# SYNOVIAL FLUID BIOMARKERS IN DIFFERENTIATING BETWEEN INFLAMMATORY AND NONINFLAMMATORY ARTHRITIS

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# ABSTRACT

INTRODUCTION: Arthritis is the initial manifestation of many joint disorder. The most common causes of arthritis are crystal,trauma and infection. Synovial fluid[S.F] analysis provides an easy, noninvasive option in differentiating inflammatory from noninflammatory arthritis.

OBJECTIVES : The aim of this study is to determine whether S.F Adenosine deaminase (ADA) and high sensitivity C-reactive protein (hs-CRP) can be useful in differentiating inflammatory from noninflammatory arthritis.

METHODS : A total of 56 patients with knee arthritis with joint effusion who were admitted in Vinayaka Mission Kirupananda Variyar Medical College and Hospital, Salem and those who attended the orthopaedic outpatient clinic during the period of October 2011 to March 2012 have been included in this study. There were 14 Rheumatoid Arthritis (RA), 7 Crystal induced arthritis, 3 Septic arthritis and 32 Osteoarthritis (OA).

RESULTS : Statistically significant difference in mean synovial fluid ADA and hsCRP concentration between inflammatory  $35.77\pm3.95$  IU/L,  $19.69\pm4.46~\mu$ g/ml and noninflammatory arthritis  $12.93\pm2.06$  IU/L,  $2.77\pm0.91~\mu$ g/ml (p values<0.0001, 0.0001) was observed. Statistically significant difference in mean synovial fluid ADA and hsCRP concentration was observed when rheumatoid arthritis ( $22.98\pm3.21$  IU/L,  $17.54\pm4.23~\mu$ g/ml), crystal induced ( $24.23\pm3.34~$ IU/L,  $19.47\pm3.77~\mu$ g/ml), septic arthritis ( $60.1\pm5.31~$ IU/L,  $22.07\pm5.40~\mu$ g/ml) were compared with osteoarthritis ( $12.93\pm2.06~$ IU/L,  $2.77\pm0.91~\mu$ g/ml)

CONCLUSION : Synovial fluid ADA and hsCRP helps to differentiate inflammatory from noninflammatory arthritis

KEYWORDS: Adenosine deaminase, hsCRP, Synovial

fluid, Rheumatoid arthritis, crystal induced arthritis, Septic arthritis

# **INTRODUCTION**

Arthritis is the bread and butter of orthopaedic practice. A comprehensive diagnostic approach is essential for early diagnosis. Synovial fluid analysis helps in this aspect. Analysis of synovial fluid has long been recommended as a routine procedure to assist in the diagnosis of arthritis. Available synovial fluid biochemical tests like glucose, protein have limited value and lack specificity in differentiating inflammatory from non inflammatory arthritis 18.

Adenosine deaminase and high sensitive C reactive protein of the SF are among the important biomarkers in recent studies in differentiating inflammatory from non inflammatory arthritis. Studies have reported that S.F ADA was higher in inflammatory arthritis (RA, Crystal induced and septic arthritis) when compared to non inflammatory osteoarthritis. ADA and CRP level are indicators 18 of immune system activation.

The present study was undertaken to estimate ADA, hsCRP, total protein, glucose concentration and total WBC count in synovial fluid obtained from patients with rheumatoid arthritis, osteoarthritis, crystal induced arthritis, septic arthritis and to assess the value of the investigation in differentiating between inflammatory and noninflammatory arthritis.

# **MATERIALS AND METHODS**

A cross sectional study was performed in the SF of patients with clinical evidence of knee arthritis and joint effusion who consented for arthrocentesis. The subjects were patients who attended the outpatient clinic and those who were inpatients in the department of orthopaedics at Vinayaka Missions Kirupananda Variyar Medical College and

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Hospital, Salem. The study was approved by the ethics committee of the Institute. Written consent was obtained from all the patients in this study.

The arthritis were diagnosed as RA, crystal induced arthritis, septic and OA based on physical examination, laboratory results and radiological findings. Synovial fluid was aspirated from the arthritic knee collected in sterile plain and heparinized tubes and analyzed for the following

- I SF was centrifuged with the speed of 1500 rpm. Supernatant was used for biochemical analysis.
  - a. SF hsCRP was quantitated by ELISA method
  - b. SF ADA quantitated based on the principle adenosine deaminase hydrolyses adenosine to ammonia and inosine, finally under alkaline medium blue indophenols complex is formed which is measured by using semiautoanalyser[microlab 300 - Merck]
  - c. SF Total protein estimated by Biuret method and SF Glucose estimated by GODPOD method were measured using semiautoanalyser[microlab 300 - Merck]
- II a. Gram staining done to see the presence of microorganism under light microscope,
  - b. Presence of crystals using polarised microscope
  - c. Microbiological SF culture were also done
- III SF collected in sterile heparinised vial, centrifuged at 1500rpm and the smear was prepared from the sedimentation part of centrifuged SF and analysed to determine the number of white blood cells.

## **STATISTICAL ANALYSIS**

Results are expressed as the mean  $\pm$ SD. Statistical comparison between the two groups were carried out using student's t test. P value less than 0.05 was considered significant.

## DISCUSSION

Arthrocentesis 10 is therapeutic in knee joint effusion as it provides pain relief, prevents

stretching of the ligaments and eliminates a potent source of infection. The synovial fluid or pus aspirated, in addition to being therapeutic provides us with a diagnostic tool. The fluid analysis should include a cell count, gram staining, culture, examination of crystals, 8,14 ADA 3 and hsCRP which helps in differentiating inflammatory from noninflammatory condition 18.

Adenosine deaminase is one of the key enzymes of the purine2 metabolic pathway17, catalyzing the irreversible deamination of adenosine to inosine22 and deoxyadenosine to deoxyinosine.22,17 During inflammatory reactions the enzyme is released into extracellular fluid7 and in serous effusion of different pathology the levels of ADA activity increases considerably. ADA exists in all nucleated cells and its concentration varies in different tissues. Thymus1 that contains T lymphocytes has major role in immune system contain highest level of ADA enzyme activity 4, therefore determination of ADA in synovial fluid also indicates lymphoreticular activities.

Adenosine is a purine nucleoside with tissue protective property against injuries. It has an antiinflammatory function by decreasing proinflammatory cytokines6, increasing antiinflammatory cytokines, cytokine modulation of macrophage and monocytes , endothelial cell inflammatory functions and neutrophil adherance to fibroblast and adherence of neutrophil to endothelial cell.

Adenosine acting through A2 receptors, exerts a potent inhibitory effect on the synthesis of leukotriene B4 and thus contribute to its antiinflammatory properties6. Adenosine, called a 'retaliatory metabolite'because it is a regulatory autocoid 18 that is generated as a result of cellular injury or stress, interacts with specific G proteincoupled receptors on inflammatory and immune cells to regulate their function.

The leukocyte count in the S.F is helpful in distinguishing inflammatory from noninflammatory arthritis.

CRP a member of the pentraxin family of proteins. 11 CRP activates the classical complement cascade and mediates phagocytosis, but it is also capable of

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regulating inflammation 12. CRP is secreted by the liver 21 in response to a variety of inflammatory cytokines. Levels of CRP increase very rapidly in response to trauma, inflammation and infection and decrease rapidly with resolution of the condition. Thus, the measurement of CRP is widely used to monitor various inflammatory states. In our study statistically significant increase was seen in ADA and hsCRP between inflammatory and in noninflammatory arthritis.

The results of Yuksel et al.,4 also showed significant increase of ADA in RA (21.5 $\pm$ 8.4 IU/L) than in OA (9.7 $\pm$ 4.9 IU/L).

The study of Nakamachi et al., showed that mean ADA concentration in the synovial fluid of patient with RA was higher than in OA17, Similar results were found in the study of Seung young Kim et al.,5

Batool Zamani et al., 22. showed significant raise in SF ADA and hsCRP in inflammatory arthritis[26.06 $\pm$ 8.96 IU/L and 12.72 $\pm$ 9.25 $\mu$ g/ml] when compared to noninflammatory arthritis[14.8 $\pm$ 2.79IU/L and 2.36 $\pm$ 2.7 $\mu$ g/ml].

Finding of this study correlates well with that of Batool zamani et al., 22., where mean of SF ADA and hs-CRP activity were increased in inflammatory arthritis (35.77  $\pm$  3.95 IU /L, 19.69  $\pm$  4.46  $\mu$ g/ml) when compared to noninflammatory arthritis  $(12.93\pm2.06 \text{ IU/L}, 2.77\pm0.91 \mu \text{g/ml})$ . SF ADA and hsCRP concentrations were higher in septic arthritis9,16 when compared to other arthritis. SF ADA was significantly raised in septic arthritis when compared to RA and crystal induced arthritis, but SF hsCRP concentration did not differ significantly when septic arthritis were compared with crystal induced and RA. SF ADA concentration helps in differentiating various inflammatory arthritis but hsCRP does not differentiate the inflammatory arthritis from each other.

SF protein19 and glucose did not show significance in differentiating inflammatory from noninflammatory arthritis.

## RESULTS

Totally 56 patients were included in this study. Noninflammatory OA group comprised of 32 patients. Inflammatory arthritis comprised of 24 patients which includes 14 RA, 7 crystal induced arthritis and 3 septic arthritis.

Table. 1 shows ADA ,hsCRP, total protein and glucose in the synovial fluid of different types of arthritis. Patients with inflammatory arthritis shows statistically significant increase in mean SF ADA and hsCRP [ $35.77\pm3.95$  IU/L,  $19.69\pm4.46$   $\mu$ g/ml ] when compared with patients of noninflammatory arthritis[ $12.93\pm2.06$  IU/L,  $2.77\pm0.91$   $\mu$ g/ml ]IP values < 0.0001,0.0001]

Figure 1 and figure2 shows the concentration of ADA and hsCRP in synovial fluid of different arthritis respectively.

Statistically significant difference was observed in mean SF ADA and hsCRP concentration of RA, crystal induced and septic arthritis when compared with OA [P<0.0001,0.0001,0.0001,0.0001, 0.0001,0.00011. Statistical significant difference was observed for SF ADA concentration between septic and crystal induced arthritis, also between septic arthritis and RA[P<0.0001,0.0001].There was no statistical significant difference between SF hsCRP concentration between septic and crystal induced arthritis, between RA and crystal induced arthritis and also between septic arthritis and RA [P=0.40,0.31,0.12] . Total WBC count of more than 2000 cells per cumm of SF were observed in inflammatory arthritis and total WBC were less than 2000 cells per cumm of SF in noninflammatory arthritis, which correlates well with the findings of H.Amer et al., and chokkalingam et al. 13,15

There was no statistical significant difference in mean SF total protein and glucose concentration between inflammatory  $(4.06 \pm 1.8 \text{ g}\%, 66.33\pm8 \text{mg}\%)$  and noninflammatory arthritis  $(3.6\pm0.5 \text{g}\%, 74\pm19 \text{mg}\%)$  [p values = 0.16, 0.07]

This study showed positive correlation between ADA, hsCRP and WBC count. This documents that ADA and hsCRP concentration in tissues rises when immune system is activated in response to infection or inflammatory reactions.

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PARAMETERS	NON INFLAMMA TORY ARTHRITIS	INFLAMMATORY ARTHRITIS		
	OA	RA	CRYSTAL INDUCED ARTHRITIS	SEPTIC ARTHRITIS
ADA (IU/ L)	12.9 3±2 .06	22.98± 3.21	24.23± 3.34	60.1±5.31
hs-CRP (μg/ml)	2.77±0. 91	17.54± 4.23	19.47± 3.77	22.07±5.40
Glucose(mg% )	74.0±19 .0	65.0±9. 0	68.0±7. 0	66.0±8.0
Protein (g%)	3.6±0.5	4.1±1.3	4.5±3.3	3.6±0.8
TABLE 1. SYNOVIAL FLUID ADA, HSCRP, GLUCOSE AND TOTAL PROTEIN CONCENTRATION IN ARTHRITIS PATIENTS				





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## CONCLUSION

Synovial fluid ADA is a biomarker in differentiating inflammatory from non inflammatory arthritis and it also helps to differentiate septic from rheumatoid and crystal induced arthritis. Further studies on comparison of synovial fluid ADA and hsCRP with serum ADA and hsCRP of different arthritis with a large sample size will throw more light on differentiating arthritis.

# REFERENCES

- A. Adams & R.A. Harkness et al., Adenosine deaminase activity in thymus and other human tissues. Clin. exp. Immunol. 1976; 26: 647-649.
- Katsuro Yagawa and Jun Okamura et al., Role of Adenosine Deaminase in Activation of Macrophages. Infection and Immunity. 1981; 32(1):394-397
- 3. Pettersson. T et al., Adenosine deaminase activity in joint effusions. Scand J Rheumatol. 1983; 17(5):365-9
- H Yuskel and T F Akoglu. Serum and synovial fluid adenosine deaminase activity in patients with rheumatoid arthritis, Osteoarthritis and reactive arthritis. Annals of the Rheumatic Disease. 1988; 47:492-495
- Seung Young Kim, MD., et al., Synovial fluid adenosine deaminase activity in the patients of Rheumatoid Arthritis, Osteoarthritis, Ankylosing Spondylitis and Gouty Arthritis. 1995; 2(1)46-51
- E. Krump, G. LemayetalAdenosine A2 receptorinduced inhibition of leukotriene B4 synthesis in whole blood ex vivo, British Journal of Pharmacology. 1996; 117: 1639-1644
- Gyorgy Hasko et al., Adenosine Receptor Agonists Differentially Regulate IL-10, TNF-, and nitric oxide production in RAW 264.7 Macrophages and Endotoxemic Mice. Journal of immunologyr. 1996;157:4634-39
- 8. Ma DT, Carroll GJ. Monoarthropathy. Could this be infection? Aust Fam Physician. 1998 Jan-Feb; 27(1-2):28-31

# D. Manivelavan et al, Synovial Fluid Biomarkers in

serum inflammatory markers. Scand J Infect Dis. 1998;30(6):591-6 10. Simon H Till, M L Snaith. Assessment, investigation and management of acute

9. Soderquist B et al., Bacterial or crystal-

associated arthritis? Discriminating ability of

- investigation, and management of acute monoarthritis, JAccid Emerg Med.1999;16: 355-361.
- 11. Du Clos TW. Function of C reactive Protein. Ann Med. 2000; 32(4): 274-8
- 12. Mortensen RF. C reactive protein, inflammation and innate immunity. Immunol Res. 2001, 24(2):163-76
- H.Amer et al., The utilization of synovial fluid analysis in the UK. Rheumatology.2001;40: 1060-1063
- 14. A Swan et al., The value of Synovial fluid assays in the diagnosis of joint disease: a literature survey. Ann Rheum Dis. 2002; 61:493-498
- Chokkalingam siva, M.D., et al., Diagnosing Acute Monoarthritis in adults: A practical Approach for the Family Physician. American Family Physician. 2003;68(1):83-90
- Levine MJ et al., Assessment of the test characteristics of C-reactive protein for septic arthritis in children. J pediatr Orthop. 2003 May-Jun 23(3); 373-7.

- Yuji Nakamachi et al., Specific increase in enzymatic activity adenosine deaminase 1 in rheumatoid synovial fibroblasts Arthritis & Rheumatism. March 2003; 48: 668-674
- Gyorgy Hasko 1,2 and Bruce N. Cronstein3 adenosine; an endogenous regulator of innate immunity. Trends in immunology. 2004;25:33-39
- Sangeeta B Kulkarni et al., Role of synovial fluid analysis and synovial biopsy in joint diseases. Bombay Hospital Journal. Oct 2004; 46: No.04
- Khalid Hassanein et al., Role of Adenosine Deaminase (ADA) in the diagnosis of pulmonary tuberculosis. Egyptian Journal of Bronchology. 2010;4(1):11-18
- 21. Praveen Hariharan et al., Sensitivity of Erythrocyte Sedimentation Rate and C reactive protein or the exclusion of septic arthritis in emergency department patients. The Journal of Emergency Medicine. 2011;40(4):428-431
- 22. Batool Zamani et al., Synovial fluid adenosine deaminase and high sensitivity C reactive Protein activity in differentiating monoarthritis. Rheumatol Int. 2012; 32:183-188

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