

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°) 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 CuSO₄, brought to a final volume of 100 ml with concentrated HCL). Then each tube was capped, vortexed, and placed in 100C° 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°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°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