/methanol (2:1;v/v) mixture was added to each tube for total lipid extraction, the tube were capped and vortexed for 30 seconds, 0.5 ml cold water was added to each tube and the tubes were centrifuged for 5 minutes at 2500 rpm at room temperature.

The upper phase (aqueous layer containing LSA) was transferred to another screw-capped tube. Fifty µl of phosphotungstic acid (1g/ml) was added to each tube .The tubes were vortexed and allowed to sit at room temperature for 5 minutes. The tubes were then centrifuged at room temperature for 5 minutes at 2500 rpm. After that the supernatants were decanted and the remaining pellets were redissolved in 1ml at 37^{o}C water by vigorously vortexing for 1 minute and sialic acid content was determined as mentioned for TSA.

Statistical analysis:

TSA and LSA sensitivity was calculated as the percentage of patients having values above cut-off level 2SD (standard deviation). Student's t-test was used to compare the levels of serum mean values in patients with normal.

Results

The aim of the present investigation is to find some typical role of TSA and LSA as a diagnostic pointer in inflammatory active disease to enable us for clinically meaningful difference with respect to serum TSA and LSA levels by comparing its levels

between normal subjects and different categories of patients with RA and related to degree of inflammation.

Normal subjects:

Comparison of the distributions of the serum TSA and LSA in both sexes, Table (3); shows that the mean serum values for TSA in female and male was (63.61±10.69) and (71.52±5.62) mg/dl respectively, while the mean serum value for LSA was (20.50±3.68) and (23.90±4.26) mg/dl for female and male respectively. The results show little differences between females and males for both TSA and LSA, so that the total means for both sexes was (67.57±7.56) and (22.20±3.29) mg/dl respectively.

Table 3: Serum levels of TSA and LSA in normal

Sex	TSA mg/dl (Mean±SD)	LSA mg/dl (Mean±SD)
Female (n=25)	63.31±10.69	20.50±5.68
Male (n=18)	71.52±8.82	23.90±4.26
Total (n=43)	67.57±7.56	22.20±3.29

Rheumatoid Arthritis (RA) patients:

TSA and LSA were analyzed with respect to degree of inflammation of the disease, so separate calculation was made for each test's levels of 43 normal and 54 patients with RA are shown in table 4. In the normal sera samples the overall TSA level was found to be (67.57±7.56) mg/dl, while the corresponding level in 54 patients sera samples was (88.25±12.96) mg/dl, this increase of 31% in TSA level was statistically significant (P<0.001).

Both in the normal and the various groups of patients with different degree of disease, there were significant difference between values obtained for females and males for each characteristics of the disease state, therefore they were grouped independent. The mean serum TSA levels was found to be (79.22±11.01) and (76.90±10.23) mg/dl for female and male with low activity; and (104.23±27.22) and (92.68±17.27) mg/dl for serum female and male with high active disease.

The magnitude of the increase in the value varied between 17% for female and 14% for male patients with low activity. In contrast, the magnitude of the increase in serum TSA level varied between 54% and 37% for female and male with active disease respectively.

Table 4: Serum TSA values of patients with RA patients.

Cases	mg/dl (Mean±SD)	Change in TSA level (%)
RA with Low activity		
Female (n=21)	79.22±11.01	+17
Male (n=8)	76.90±10.23	+14
RA with High activity	104.23±27.22	+54
Female (n=16)	92.68±17.26	+37
Male (n=9.0)		
Total (n=54)	88.25±12.96	+31
Normal (n=43)	67.57±7..56	-

The sensitivity of TSA observed in our study fell from 38% (8 of 21) to 25% (2 of 8) for female and male with low activity disease, while the values reached 75% (12 of 16) to 56% (5 of 9) for female and male with high activity disease figure 1, this increase of sensitivity might contribute to the development of RA and to the progression of the disease itself.

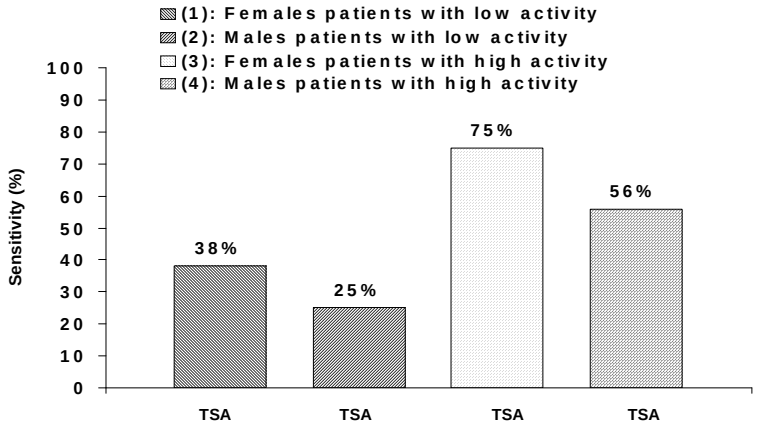

Figure 1: Sensitivity of TSA in patients with (RA)

Striking differences were found in the levels of serum LSA (Table 5). The mean LSA level in 43 normal was found to be (22.20±3.29) mg/dl, while the mean LSA in overall sera patients with RA was (30.92±4.11) mg/dl, this 39% increase in the LSA level was statistically significant (P<0.001).

The results show significant change between values obtained for both sexes, so the increase in the LSA serum level was more pronounced change when the patients were grouped according to the high activity of the disease and the extent of the increase varied between 62% for female and 45% for male with active disease.

Table 5: Serum LSA values of patients with RA patients

Cases	mg/dl (Mean±SD)	Change in LSA level (%)
RA with low activity		
Female (n=21)	28.69±5.38	+29
Male (n=8)	26.71±3.41	+20
RA with high activity		
Female (n=16)	36.05±8.09	+62
Male (n=9)	32.26±6.95	+45
Total (n=54)	30.92±4.11	+39
Normal (n=43)	22.20±3.69	-

Figure 2 shows the distribution and the sensitivity of LSA in the serum normal and in the RA patients according to the activation and severity of the disease. The mean serum LSA level in the total groups of RA was significantly elevated than normal value and the sensitivity fell from 43% (9 of 21) to 37% (3 of 8) for female and male with low activity and 56% (9 of 16) to 44% (4 of 9) for female and male patients with high activity disease.

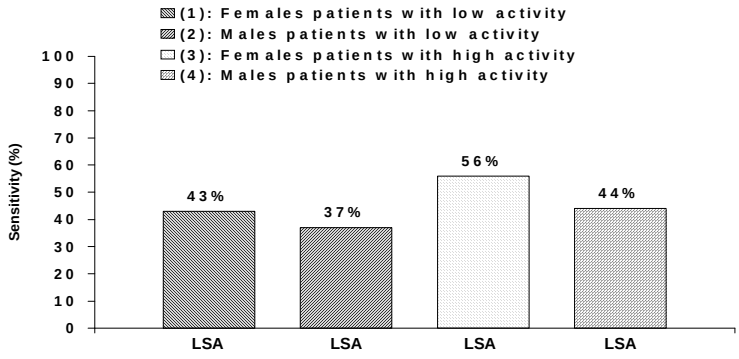

Figure 2: Sensitivity of LSA in patients with (RA)

Discussion

The present study demonstrates that the mean serum TSA and LSA levels in patients with RA was significantly increased compared to normal, this results confirm those of studies that observed high plasma or serum TSA and LSA in patients with cardiovascular disease, inflammatory reaction and different types of cancer[14-17].

TSA includes a small amount of free sialic acid as well as glycoprotein and glycolipid-bound sialic acid, these oligosaccharide chains, with sialic acid on the N-terminal position, are present on the cell surface.Sialic acid induces an electronegative charge[12] because, as a relatively strong acid (pKa=2.6), it is completely ionized at physioiogical pH[18].

This ionization plays a major role because the distribution of cell surface dense anionic sites is correlated, in vitro, with abnormal cell aggregation[19]. The mechanism of increased serum SA level in inflammatory conditions is unclear, but several explanations have been proposed these include: (a) spontaneous release of aberrant SA-containing cell surface glycoconjugates, (b) increased levels and /or glycosylation of normal serum glycoproteins[16].

One of hypothesis of increase of plasma or serum TSA levels in patients with inflammatory state could be explained by an increased output of serum proteins from the liver due to some type of acute phase protein. This also requires an increase in the activity of sialidase, which will catalyze and remove the sialic residues from an acute proteins[20].

References

1. Bhavanandan VP, and Sheykhazari M: Adaptation of the periodate-resorcinol method for determination of sialic acids to a microassay using microtiter plate reader. Anal Biochem, 1993; 213: 438-40.
2. Warren L: The distribution of sialic acids within the eukaryotic cell. In: Biological Roles of Sialic Acid (Rosenberg A, Schengrund C, Eds), plenum Press, New York 1976; pp103-121.
3. Jeanloz RW, and Codington JF. The biological role of sialic acid at the surface of the cell. In: Biological Roles of Sialic Acid (Rosenberg A, Schengrund C, Eds), Plenum Press, New York, 1976; pp201-238.
4. Varki A: Sialic acids as ligands in recognition phenomena. FASEB J, 1997; 11: 248-55.
5. Lopez-Saez JJB, and Senra-Varela A: Evaluation of lipid-bound sialic acid as a tumor marker. J Biol Markers, 1995; 10: 174-9.
6. Taysis, Akcay F, Uslu C, Dogru Y, and Gulcin I: Trace elements and some extracellular antioxidant proteins levels in serum of patients with laryngeal cancer. Biol Trace Elem Res, 2003; 91: 11-8.
7. Frang-Kirscher SG: Comparision of sialic acids axcretion in spot urines and 24-hour-urines of children and adults. Eur J Clin Chem, 1997; 35: 47-52.
8. Shauer R: Sialic acids and their role as biological markers. Trends Biochem Sci, 1985; 10: 357.
9. Haq M, Hag S and Tutt P: Serum total sialic acid and lipid associated sialic acid in normal subjects and those having a myocardial infraction and their relation to acute phase proteins. Ann Clin Biochem, 1993; 30: 383-6.
10. Tentative EULAR criteria for disease activity measurement of rheumatoid arthritis (EULUR Core Set). Rheumatol Eur, 1994; 23: 37-9.
11. Svennerholm L: Quantitative estimation of sialic acids. A colorimetric resorcinol-hydrochloric acid method. Biochem Biophys Acta, 1957; 24: 604-11.
12. Katopodis N, Hirshaut Y, and Geller NL: Lipid-associated sialic acid test for the detection of human cancer. Cancer Res, 1982; 42: 5270-5.
13. Katopodis N, and Stock CC: Improved method to determine lipid-bound sialic acid in plasma or serum. Res Commun Chem Path Pharmacol, 1980; 30: 171-80.
14. Crook M, Lump P, Andrews V, and Swaminathan R: Serum total sialic acid, a reputed cardiovascular risk factor, and its relationship to lipids, plasma fasting insulin, blood pressure and body mass index in normal individuals. Clin Sci, 1999; 95: 535-42.
15. Rogers M, Williams D, and Niththananthan R: Decrease in erythrocyte glycophorin sialic acid content is associatet with increased erythrocyte aggregation in human diabetes. Clin Sci, 1992; 82: 309-13.
16. Stefenelli N, Klotz H, Engel A, and Bauer P: Serum sialic acid in malignant tumors, bacterial infections and chronic liver disease. J Cancer Res Clin Oncol, 1985; 109: 55-9.
17. Pervin V, Mukaddes C, and Dilek SL: Total and lipid-bound sialic acid levels in actinin keeatpsis and basal cell carcinoma. J Med Sci, 1999; 29: 419-23.
18. Jaques LW, Brown EB, Barrett JM, Brey WS, and Weltner WJ: Sialic acid: A calcium binding carbohydrate. J Biol Chem, 1977; 252: 4533-8.

19. Raz A, Bucana C, Mclellan W, and Fidler IJ: Distribution of membrane anionic sites on B16 melanoma variants with differing lugs colonsing potential. Nature, 1980; 284: 363-4.
20. Wakabayashi I, Sankamoto K, and Yoshimato S: Relation of serum sialic acid to lipid concentration. BMJ, 1992; 305: 562-3.