IMPORTANCE OF BIOCHEMICAL MARKERS - ADA AND CRP IN THE BODY FLUIDS FOR THE DIAGNOSIS OF TB AND THEIR CORRELATION IN THE TUBERCULOSIS PATIENT.

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ABSTRACT

Tuberculosis (TB) has been a major global public health problem. Tuberculosis is the commonest opportunistic infection among people living with HIV and in several instances HIV and TB co-exist. Tuberculosis (TB) is a major cause of pleural effusion, which in TB usually has lymphocytic and exudative characteristics. Tuberculosis produces a spectrum of histopathogical reaction in the bone marrow in Patients with poor nutritional status. They range from normal marrow hyperplasia and necrosis of the marrow. There is great excitement in the tuberculosis (TB) scientific community over the introduction of new tools into TB control activities. The development of new tools is an important component of the Global Plan to Stop TB and the World Health Organization’s new global Stop TB Strategy. TB is no longer the scourge it once was, but it remains an important cause of morbidity and mortality worldwide. Tuberculous effusion is usually diagnosed by pleural biopsy as bacteriological examination of pleural fluid, sputum and laryngeal swab specimens is of limited value because of the low diagnostic yields (9-32%) with these methods. In this research we have found two biochemical markers-ADA and CRP which are important in the diagnosis of TB. Adenosine deaminase (ADA) test can be used for early TB detection where TB is endemic or other diagnostic means are expensive in adult population. Adenosine deaminase analysis is a simple and inexpensive colorimetric test that can be performed on serum and body fluids. Use of CRP as a marker for detection helps us not only in diagnosis but also helps us to evaluate progression & effectiveness of drugs used to cure it. The test is under the common men pocket.
KEYWORDS: ADA, CRP, AFB, purine, peritoneal, pericardial.

INTRODUCTION
Tuberculosis (TB) is a bacterial disease caused by the tubercle bacilli which includes *Mycobacterium tuberculosis*.\(^1\) TB has been a major global public health problem from times immemorial. Recent estimates are that 8-10 million new tuberculosis (TB) cases occur each year in the world, 2-3 million die. World Health Organization (WHO) estimates shows that globally there are 8.6 million incident cases of TB of which 80% are in 22 countries, with India ranked as the highest burden country.\(^2\) India is the second-most populous country in the world; India has more new TB cases annually than any other country. In 2011, out of the estimated global annual incidence of 9 million TB cases, 2.3 millions were estimated to have occurred in India, accounting for approximately one fifth of the global incidence.\(^3\) Tuberculosis is the commonest opportunistic infection among people living with HIV and in several instances HIV and TB co-exist. The possibility of HIV infection in cases of tuberculosis and Vice-Versa should be considered at all times. In 2011 worldwide 430,000 people were estimated to have died of TB and HIV co-infection, in addition to the 990,000 people who died from TB alone. It has been seen that AIDS with a co-infection of tuberculosis exist more predominantly in the lower economic group of people.\(^3\)

Infectious disease and malnutrition are major health problems in the developing world. Malnutrition can predispose to the development of a variety of infections. Malnutrition and tuberculosis are both problems of considerable magnitude in most of the underdeveloped regions of the world. It is important to consider, how these two problems tend to interact with each other. The term consumption has been virtually synonymous with tuberculosis throughout the history and the link between tuberculosis and malnutrition has long been recognized; malnutrition may predispose people to the development of clinical disease and tuberculosis can contribute to malnutrition. Before the advent of antituberculosis chemotherapy, a diet rich in calories, proteins, fats, minerals, and vitamins was generally considered to be an important, if not essential factor in treatment of tuberculosis. Tuberculosis adversely affects the nutritional status in several ways. These effects are more profound in the setting of co-infection with HIV. Both HIV status and malnutrition significantly contribute to atypical presentation of pulmonary tuberculosis.\(^4\)

Tuberculosis (TB) is a major cause of pleural effusion, which in TB usually has lymphocytic and exudative characteristics. Tuberculosis produces a spectrum of histopathological reaction...
in the bone marrow. They range from normal marrow hyperplasia and necrosis of the
marrow. The bone marrow in patients with untreated tuberculosis may show megaloblastic
changes and this could be a reflection of poor nutritional status of these patients. Iron stores
estimated on bone marrow aspirates bear a good correlation with serum iron level. Patients
with poor nutritional status may have decreased iron status on bone marrow examination.[4]

**ADENOSINE DEAMINASE**

Adenosine deaminase (ADA) is a hydrolase enzyme, polymorphic and actively participates in
the metabolism of adenine nucleotides. ADA is an enzyme involved in the conversion of
adenosine to inosine. The cell differentiation of the immune system in humans, due to the
interaction of the mycobacterium with the host factors, is the source of ADA activity. This
enzyme catalyzes hydrolytic de-amination of adenosine and deoxyadenosine to inosine and
deoxyinosine respectively; in this process ammonia is released. ADA modulates the
concentration of adenosine which is both a metabolic precursor for nucleic acids
(intracellular) and significant signaling molecule involved in the regulation of various
physiological processes. Lymphoid tissue has 10 to 20 times higher Adenosine Deaminase
concentration than the other tissues.[3]

Human adenosine deaminase (ADA ; EC 3.5.4.4; an enzyme of purine catabolism) activity
has been found to be increased in various diseases such as tuberculosis, HIV, typhoid,
infectious mononucleosis and certain malignancies especially those of hemopoietic origin.
ADA assay in various body fluids had established its usefulness in the laboratory diagnosis of
extrapulmonary TB (such as meningeal, pleural, peritoneal and pericardial TB), smear-
positive TB and SNPTB.[5] High ADA levels can also be found in pleural effusions secondary
to other processes or lesions especially pneumonia, emphysema, lymphoma, neoplasia and
systemic lupus erythematosus.[6] ADA has two principal isoenzymes, ADA-1 and ADA- 2,
which have different optimal pH, Michaelis constants and relative substrate specificity
patterns has roughly equal affinities for adenosine and 2'-deoxyadenosine, with a 2'-
deoxyadenosine deaminase/ ADA activity ratio of approximately 0.75; it is found in many
tissues. ADA-2 has much greater affinity for adenosine (2'- deoxyadenosine deaminase/ADA
activity ratio approximately 0.25), and is found only in macrophages, which release it when
stimulated by the presence of live micro-organisms in their interior.[7] While human tissue
extracts contained ADA1 predominantly, ADA2 was the main component of serum ADA.
Therefore, ADA activity measured in serum reflects ADA2 activity. The high ADA activities in tuberculous pleural effusions are largely due to ADA-2.\(^6\)

The ADA, isoenzymes are found highest activity in lymphocytes and monocytes, whereas ADA, isoenzyme gene products appears to be found only in monocytes. The assay of ADA activity in pleural and other effusion is very useful in differential diagnosis especially is the case of tuberculosis which is characterized by an increase in activity. Human ADA exists in at least three molecular forms. ADA is a monomeric protein with a molecular mass of n35 kDa (gene assignment, chromose 0). ADA2+CP (molecular mass ~ 280 k Da) is composed of two ADA1 molecules connected via a combining protein (CP; binding protein) (gene assignment, chromosome 2 and 6). Third isoenzyme, ADA2 appears to be coded by a separate gene locus of unknown chromosomal. Serum ADA activity is increased in various diseases such as liver disease, tuberculosis typhoid, infective mononucleosis and certain malignancies especially those of hemopoetic origin. The origin of serum ADA and the mechanism by which serum activities are increases have not been fully elucidated. Usually total ADA activity is measured without determining the contribution of each isoeyzymes.\(^4\)

The activity of ADA is greater in lymphocytic cell and than in erythrocytes and, in relation to the former is greater in T-lymphocytes than in B-lymphocytes and varies during T-cell differentiation with significant increases of its level in immature or undifferentiated states.\(^4\) Cellular immunity mediated by T-lymphocytes constitutes a major defence against tuberculosis.

Adenosine deaminase has shown promising results in the diagnosis of tuberculous pleural, peritoneal and pericardial effusions and tuberculous meningitis. In CSF, ADA is elevated in cases of tuberculous meningitis.\(^4\)

ADA is widely distributed in lymphoid tissue, especially in the thymus, lymph nodes, spleen, and gastrointestinal tract.\(^8\) The enzyme is found in a higher concentration in T-lymphocytes\(^9\) than in the B-lymphocytes in TB and other infections the activity of ADA is increased after activation of T-lymphocytes by antigenic stimulation.\(^10,11\) The increased ADA activity is thought to be due to a local activity of T-lymphocytes and monocytes.\(^12\)

Since 1978, when ADA activity was found to be high in tuberculous pleural exudates, ADA has been used in the diagnosis of tuberculous pleural effusions; overall, its sensitivity in this
role has been 99% and its specificity 93%. Thus the only test left is Adenosine deaminase Activity (ADA). This is a single test which is sensitive and specific and at the same time inexpensive and easy to perform.\cite{3}

Diagnosis or, the etiology can be difficult in spite of a careful clinical examination of the patient aided by investigative procedures and laboratory tests. TB and malignancy are the two most common causes of exudative pleural effusion which should always be considered in the differential diagnosis. Tuberculous effusion is usually diagnosed by pleural biopsy as bacteriological examination of pleural fluid, sputum and laryngeal swab specimens is of limited value because of the low diagnostic yields (9-32%) with these methods.\cite{13,14} However, the limitations of pleural, pericardial or peritoneal biopsy can be circumvented by using a laboratory test to detect tuberculous activity in body fluids. The measurement of ADA activity in body fluids has been reported to be useful in the diagnosis of tuberculous effusion. This test will be of particular value in situations where it is not possible to do a pleural biopsy because of the small extent of the effusion or when standard diagnostic procedures including pleural biopsy have given negative results.\cite{15,16}

In the presence of a predominantly neutrophilic infiltration (eg. emphysema), rheumatoid arthritis and lymphoma, high body fluid, ADA activity is not reliable as a marker of tuberculous infection. The assay of ADA can be recommended as a useful diagnostic test of tuberculous pleurisy. Unlike pleural biopsy, ADA is not affected by sampling error e.g. when a non-involved area of the pleura is biopsied, and only a small volume of pleural fluid (5 ml) is required to perform the assay, which can be done in most clinical laboratories.\cite{17}

**CRP (C-reactive protein)**

CRP (C-reactive protein) was originally discovered by Tillett and Francis in 1930 as a substance in serum of patients with acute inflammation that reacted with C-polysaccharides of *pneumococcus*. Initially, it was thought an ohogenouscretion as it is elevated with variety of illness including carcinomas. Discovery of hepatic synthesis and secretion of CRP closed that debate. It is thought to bind to sphocholine, thus initiating recognition and phagocytosis of damaged cells. CRP gene is located on first chromosome. CRP is 224 residue proteins with monomer molar mass of 25106 Da, annual pentameric disc shaped. CRP is produced by liver and its level rises when there is a widespread systematic inflammation. CRP test is one of the many diagnostic tests used within centralized hospital laboratories to monitor a series of inflammatory conditions. At present, CRP analysis is predominantly employed to monitor the
extent of activity of diseases and effect of treatment. CRP analysis is a useful test for supporting the diagnosis of infection and has been proven as a valuable tool in follow-up and differential diagnosis such as in case of cancer, connective tissues diseases, heart attack, inflammatory bowel diseases (IBS), lupus, pneumonia rheumatoid arthritis, rheumatic fever and TB. Low level of CRP does not always mean that there is no inflammation present, more sensitive CRP test is called high sensitive CRP (hs-CRP).

As mentioned earlier, the Mycobacterial culture though it is definite method however, it is a time consuming (4-6 weeks at least) and requires lot of manual work and stringent conditions. Thus, CRP estimation is easy simple test to support diagnosis of TB infection. It also reflects the course of diseases and effectiveness of drug.

CRP is the classic acute phase reactant. Its concentration in serum rise up to a thousand fold in response to most forms of tissue damage, inflammation and infection. As increased hepatic production of C reactive protein is a rapid and sensitive response to most forms of microbial infection, the value of measurement in the diagnosis and management of various infective conditions has been established.

CRP estimation is more helpful in culture negative patients of partially treated bacterial meningitis.\[18\] CRP levels were found to be significantly higher in sputum-positive group as compared with the sputum-negative group. Statistically, the difference was found to be significant (P < 0.0005).\[19\]

The CRP levels decrease as the lesion become inactive or as the severity of the disease decreases CRP is a proper test for evaluation and prognosis of TB. It can reflect the course of the disease and also the effectiveness of the disease. CRP seems to be reactive in those patients in whom the disease is in the active stage regardless of the extension and site of involvement. The rapid decline in serum CRP concentration after treatment in most patients with tuberculous patients is of diagnostic value. Serum CRP levels have a role in identifying the advanced and extensive disease patients thereby indirectly helping the health workers to pick up delayed convertors/potential defaulters, so as to guide them to put in extra efforts on these groups, in TB control programs.\[20\]
MATERIALS AND METHOD
The present study "Biochemical Studies in Blood and Body Fluids of Tubercular Patients" was carried out in Department of Pathology of SAAII College of Medical Science & Technology, Kanpur from Dec. 2010 to October 2012. Basic infrastructures available in SAAII College of Medical Science & Technology, Kanpur as well as test available in Dept, of Biochemistry of SAAII College of Medical Science & Technology, Kanpur are used for the study. The patients diagnosed as suffering from pulmonary TB as well as TB of various organs of body are the subject of study. Patients are drawn from indoor wards and out-patients department of L.L.R Hospital and associated Hospital, Kanpur and T.B Hospital Azad Nagar, Kanpur.

Group A consisting of 100 normal healthy age and sex matched controls, 100 authentic pulmonary TB patients were chosen for study in group B, while group C consisting of 100 cases of non-tubercular diseases. The clinical features and detailed history of each case is recorded in a standard format including exposure to infection, physical examination and chest radiography.

Criteria for selection
Control Healthy Subjects (Group A)
(Age and sex match group)
Inclusion criteria: Healthy individuals in age group of >30 years.
Exclusion criteria
1) Individuals with evidence of any medical disorder of infectious and non infectious origin.
2) Individuals <30 yrs of age
3) Individuals having immunocompromised status
4) Individuals with past history of TB

Cases (Group B)
TB Inclusion criteria
1) Patients > 30 years of age.
2) Patients with steady state of the disease at the time of study.
3) Patients with both sexes were included.
4) Patients with pleural effusion, pericardial effusion, ascitis secondary to TB were also included in study.
5) Patients with pulmonary and extra-pulmonary TB.

**Exclusion criteria**
1) Patients with any other type of medical disorder.
2) Patients with any crises at the time of study.

**Cases (Group C)**

**Non-tubercular Disorder**

**Inclusion criteria**
1) Patients > 30 years of age.
2) Patients with steady state of the disease at the time of study,
3) Patients with both sexes were included.
4) Patients with pleural effusion, pericardial effusion, ascitis secondary to non-TB disease (malignancy, liver diseases, respiratory diseases, septic and viral meningitis, etc) were included in study.

**Exclusion criteria**
1) Patients with any other type of TB.
2) Patients with any crises at the time of study.
3) Patients with past history of TB.
4) Patients with history of exposure TB.

**Specimen Collection**
A) Blood samples of the controls/study group was collected for various biochemical analyses by vein puncture and transferred to EDTA vacutainer and used for hematological tests and serum was used for analysis of ADA and CRP.
B) Body fluids like pleural, peritoneal, and pericardial and CSF fluids all were collected in sterile tubes.

**Routine Investigation:** Following investigations are carried out in each patient.

**A) Hematological Tests**[21]
1) Total white blood cell count was done by automated cell counter.
   Normal Range 4.0-10.0 x 10^9
2) Differential leukocyte count, by stain blood film.
Normal Range

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Normal Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophil</td>
<td>$2 - 7.5 \times 10^3 / \mu l$</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>$1 - 3.5 \times 10^3 / \mu l$</td>
</tr>
<tr>
<td>Monocytes</td>
<td>$0.2 - 1.0 \times 10^3 / \mu l$</td>
</tr>
<tr>
<td>Eosinophil</td>
<td>$0.0 - 0.1 \times 10^3 / \mu l$</td>
</tr>
</tbody>
</table>

3) ESR by Wintrobe's method
Normal value: Male: 0-10 mm at the end of one hour.
Female: 0-15mm at the end of one hour.

4) Ziehl Neelsen's (Z-N) staining for AFB: In case of all sputum, fluids will be subjected to staining for AFB by Z-N Technique.

Ziehl-Nielsen Stain for AFB\(^{[22]}\)

**Principle**
The organism such as *Mycobacterium tuberculosis* that causes tuberculosis and *mycobacterium leprae* that causes leprosy are extremely difficult to stain by ordinary method because of lipid containing cell wall. They bind carbol fuchsin tightly and resist distaining with strong decolorizing agent such as alcohol and strong acid. Acid fast negative bacteria (other bacteria) readily lose the stain when treated with acid – alcohol solution. Heat is applied in Ziehl Nielsen hot stain method for detection of *M. tuberculosis*.

**Method**
(1) Smears were prepared from the sputum specimen on glass slide and were fixed by heating on flame.
(2) The heat fixed slides were placed on staining rack and the smear was flooded with working carbol - fuchsin stain.
(3) The stain was not allowed to dry by continuous gentle heating
(4) The stain of slides was washed with running continuous water until the colour changes colourless.
(5) The slide was covered with 20% sulphuric acid for about 1 minute (until the yellow coloured complex was drained out completely).
(6) The slide was covered with methylene blue stain for 1 minute.
(7) The slides were washed with tap water and the water was allowed to drain out.
(8) The slides were examined under the low power objective and then examined under oil-emersion objective.
RESULTS
Acid Fast Bacilli red tubercular bacilli in the form of short rods were observed.

Reagents
(1) **Soft carbol fuchsin**
New Fuchsin (CI42520) 1.5 gm
Absolute alcohol 10 ml
Distilled water 80 ml
Glycerol 10 ml
Triton X100 0.75 ml
New fuchsin is added to the alcohol in a 100 ml flask and mixed, on a magnetic stirrer for 30 minutes. Glycerol is then added and the Triton X100 to the water and mixed till it dissolved. This is then added to the New fuchsin mixture and continue mixing on the stirrer overnight. It is then filtered and store in a brown glass bottle.

(2) **Stock methylene blue**
Methylene Blue (CI52015) 2 gm using a magnetic stirrer dissolve in 100 ml distilled water. Then absolute alcohol 100ml was added.

(3) **Working Methylene Blue**
Stock Methylene Blue 40 ml
Distilled water 60 ml
Glacial acetic acid 0.5 ml

(4) **0.5% Acid Alcohol**
Distiller water 700 ml
Absolute alcohol 300 ml
Hydrochloric acid 5 ml

(5) **5% Sulphuric Acid**
Distilled water 475 ml
Sulphuric acid 25 ml

B) **Biochemical Tests**
1) Serum ADA
2) Serum CRP
4) Analysis of ADA in Body Fluids (Pleural, Pericardial, CSF, Peritoneal)
5) Estimation of CRP in Body Fluids (Pleural, Pericardial, CSF, Peritoneal)

C) Radiological Investigation
1) Chest X-ray

Details of experiment, on ADA and CRP in the Present study

Measurement of ADA Activity\[^{[23]}\]

The principle of this technique is that ADA hydrolyses adenosine to ammonia and inosine. Ammonia thus formed further reacts with a phenol and hypochlorite in an alkaline medium to form a blue indophenol complex with sodium nitroprusside acting as a catalyst. Intensity of the blue colored indophenol complex formed is directly proportional to the amount of ADA present in the sample.

\[
\text{ADA} \\
\text{Adenosine + H}_2\text{O} \rightarrow \text{Ammonia+Inosine} \\
\text{Alkaline} \\
\text{Ammonia + Phenol + Hypochlorite} \rightarrow \text{Blue Indophenol Complex}
\]

Reagents and Solutions
a) Buffer reagent-L1.
b) Adenosine reagent-L2.
c) Phenol reagent-L3.
d) Hypochlorite reagent-L4.
e) ADA standard -S.

Preparation of Working Reagent
Working Phenol reagent: - 1 part of reagent + 4 part of distilled water. Working Hypochlorite reagent: - 1 part of reagent + 4 part of distilled water

Test Procedure
1. All the reagents and samples were brought to room temperature before use.
2. Working phenol reagent and working hypochlorite reagent were prepared before use as stated above.
3. The spectrophotometer filter was set up at 630 nm at 37 \(^{\circ}\)C.
4. Test tubes were cleaned and dried and was labeled as Blank (B), Standard (S), Sample Blank (SB) and Test (T). Additions were done as follows.

<table>
<thead>
<tr>
<th>Addition Sequence</th>
<th>B</th>
<th>S</th>
<th>SB</th>
<th>T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer Reagent (ml)</td>
<td>0.20</td>
<td>0.20</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Adenosine Reagent (ml)</td>
<td>-</td>
<td>-</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>Deionized Water (ml)</td>
<td>0.02</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Standard (ml)</td>
<td>-</td>
<td>0.02</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sample (ml)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.02</td>
</tr>
</tbody>
</table>

After being mixed well and incubated at 37° C for 15 minutes or at room temperature for 30 Minutes. Then the following additions were done.

<table>
<thead>
<tr>
<th>Working Phenol Reagent</th>
<th>1.00</th>
<th>1.00</th>
<th>1.00</th>
<th>1.00</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td></td>
<td></td>
<td></td>
<td>0.02</td>
</tr>
<tr>
<td>Working Hypochlorite Reagent</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
</tbody>
</table>

The absorbance of Blank (Abs. B) Standard (Abs. S), Sample Blank (Abs. SB) and Test (Abs. T) was measured against distilled water.

**Estimation of C - Reactive Protein**[24]

C Reactive phase protein synthesized in the liver. Its rate of synthesis increases within hours of acute injury or the onset of inflammation and may reach as high as 20 times the normal levels. A rapid fall in CRP level indicates recovery.

**Principles**

CRP-UV is a turbidometric immunoassay for the determination of C reactive protein in human serum and is based on the principle of agglutination reaction. The test specimen is mixed with activation buffer CRP- UV reagent is then added allowed to react. Presence of CRP in the test specimen results in the formation of a soluble complex producing a turbidity, which is measured at 340 nm wavelength. The increase in turbidity corresponds to the concentration of CRP in the test specimen.

**Reagents**

1) Activation buffer
2) Solution of CRP antibody.
3) Calibrator
Specimen Collection and Preparation

Only serum was used for testing. The samples were stored at 2 - 8°C before analysis. Hemolysed, icteric or highly turbid serum was not used for analysis. Turbid or particulate serum samples were clarified by centrifugation at 2000 rpm for 15 minutes and clear supernatant was used for testing.

Assay Conditions

All reagents were brought at room temperature before use.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength</td>
<td>340nm</td>
</tr>
<tr>
<td>Reaction Temperature</td>
<td>37°C</td>
</tr>
<tr>
<td>Cuvette</td>
<td>1 cm path length</td>
</tr>
</tbody>
</table>

Test procedure

1. The instrument was set to zero with distilled water.
2. 500 IJI of CRP UV activation buffer (R1) and 50 pi of serum were pipetted in the measuring cuvette. Mixed well and incubated for 5 minutes at 37°C.
3. Absorbance (A1) was read.
4. 501 of CRP UV reagent (R2) (pre-incubated at 37°C) was then added to cuvette, mixed gently and the stopwatch was started simultaneously.
5. Absorbance (A2) was read at the end of exactly five minutes.
6. AA (A2-A1) was calculated for the test specimen.

RESULT

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>GROUP A</th>
<th>GROUP B</th>
<th>GROUP C</th>
<th>p VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEAN + S.D. OF AGE</td>
<td>20.35±5.09</td>
<td>20.30±4.59</td>
<td>20.25±3.78</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>MEAN + S.D. OF ESR (MALE)</td>
<td>19.51±5.01</td>
<td>31.93±6.4</td>
<td>17.08±5.44</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>MEAN + S.D. OF ESR (FEMALE)</td>
<td>21.36±4.95</td>
<td>30.36±6.86</td>
<td>23.52±6.78</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>SPUTUM POSITIVE</td>
<td>0</td>
<td>83</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>SPUTUM NEGATIVE</td>
<td>100</td>
<td>17</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>X-RAY POSITIVITY</td>
<td>0</td>
<td>89</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>X-RAY NEGATIVITY</td>
<td>100</td>
<td>11</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>MEAN + S.D. OF SERUM ADA</td>
<td>10.11±0.39</td>
<td>19.32±2.15</td>
<td>10.55±0.9</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>MEAN + S.D. OF SERUM CRP</td>
<td>0.4±0.07</td>
<td>2±0.39</td>
<td>0.7±0.39</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>MEAN + S.D. OF PLEURAL FLUID ADA</td>
<td>0±0</td>
<td>29.61±3.93</td>
<td>10.33±1.28</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>MEAN + S.D. OF PERICARDIAL FLUID ADA</td>
<td>0±0</td>
<td>28.53±1.02</td>
<td>10.11±0.53</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>MEAN + S.D. OF PERITONEAL FLUID ADA</td>
<td>0±0</td>
<td>27.91±2.23</td>
<td>10.46±1.00</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>MEAN + S.D. OF CSF ADA</td>
<td>0±0</td>
<td>28.40±1.86</td>
<td>10.07±0.88</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>MEAN + S.D. OF PLEURAL FLUID</td>
<td>0±0</td>
<td>2.05±0.04</td>
<td>0.66±0.02</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Parameters</td>
<td>Positive Case</td>
<td>p Value</td>
<td>Negative Case</td>
<td>p Value</td>
</tr>
<tr>
<td>------------</td>
<td>---------------</td>
<td>---------</td>
<td>---------------</td>
<td>---------</td>
</tr>
<tr>
<td>MEAN + S.D. OF PERICARDIAL FLUID CRP</td>
<td>0±0</td>
<td>1.79±0.07</td>
<td>0.74±0.04</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>MEAN + S.D. OF PERITONEAL FLUID CRP</td>
<td>0±0</td>
<td>1.74±0.08</td>
<td>0.69±0.03</td>
<td>&lt;0.05</td>
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<tr>
<td>MEAN + S.D. OF CSF CRP</td>
<td>0±0</td>
<td>0.67±0.32</td>
<td>0.49±0.02</td>
<td>&lt;0.05</td>
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</tbody>
</table>

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<tr>
<th>Parameters</th>
<th>Positive Case</th>
<th>p Value</th>
<th>Negative Case</th>
<th>p Value</th>
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<tr>
<td>MEAN + S.D. OF ADA IN SPUTUM</td>
<td>19.31±0.24</td>
<td>&lt;0.05</td>
<td>16.30±0.13</td>
<td>&lt;0.05</td>
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<td>MEAN + S.D. OF SERUM CRP IN SPUTUM</td>
<td>1.98±0.04</td>
<td>&lt;0.05</td>
<td>1.73±0.03</td>
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<td>MEAN + S.D. OF PLEURAL FLUID ADA IN BACTERIAL INFECTION</td>
<td>34.75±1.08</td>
<td>&lt;0.05</td>
<td>29.61±0.90</td>
<td>&lt;0.05</td>
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<td>MEAN + S.D. OF PERICARDIAL FLUID ADA IN BACTERIAL INFECTION</td>
<td>29.09±0.40</td>
<td>&lt;0.05</td>
<td>27.18±0.37</td>
<td>&lt;0.05</td>
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<td>MEAN + S.D. OF PERITONEAL FLUID ADA IN BACTERIAL INFECTION</td>
<td>28.27±0.66</td>
<td>&lt;0.05</td>
<td>26.48±0.20</td>
<td>&lt;0.05</td>
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<tr>
<td>MEAN + S.D. OF CSF FLUID ADA IN BACTERIAL INFECTION</td>
<td>29.31±0.39</td>
<td>&lt;0.05</td>
<td>27.50±0.36</td>
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<td>MEAN + S.D. OF PLEURAL FLUID CRP IN BACTERIAL INFECTION</td>
<td>2.28±0.07</td>
<td>&lt;0.05</td>
<td>2.05±0.05</td>
<td>&lt;0.05</td>
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<tr>
<td>MEAN + S.D. OF PERICARDIAL FLUID CRP IN BACTERIAL INFECTION</td>
<td>2.20±0.07</td>
<td>&lt;0.05</td>
<td>1.74±0.10</td>
<td>&lt;0.05</td>
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<tr>
<td>MEAN + S.D. OF PERITONEAL FLUID CRP IN BACTERIAL INFECTION</td>
<td>2.09±0.10</td>
<td>&lt;0.05</td>
<td>1.63±0.11</td>
<td>&lt;0.05</td>
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<td>MEAN + S.D. OF CSF FLUID CRP IN BACTERIAL INFECTION</td>
<td>0.74±0.07</td>
<td>&lt;0.05</td>
<td>0.58±0.01</td>
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**Graph No. 1**

**Graph No. 2**
DISCUSSION

The subjects included in our study are age and sex matched. The sex distribution consisting of 58% of males and 42% of female in cases i.e. group B. Same distribution was followed while selecting healthy control i.e. group A as well as while selecting the study group having non-tuberculous diseases i.e. group C for comparison. Number of males in our study exceeds the number of female. This is due to the sex ratio in our society is more towards male than female. The health problems of males in our society are more looked after than female health problems. All this is attributed to the structure of our society as built since long time. Also, the prevalence of TB is more in males as compared to that with females.

The patients present in group B are between 21 - 40 years, which is further divided into two groups i.e. from 21-30 years and from 31-40 years. The distribution of age in this group suggests that the TB is more prevalent in fourth decade of life as the group is more vulnerable for external atmosphere. Similarly, age match controls were selected and those with non-tuberculous disease cases were selected for comparison. The mean age and SD for TB case were 20.35±5.09 and for control is 20.30±4.59 and that for non-TB cases were 20.25±3.78. There was no statistically significant difference between the age of the control and cases that were selected for the study.

In preliminary laboratory investigations, ESR was done in both controls A and C and case group B and was subjected for statistical analysis for comparison. The results also are compared separately for males and females as the levels of ESR normally differ in males and females. The ESR values are found significantly different in tuberculous cases as compared to healthy controls and non-tuberculous cases.
ESR is one of the indicators of acute inflammation. The ESR is raised in many other conditions of acute inflammation. Though the rise is significant, in cases as compared to the controls and non-tuberculous cases but as ESR is increased in many other inflammatory conditions it is not a specific parameter for the diagnosis of tuberculous infection. It alone has no significant value in diagnosis of the tuberculous infection. So, it is not considered for the diagnostic purpose but as a supportive laboratory investigation in diagnosis of tuberculous infection. In present study, body fluids in some selected tuberculous cases and non-tuberculous cases for biochemical parameters such as ADA and CRP in some selected cases were also studied. The types of fluid examined are pleural, pericardial, peritoneal and CSF and was compared with the non-tuberculuous cases. The fluids were analyzed for all these parameters. A total 53 patients were analyzed for pleural fluid, 21 each or pericardial and peritoneal fluids and 29 for CSF. The fluid findings were compared that with the corresponding non-tuberculous cases.

The sputum and body fluids were also examined for the bacterial positivity by microscopy with Z-N staining. It was observed that, 23 of 53 pleural fluid, 11 each of pericardial and peritoneal fluid out of 23 fluids studied and 15 out of 29 CSF studied were positive for tuberculous bacteria. Here, low positivity for acid fast bacilli (AFB) in body fluids is noted.

The bacterial positivity when compared with levels of biochemical parameters estimated in body fluids such as ADA and CRP, it was observed that the levels of ADA and CRP are higher in those patients. Who’s the body fluid is positive for the AFB. That level observed were significantly higher in these patients as compared to those in which body fluids are negative for AFB. This shows that the disease is more advanced and extensive affecting biochemical constants. Thus, suggesting that active microbiological status affects the biochemical parameters.

Sputum was also studied in the all three study group for the presence of tubercular bacteria by Z-N staining. Out of the 100 TB patient studied for sputum positivity, 83 showed AFB positivity for tubercular bacteria while 17 were AFB negative in sputum. This shows that the sputum negative 17 patients were of pulmonary TB while the remaining 83 were consist of bacterial positive pulmonary TB cases. Such high percentage of AFB positivity suggests that patients of the present study were of advanced pulmonary TB. Also, the high AFB chances of catching the AFB positivity in this group can be attributed to the fact that successive three days examination of sputum was performed in all negative cases which resulted in to high
sensitivity. The result also emphasizes the need of successive 3 days sputum test for AFB positivity in all negative cases of suspected pulmonary TB, as well as of those who are having pulmonary and extra-pulmonary TB cases.

Chest X-ray was also done as a part of routine investigation in TB diagnosis. It was found that 90 of the 100 tubercular cases were also having positive chest X-ray finding for the TB. These cases are the chest X-ray positive tubercular cases.

**ADENOSINE DEAMINASE**

Pleural, pericardial, peritoneal fluid and CSF ADA levels are 29.61±3.93, 28.53±1.02, 27.91±2.23, 28.40±1.86, which is significantly higher in Group B than and Group C. In Group B the pleural fluid ADA to serum ADA ratio in our study is 2.34±0.07, pericardial fluid ADA to serum ADA 1.81±0.09, peritoneal fluid ADA to serum ADA 1.68±0.09, CSF ADA to serum ADA which significantly differs from that compared to Group C and Group B.

In the present study, it is found that, the sensitivity and specificity for serum ADA concentration 98% and 100% and for pleural fluid/serum ADA concentration 100% and 96%, respectively. Also, the value of fluid and serum ADA, as well as fluid/serum ADA ratio were higher in patients with exudates (i.e. TB). Thus, the results of present study confirm that ADA activity is a useful parameter for differentiating exudates from transudates. In the present study, it is found that, at a cut-off point of 15 IU/L, the sensitivity and specificity of pleural ADA concentration for the diagnosis of exudates to be 98% and 100%, respectively at a cut-off point of 1.0 for pleural fluid/serum ADA concentration 100% and 96% respectively. Hence, the cut-off point established in this study yielded a better sensitivity and specificity as compared to the other study. Pleural effusion is a common clinical disorder with many diverse causes.

The present results confirm the high sensitivity and specificity of ADA test for early diagnosis of TB in cases of serous effusions. ADA has been proposed to be a useful surrogate marker for TB in pleural, pericardial and peritoneal fluids.

**C - REACTIVE PROTEIN (CRP)**

In present study, the serum CRP level was compared with that of non-tubercular disease patients and control group with that of tubercular group. The serum CRP levels were
estimated and compared among all the three groups using one-way ANOVA and a significant difference was found in these study groups. The mean CRP level was found to be in Group A as 0.4±0.07mg/dl in Group B as 2.02±1.36 mg/dl and in Group C as 0.7±0.39mg/dl. The p value is significant and is found <0.01. In present study we also found that those with active lesion have high levels of serum CRP than those with mild or inactive disease. This indicates that it is also a good indicator of the severity and progress of the tuberculosis.

In present study also, CRP estimation was done from the fluids of subjects in group B and group C and it was found that there was significant difference between the CRP levels of body fluids. pleural, pericardial, peritoneal and CSF in TB were found to be 2.05+0.04, 79+0.07, 1.74+0.08 and 0.67+0.32 respectively.

For this reason, in addition to the clinical and radiological characteristics, the cytological and biochemical findings can aid in diagnosing tuberculous pleuritis.

From present study, it is found that measurement of CRP level in pleural fluid can be useful; with the advantage that CRP can be determined in conventional auto analyzers and is an inexpensive test. In patients with lymphocytic pleural effusion, values of CRP in the pleural fluid >50 mg/l should strongly suggest the possibility of tuberculous pleuritis. On the contrary, low CRP levels (<30 mg/l) virtually ruled out this possibility.

Tuberculous pericarditis is seen in 1-8% of these patients. The route of spread to pericardium is usually from mediastinal or hilar nodes or from lung and rarely as part of miliary TB. Tuberculous pericarditis can present with recurrent pericardial effusion without any history or symptoms of TB. It can also be diagnose with biochemical marker such as CRP with great sensitivity and specificity.

In present study, it is also seen that the CSF CRP levels are significantly higher in tuberculous meningitis patients than in non-tuberculous patients of meningitis. Only in 4 of non tuberculous cases the higher levels are found than the TB meningitis cases. This may be attributed to that these cases may be of bacterial meningitis type where the CSF CRP values were 'higher than TB meningitis and viral meningitis. So the level of CSF CRP lies in TB meningitis lies in between bacterial meningitis and viral meningitis type of variety.
CRP is used mainly as a marker of inflammation. Apart from liver failure, there are few known factors that interfere with CRP production. CRP is mainly synthesized in the liver. But studies show that CRP can also be synthesized in the neurons and lipopolysaccharide-S can induce CRP synthesis in extra hepatic sites. CRP levels are affected by factors such as hepatic dysfunction, dyslipidaemia, females on oral contraceptive pills, and patients on steroids measuring and charting CRP values can prove useful in determining disease progress or the effectiveness of treatments. High levels of CRP in the blood mean that there is inflammation somewhere in the body. Other tests are needed to determine the cause and location of the inflammation. The CRP pleural fluid level adds significant diagnostic information that should be considered together with all the other epidemiological, clinical, cytological and biochemical data.

CONCLUSION

The present study is conducted with an aim to find out the usefulness of ADA, and CRP levels in blood as well as in various body fluids for the diagnosis of TB. The inter-relationship of all these parameters, there co-relation and comparison is also undertaken to find out there diagnostic and prognostic importance.

We conclude that a correlation exists between body fluid ADA levels with body fluid CRP levels. The correlation is positive type and is statistically significant. Thus, by combining all these parameters i.e. ADA and CRP the diagnosis of the tuberculous infection becomes more easy, accurate and fast emphasizing the need of their continued use and to differentiate pulmonary and extra pulmonary tubercular infection as well as helpful in finding out the LTBI (Latent TB Infection) . The combined estimation of parameters such as ADA and CRP in serum as well as body fluids is of great value in diagnosis and prognosis of these lesions.

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