

# Serum Levels of Tartrate Resistant Acid Phosphatase in Ankylosing Spondylitis

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## ABSTRACT

Ankylosing spondylitis, a chronic inflammatory disease, is a burden on the patient and society. Serum levels of Tartrate Resistant Acid Phosphatase were assessed in 61 patients, who had mild AS, and 65 age and sex-matched patients with moderate AS. Tartrate Resistant Acid Phosphatase was significantly increased in patients with moderate disease compared with patients having mild disease. The data from the present study indicate that TRAP might be a marker for AS.

**Keywords:** Ankylosing spondylitis, Tartrate Resistant Acid Phosphatase

## INTRODUCTION

Ankylosing spondylitis (AS) is a chronic inflammatory disease of unknown cause, characterized by sacroiliitis and spondylitis. <sup>(1)</sup> AS is the most frequent and most severe subtype of spondyloarthritis. <sup>(2)</sup> The estimated number of AS cases is about 4.63 to 4.98 million in Asia. <sup>(3)</sup> AS affects the axial skeleton, causing characteristic inflammatory back pain, which can lead to structural and functional impairments and a decrease in quality of life. <sup>(4)</sup> The first symptoms of AS usually appear in late adolescence. The initial symptom is typically a dull pain that is insidious in onset, felt deep in the buttock and/or in the lower lumbar regions and is accompanied by morning stiffness that lasts for a few hours, improves with activity, and returns with inactivity. The pain becomes persistent and bilateral within a few months and is usually worse at night. Immune

mediated mechanisms are involved in the pathogenesis, evidenced by inflammatory histology, raised serum levels of IgA and acute phase reactants, and the close relationship between HLA-B27 and AS. <sup>(5)</sup>

AS is the prototypical member of the family of spondyloarthropathies, and is characterized by seronegativity, axial predominance and new bone formation, which underlie symptoms of inflammatory, back pain, enthesopathy and extra-articular manifestations, including anterior uveitis, psoriasis and colitis. Patients with AS typically experience a wide variety of morbidities. These include both morbidities related to the disease itself- most prominently progressive, irreversible, structural damage to the axial or peripheral skeleton and morbidities stemming from treatments for the disease, including toxicities from NSAID use, and increased risk of infections and immunogenicity concerns with biologics. <sup>(6)</sup>

Due to chronicity of the disease and its development in early adulthood, the burden of the disease is consequently substantial on both patients and society.

Several biochemical markers have been studied to assess progress or prognosis of the disease, for example interleukin-6, <sup>(7)</sup> matrix metalloproteinase 3, <sup>(8)</sup> circulating protein fragments of cartilage and connective tissue degradation, <sup>(9)</sup> etc. But all markers have their respective drawbacks in predicting the course of the disease. So, the present study was undertaken to assess the serum levels of Tartrate Resistant Acid

Phosphatase (TRAP) in AS to find out whether there is any change of the above mentioned parameter, and if it is significantly related to AS.

## MATERIALS AND METHODS

The present study was a hospital-based study conducted in the department of Biochemistry of a tertiary care medical college and hospital of West Bengal. The study was approved by the local ethical committee and all patients gave their informed consent to take part in this investigation.

The present study period was 8 months and included 126 AS patients attending the outpatient department (OPD), duration of disease ranging from 1 year to 7 years. The patients were in the age range of 19 to 32 years. The patients were divided into Group A, having 61 patients who had mild disease, and Group B, having 65 age and sex-matched patients with moderate disease. Complete history and physical examination of all cases were undertaken. Exclusion criteria included subjects having hairy cell leukemia, metastatic bone cancers, osteoporosis, Paget's disease, osteogenesis imperfecta, multiple myeloma, myeloproliferative disorders.

Venous blood sample was collected from each case after 12 hours of fasting. All samples were coded and assayed in a blind fashion by an investigator who was unaware of the subjects' clinical status.

Serum TRAP was determined as follows: p-Nitrophenyl phosphate (pNPP; 7.6 mmol/L) was used as substrate in a buffer containing 100 mmol/L sodium acetate and 50 mmol/L sodium tartrate (pH 5.5). Samples (50  $\mu$  L) were added to 150  $\mu$  L of substrate and incubated at 37 °C for 60 min. The reaction was stopped by the addition of 50  $\mu$  L of 3 mol/L NaOH; the absorbance was read at 405 nm. The activity was estimated in U/L, using solutions of p-nitrophenolate as calibrators. <sup>(10)</sup>

Statistical analysis of the data was performed by using Statistical Package for Social Sciences (SPSS) and inferences were

drawn.  $P < 0.05$  was considered to be significant and  $p < 0.001$  highly significant.

## RESULTS

Table 1. Serum levels of TRAP (in Mean+SD) in cases

	Group A	Group B
TRAP (U/L)	6.3±0.81	6.7±0.92

p value and statistical significance:

The two-tailed p value equals 0.0109

By conventional criteria, this difference is considered to be statistically significant.

Confidence interval:

The mean of Group A minus Group B equals -0.4000

95% confidence interval of this difference: From -0.7064 to -0.0936

Intermediate values used in calculations:

$t = 2.5836$

$df = 124$

standard error of difference = 0.155

SEM values of groups A and B are respectively 0.1037 and 0.1141

## DISCUSSION

In AS there is synovitis, which tends to produce articular erosion, and an inflammatory enthesopathy, which results in capsular ossification in diarthrodial joints and syndesmophyte formation in cartilaginous joints, both of which are primarily responsible for bony ankylosis. Nonspecific secondary mechanisms contribute to the final picture. These include enchondral ossification, which produces synostosis, osteoporosis and altered stress distribution. <sup>(11)</sup> AS is characterized by both increased bone formation and increased bone resorption. <sup>(12)</sup> Osteoporosis is observed in parallel with increased bone resorption. <sup>(13)</sup>

TRAP is highly expressed in osteoclasts and, therefore, used as a specific histochemical marker for these cells. <sup>(14)</sup> TRAP prompts the dephosphorylation of bone matrix phosphoproteins like osteopontin and bone sialoprotein and was originally shown to be important for a

normal endochondral bone formation. (15,16) Occurrence of the enzyme in the cartilage canals exactly at the onset of their formation suggests a possible role in the establishment of the vascular network. (17,18) TRAP can dephosphorylate a number of substrates, including osteopontin, bone sialoprotein, casein, and mannose 6-phosphate. (19,20) Moreover, TRAP is abundantly expressed on osteoclasts and plays an important role in osteoclastic bone resorption. For example, the resorbed bone matrix, such as type I collagen, is endocytosed into osteoclasts and is likely to be further degraded by reactive oxygen species (ROS) derived from TRAP. (21) In addition, TRAP seems to be secreted into the resorption lacuna and dephosphorylates bone matrix osteopontin, resulting in enhanced migration of osteoclasts. (19,22) By far the greatest interest in the biochemistry of mammalian TRAP relates to its use as a marker for osteoclasts, the cells that resorb bone. The relative specificity of TRAP as an osteoclast marker, its proposed involvement in the resorptive process, and its abundance has suggested that it could be used as a serum marker for bone resorptive activity in pathological states. (23)

In the present study, AS cases had increased levels of TRAP compared to healthy people; group B subjects, having moderate disease, had significantly higher levels of TRAP compared to group A subjects, having mild disease (table 1). Serum TRAP has been indicated as a disease associated marker for the clinical diagnosis of excessive bone resorption. (14,24) Furthermore, it increases with the rate of resorption taking place. (10) Also, there is a direct relation between excessive osteoclast facilitated bone resorption and the arrival of increased amounts of TRAP in the circulation. Thus, in the present study, increased TRAP levels might indicate bone resorption in AS. Other biochemical markers of bone resorption were found by Grisar et al to be significantly increased in patients with AS. (25)

But, there are certain limitations in the present study. Number of patients in the study groups was not large. Thus, care must be taken in extrapolating the present findings to other populations. Patients were taking a number of medications to control AS. However, the drugs are characteristic of patients with AS and do not affect serum TRAP levels. Despite these limitations, we believe that our study points towards using TRAP as an important, promising marker for AS. As our findings point to an increase in TRAP, the problem of bone resorption in AS should also be further investigated in a larger number of patients, and other markers should be assessed.

## CONCLUSION

The present study indicates that TRAP might be used as marker for AS. Still, other aspects of AS should be assessed, with a bigger study group, more parameters and better, sophisticated tools, to validate the present data, and continue the line of research.

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**Conflict of interest:** None declared

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