Performance Evaluations

ADA-MTB®
For the determination of ADA activity in serum, plasma and biological fluids

ISO 9001: 2008
EN ISO 13485: 2012
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<td>ADA-MTB as a Tuberculosis Marker</td>
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<td>2.</td>
<td>Asian Pacific Journal of Tropical Disease 2013, 3 (1)</td>
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<td>3.</td>
<td>Medica Innovatica, June 2014, Volume 3 - Issue 1</td>
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<td>109-114</td>
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<td>International Journal of Basic and Applied Medical Sciences ISSN: 2277-2103</td>
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<td>International Journal of Biomedical And Advance Research (2013) 04 (05)</td>
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<td>IOSR Journal of Dental and Medical Sciences (IOSR-JDMS) e-ISSN: 2279-0853, p-ISSN: 2279-0861. Volume 13, Issue 1 Ver. IX. (Feb. 2014),</td>
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<td>Journal of Microbiology and Infectious Diseases/JMID,2013; 3(3)</td>
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**ADA-MTB®**

For the determination of ADA activity in serum, plasma and biological fluids
Performance Evaluations

AS A REFERENCE PRODUCT

ADA-MTB®
For the determination of ADA activity in serum, plasma and biological fluids
AS A MARKER FOR TUBERCULOSIS
Cerebrospinal fluid adenosine deaminase levels as a diagnostic marker in tuberculous meningitis in adult Nepalese patients

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Department of Microbiology, Kathmandu Medical College Teaching Hospital, Sinamangal / Duwakot, Kathmandu, Nepal

Objective: To study the cerebrospinal fluid (CSF) adenosine deaminase (ADA) levels in tuberculous meningitis (TBM) and non–TBM – viral meningitis cases and to determine its diagnostic significance as a biochemical marker of TBM infection.

Methods: The study population comprised two different patient groups. TBM – group I – 28 cases and non–TBM–viral meningitis – 22 cases. These were enrolled consecutively in the study and CSF specimens were collected from them. ADA estimation was carried out by spectrophotometry.

Results: ADA levels (mean ± SD) in the TBM and non-TBM groups were 16.46 ± 6.24 U/L and 5.13 ± 2.96 U/L, respectively (highly significant P < 0.001). Using a CSF ADA cut off reference value of >10 IU/L, the test showed a good sensitivity of 82.14% (95% CI 64.41–92.12) and a high specificity of 90.91% (95% CI 72.19–97.47).

Conclusion: CSF ADA levels are elevated in the TBM cases as compared to the non-TBM – viral meningitis cases with a good sensitivity and a high specificity. It is a simple and inexpensive diagnostic adjunctive test in the rapid and early diagnosis of TBM.
2. Materials and Methods

2.1. Setting

This prospective study was conducted from January 2009 to June 2010 at a large centrally located tertiary care hospital in Kathmandu, Nepal. The study protocol was approved by the institute ethical review committee and informed consent was obtained from the patients prior to inclusion in the study.

2.2. Patients

A total of consecutive 50 cases of clinically suspected of meningitis admitted in the medical ward of the hospital were selected. Out of these 50 cases of meningitis—28 cases were of TBM and 22 cases of non-TBM-viral meningitis.

2.2.1. Diagnostic criteria

All the cases were thoroughly examined clinically and investigated. Diagnosis of TBM was based on the clinical criteria, lymphocytic pleocytosis in the CSF with raised protein level and a low glucose level (<40% of matched blood glucose), negative bacterial and fungal cultures, a positive finding on ZN stain and/or a positive response to anti tubercular treatment for the duration of two months. Viral meningitis was diagnosed on the basis of acute onset of symptoms and signs of meningeval irritation, biochemical examination of CSF, negative results in gram stain, ZN stain and India Ink stain microscopic examinations of CSF, negative bacterial and fungal cultures and complete response to symptomatic treatment without antibacterials.

2.2.2. Exclusion criteria

Exclusion criteria for the study were the cases with age less than 14 years and cases who have undergone prior treatment outside the hospital and cases with CSF showing turbidity, hemorrhage and/or were xanthochromic.

2.3. Specimen collection

CSF specimens were obtained by lumbar puncture performed by a trained medical officer. A total of 3 ml of CSF was collected and distributed in three sterile vials and was subjected to various laboratory investigations involving biochemical, cytological and microbiological procedures.

2.4. ADA Assay

ADA estimation was carried out by spectrophotometry method based on the principle of Guisti and Galanti method of enzymatic analysis[15]. ADA MTB diagnostic kit from Microexpress—a division of Tulip Diagnostics Pvt. Ltd., India was used according to the manufacturer’s instructions. A cut off reference value of >10 IU/L CSF ADA was considered to be positive as per the guidelines provided in the test kit literature.

2.5. Data analysis

The results were expressed as mean ± SD. Statistical comparison was carried out by using the Student’s t test. A two-tailed P value of <0.001 was taken as statistically significant. Diagnostic test 2×2 contingency tables were made. Sensitivity, specificity, positive and negative predictive value, positive and negative likelihood ratio and diagnostic accuracy were calculated. All parameters were estimated with 95% confidence interval using the Stata 10.1 statistical software package (Stata Corp. College Station, Tx).

3. Results

Based on the diagnostic criteria, the patients were divided into two groups as TBM cases and non-TBM—viral meningitis cases. In TBM cases the age (mean±SD) and sex ratio (male:female) were 36.21±15.21 and 1.15:1, respectively and in non-TBM—viral meningitis cases the age (mean±SD) and sex ratio (male:female) were 38.59±18.30 and 1.44:1, respectively (Table 1).

<table>
<thead>
<tr>
<th>Study Group</th>
<th>No. of patients</th>
<th>Age (years)</th>
<th>Sex Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBM</td>
<td>28</td>
<td>36.21±15.21</td>
<td>1.15:1</td>
</tr>
<tr>
<td>Non-TBM—viral meningitis</td>
<td>22</td>
<td>38.59±18.30</td>
<td>1.44:1</td>
</tr>
</tbody>
</table>

The ADA levels (mean±SD) in TBM and non-TBM were 16.46±6.24 IU/L and 5.13±2.96 IU/L, respectively. The difference between the two groups of patients studied was found to be highly significant (Table 2).

<table>
<thead>
<tr>
<th>Study Group</th>
<th>No. of patients</th>
<th>P value comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBM</td>
<td>28</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Non-TBM—viral meningitis</td>
<td>22</td>
<td></td>
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</tbody>
</table>

Out of 28 TBM patients, 23 were found to be having CSF ADA above the cutoff value while 5 patients had values below the cut off. Of the 22 non-TBM—viral meningitis
patients, two were found to be having CSF ADA values above the cut off. The CSF ADA test in TBM patients showed sensitivity, specificity, positive and negative predictive values, positive and negative likelihood ratios and accuracy of 82.14%, 90.91%, 92.00%, 80.00%, 9.03%, 0.19% and 86.00% respectively in differentiating from non-TBM patients (Table 3).

4. Discussion

Differentiating TBM from non-TBM meningitis especially viral meningitis, by current laboratory methods is a major diagnostic challenge in clinical practice. There is considerable urgency in establishing the correct diagnosis in patients with TBM because specific therapy is most effective when instituted early in the course of illness. Rapid diagnostic tests with good sensitivity and specificity are required to aid the presumptive diagnosis of TBM[16]. ADA had been of interest for many years in TB diagnosis[17]. The determination of ADA activity in body fluids such as pleural fluid, peritoneal fluid and CSF has been reported to be a valuable marker in the diagnosis of extra-pulmonary TB[13]; It is of great relevance to evaluate the efficiency of the determination of ADA activity, which can be used in countries where TB prevalence is high. In addition, the determination of ADA activity can, in many cases favour the diagnostic confirmation, replacing biopsy, laparoscopy and other tests that definitely confirm the diagnosis but are more sophisticated, expensive and in many health care facilities, unavailable[18].

The results of this study showed that the CSF ADA value (mean±SD) in TBM cases was 16.46±6.24 while in non-TBM cases, it was 5.13±2.96, respectively (highly significant $P<0.001$). At a cut off value of 10 IU/L, the sensitivity and specificity of the test were 82.14% and 90.91% respectively. The positive and negative predictive values were 92% and 80%, respectively while the positive and negative likelihood ratios and accuracy were 9.03, 0.19 and 86% respectively. A positive likelihood ratio of 9.03 suggest that TBM patients have an approximately 9-fold higher chances of being ADA assay positive as compared to patients without TBM. However, if the ADA assay result is negative, the probability that the patient has TBM is approximately 19%, which is not low enough to rule out TBM. These results suggest that a negative ADA assay result should not be used alone as a justification to exclude or discontinue anti-TB treatment. The choice of therapeutic strategy should be based on the results of microscopic examination of a smear or culture for Mycobacterium tuberculosis, as well as other clinical data, such as response to anti-TB treatment[19].

Several studies had shown that ADA levels were found to be significantly high in TB group as compared to bacterial and viral meningitis[5,7]. Gupta et al. reported CSF ADA level 10 IU/L, as a cut off value showed 94.73% sensitivity and 90.47% specificity in differentiating TBM from non-TBM cases[10]. Agarwal S, describes a sensitivity and specificity of 99.90 and 87.5% respectively using a cut off value of 10 IU/L[12]. Gautam et al. demonstrated a sensitivity and specificity of CSF ADA activity as 85.0% and 88.0% respectively at a cut off value of 6.97 IU/L to diagnose TBM in CSF[14]. In a study done in Gujarat, India the sensitivity and specificity was 73.9% and 92.6% respectively when a cut off value of ADA of 10 IU/L was used[21]. Mehta et al. described a sensitivity and specificity of CSF ADA for TBM diagnosis to be 78% and 98% respectively at cut off value of 11 IU/L[22]. The value of cut off has a great importance in the evaluation of the sensitivity and specificity of CSF ADA test. The amount of this cut off is controversial at the present time[23]. The standardized cut off of ADA values for the diagnosis of TBM have not been established and the values used in various studies ranged from >5.0 to >15.0 IU/L making the practical use of this assay more difficult[13,24]. Multicenter studies in different populations are needed to determine standard CSF ADA values[11].

Due to the difficulty of establishing the diagnosis of TBM using clinical, radiological (magnetic resonance imaging or computed tomography), cytological, biochemical and even microbiological approaches, additional tests have been developed. The use of ADA as a diagnostic marker is increasing because it is simple and affordable[5]. Currently, TBM and its early diagnosis is a global issue and is becoming more and more crucial. All relevant studies share the view that ADA is a useful test in early differential diagnosis of TBM[11].

In conclusion, our results suggests that CSF ADA levels are elevated in TBM patients as compared to non-TBM (viral meningitis) patients and thus estimating CSF ADA is a useful differential diagnostic marker in these cases and can help the clinician make an early diagnosis of TBM. It is a simple, rapid and an inexpensive diagnostic tool that can be easily made available and performed with minimal training especially useful in resource–limited settings.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

The authors thank the Director, Bir Hospital (National Academy of Medical Sciences) for providing the infrastructure and support during the period of the study. The authors also heartily appreciate the help received from the technical personnel of the Sankata Pathology Laboratory, New Road, Kathmandu, in carrying out the laboratory investigations.

Comments

Background

Tuberculous meningitis (TBM) is an endemic disease in developing countries. Multidrug resistance in tuberculosis and acquired immuno-deficiency syndrome (AIDS) further worsen the outcome of this disease. Delay in diagnosis and in the start of effective treatment results in poor prognosis and sequelae. Available methods of diagnosis of TBM were evaluated and all of them were found to have low sensitivity and specificity. Adenosine deaminase (ADA) is an enzyme in the purine pathway and an elevated ADA level in body fluids have been considered by several researchers to differentiate tuberculosis disease from non-tuberculosis disease.
Research frontier

A reliable and rapid test which can be performed in any standard laboratory, could be of immense help in the diagnosis of TBM. Any test which facilitates a correct and simplified diagnosis of TBM should be very valuable. Estimating CSF ADA levels in TBM cases and its role in differentiation from non-TBM cases will provide one such alternative to us. Several studies are being carried out to establish its usefulness in different geographical areas.

Related reports

Several studies had shown that ADA levels were significantly elevated in TBM group as compared to non-TBM cases. The results of this study compare favorably with that reported by Gautam et al (2007) and Belagavi and Shalini (2011).

Innovations & breakthroughs

Reports and investigations about role of ADA levels in TBM are limited. This study has shown that CSF ADA levels are elevated in TBM cases as compared to non-TBM viral meningitis cases with a good sensitivity and a high specificity.

Applications

This test is simple to perform and is also inexpensive that should be included in the diagnostic work-up of TBM cases on a routine basis. This is critically important and would greatly help in the rapid and early diagnosis of TBM in clinical practice.

Peer review

This is an excellent and a very significant study designed to investigate the diagnostic role of CSF ADA levels in TBM cases and to differentiate it from non-TBM viral meningitis cases. A simple, rapid and inexpensive test is critically important and an urgency in clinical practice especially in developing countries to diagnose a case of TBM. The results of this study, confirms that CSF ADA levels are significantly elevated in TBM cases as compared to viral meningitis cases with a good sensitivity and a high specificity.

References


Clinico-diagnostic and prognostic markers of pulmonary tuberculosis – A case control study

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Abstract

Introduction: India continues to have the highest number of tuberculosis (TB) cases in the world and around 2 million cases are reported in India every year. Present study was undertaken to assess the usefulness of serum adenosine deaminase (ADA) and C-reactive protein (CRP) as diagnostic and prognostic marker of pulmonary tuberculosis.

Materials and methods: Present study comprises of 72 subjects of which, serum ADA was estimated in 36 confirmed pulmonary TB patients and 36 control group. All 36 patients were treated with anti tubercular therapy for six months and again the serum ADA was estimated. Along with that body mass index (BMI), erythrocyte sedimentation rate (ESR), peripheral blood leukocyte and lymphocyte counts were measured.

Results: In our study serum ADA levels at the beginning of the treatment was 47.83 ± 12.71 IU/L and in controls was 23.60 ± 5.19 IU/L. The levels of serum ADA were significantly higher (p < 0.001) in the pulmonary tuberculosis patients than in controls. C-reactive protein was significantly increased (p < 0.001) in pulmonary TB cases as compared to controls. The mean serum ADA levels after completion of treatment was 28.22 ± 6.04 IU/L. The difference in serum ADA levels between before treatment and after treatment was statistically significant (p < 0.001). There is also a significant difference in the BMI, CRP, ESR levels and peripheral leukocyte and lymphocyte count.

Conclusion: The estimation of serum ADA can be used as noninvasive and rapid diagnostic and prognostic marker of pulmonary tuberculosis. Estimation of CRP helps as an adjuvant supportive diagnostic tool in pulmonary tuberculosis.

Keywords: Serum adenosine deaminase; C-reactive protein; pulmonary tuberculosis.
cause pulmonary tuberculosis

Early and accurate diagnosis is the prior critical step in controlling TB. Any person with productive cough more than two weeks, evening rise of temperature and weight loss should be evaluated for TB. Despite the presence of standard diagnostic methods, diagnosis of TB is still problematic. Although the diagnostic keys of pulmonary TB are good old methods like based on clinical signs and symptoms, chest radiograph findings, AFB in sputum smears and culture and isolation of bacilli in sputum sample. Even though chest radiograph is done routinely in all institutions it is not the specific for pulmonary TB [4]. The sputum smear is positive only if bacilli should be more than 10,000/ mL of sputum, the sensitivity of sputum smear is only 40-70%. Culture and isolation of bacilli is a more sensitive method among all other methods but it may take up to four to eight weeks, delay is unacceptable in emergency situations [5]. However, molecular or nucleic acid amplification test (NAAT) and polymerase chain reaction (PCR) is a rapid diagnostic methods for pulmonary TB but it is not cost-effective and only few centers use it [6]. Detection of smear positive cases is the highest priority in TB control programme, as these cases are infectious and contribute substantially to the transmission of disease.

Thus it is necessary to find faster methods with higher sensitivity, different methods are tried like biochemical tests, genetic and serodiagnostic tests for rapid and accurate diagnosis of tuberculosis. Among such biochemical tests measurement of serum adenosine deaminase (ADA) activity can be used for rapid and early diagnosis and prognosis of tuberculosis.

The increase in serum ADA levels and its value for early diagnosis has been shown in many studies [7-9], where cell mediated immunity is stimulated. Many studies have confirmed the high sensitivity and specificity of ADA for early diagnosis of extra pulmonary TB such as tuberculous pleuritis, pericarditis and meningitis. The studies also showed decrease of serum ADA activity after completion of treatment [10]. C-reactive protein (CRP) a positive acute phase protein. Levels of this protein increased in pulmonary tuberculosis patients and significantly decrease after treatment.

Hence the present study was undertaken to compare serum ADA and CRP levels between the normal healthy controls and pulmonary tuberculosis patients and also to compare the serum ADA and CRP levels in pulmonary tuberculosis patients before and after treatment.

Material and methods

Study was conducted in the department of Biochemistry at Navodaya medical college and Hospital research centre, Revised National Tuberculosis and Control Programme a Directly Observed treatment-Short course Chemotherapy centre, Raichur, Karnataka, India in the year 2010. Present study comprises of 72 subjects out of which 36 healthy controls and 36 pulmonary tuberculosis patients. Informed consent was taken from all the study participants. The study was approved by the institutional ethical committee.

History and clinical examination of all the participants was done. Clinically, microbiologically and radiographically confirmed new cases of pulmonary tuberculosis were included in the study. Healthy individuals as controls were selected from volunteers among blood donors and hospital staff. Patients suffering from extra pulmonary tuberculosis, enteric fever, leprosy, infectious mononucleosis, HIV, diabetes, hypertension, chronic diseases and other respiratory disorders are excluded from the present study. About 5 mL of venous blood was collected under aseptic precaution from cubital vein. 3 mL is used for biochemical parameters and 2mL with EDTA sample used for ESR and cell count. All the parameters are estimated on the same day.

Serum ADA, CRP, ESR, peripheral blood leukocyte and lymphocyte count was carried out for all participants. Serum was separated by centrifugation and the serum ADA estimated within 2-4 hours. Serum ADA was estimated by Giusti and Galanti method of enzymatic analysis [11] (E) using ADA-MTB kit from Microexpress a division of Tulip diagnostics (P) Ltd. Optical density (OD) measured at 570-630 nm in spectrophotometer, intensity of blue color directly proportional to the amount of ADA present in the given sample and expressed as IU/dL. C-reactive protein (CRP) by Turbidimetric immunoassay [12] from Erba.

All 36 patients came for follow up study, who had taken antitubercular therapy (ATT) for six month according to RNTCP, DOTS regimen and again
serum ADA, CRP, ESR and peripheral blood leukocyte and lymphocyte count was estimated. 

**Statistical analysis:** All the results were expressed as mean ± SD and student’s t-test (paired) applied for quantitative data using OPEN EPI software. The p value (p < 0.001) was considered as statistical significance.

**Results**

The demographic characters are shown in table-1. Serum ADA, CRP, ESR, leukocyte and lymphocyte count significantly increased in cases than controls where as BMI was significantly decreased in cases than controls table-2.

Table-3 shows the mean values of different parameters before and after treatment. In cases there was a significant decrease in the serum ADA activity after the completion of antitubercular therapy (p < 0.0001) as compared to before treatment. There is decrease in CRP, ESR and leukocyte count and a slight increase in BMI and lymphocyte count.

It was observed that there was a difference in the serum ADA levels between after treatment (six month) therapy and control group (p=0.01).

<table>
<thead>
<tr>
<th>Characters</th>
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<th>P</th>
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<td>No of subjects</td>
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<td></td>
<td></td>
<td></td>
</tr>
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<td>36</td>
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<td>Female</td>
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<td>Female</td>
<td>13</td>
<td>07</td>
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**Table No 1. Table showing the demographic characters of controls and cases**

**Table No 2. Comparison of biochemical parameters in controls and cases using unpaired 't' test**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Controls</th>
<th>Cases</th>
<th>t</th>
<th>P</th>
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</tr>
</thead>
<tbody>
<tr>
<td>BMI in Kg/m²</td>
<td>21.7 ± 2.3</td>
<td>17.7 ± 1.8</td>
<td>8.49</td>
<td>0.0001</td>
<td>HS</td>
</tr>
<tr>
<td>ADA in IU/L</td>
<td>23.6 ± 5.19</td>
<td>47.8 ± 12.71</td>
<td>10.58</td>
<td>0.0001</td>
<td>HS</td>
</tr>
<tr>
<td>CRP in mg/L</td>
<td>8.56 ± 1.51</td>
<td>72.97 ± 11.4</td>
<td>33.5</td>
<td>0.0001</td>
<td>HS</td>
</tr>
<tr>
<td>ESR after 1hr</td>
<td>14.3 ± 4.54</td>
<td>59.5 ± 9.78</td>
<td>25.13</td>
<td>0.0001</td>
<td>HS</td>
</tr>
<tr>
<td>Leucocytes /mm³</td>
<td>8544 ± 1239</td>
<td>11034 ± 1619</td>
<td>7.32</td>
<td>0.0001</td>
<td>HS</td>
</tr>
<tr>
<td>Lymphocytes /mm³</td>
<td>1696 ± 310</td>
<td>1718 ± 266</td>
<td>10.96</td>
<td>0.001</td>
<td>S</td>
</tr>
</tbody>
</table>

**HS = Highly sensitive   S = Sensitive**
Table No 3. Change in biochemical parameters after ATT in cases using paired 't' test

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before treatment</th>
<th>After treatment</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI in Kg/m²</td>
<td>17.7 ± 1.8</td>
<td>19.58 ± 2.32</td>
<td>5.16</td>
<td>0.0001</td>
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<td>ADA in IU/L</td>
<td>47.83 ± 12.71</td>
<td>28.22 ± 6.04</td>
<td>11.86</td>
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<tr>
<td>CRP in mg/L</td>
<td>72.97 ± 11.4</td>
<td>9.79 ± 2.84</td>
<td>32.49</td>
<td>0.0001</td>
</tr>
<tr>
<td>ESR after 1 hr</td>
<td>59.5 ± 9.78</td>
<td>22.69 ± 6.38</td>
<td>20.02</td>
<td>0.0001</td>
</tr>
<tr>
<td>Leucocytes/mm³</td>
<td>11034 ± 1619</td>
<td>8171 ± 1033</td>
<td>14.31</td>
<td>0.0001</td>
</tr>
<tr>
<td>Lymphocytes/mm³</td>
<td>1718 ± 266</td>
<td>1813 ± 331</td>
<td>6.81</td>
<td>0.001</td>
</tr>
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</table>

HS = Highly sensitive    S = Sensitive

Discussion

In present study there was significant increase (p < 0.001) in serum ADA levels in pulmonary TB cases as compared to controls. The findings are in accordance with the study of Mishra OP [13], Kuyaku N [14] and Meena verma [15]. Adenosine deaminase an enzyme of purine catabolism belongs to hydrolase group (EC: 3.5.4.4 - Adenosine aminohydrolase). It is involved in the maturation, propagation and differentiation of lymphocytes mainly T-lymphocytes, hence high concentration of ADA activity is found in lymphocytes. The serum ADA level increases in diseases result from the intracellular microorganisms as well. ADA is produced after alveolar macrophages are infected by M. tuberculosis and is determined in serum during the active pulmonary TB. Lymphocytes, particularly T-lymphocytes have significant roles in the control of tuberculosis infection, in which lymphocytes turnover increases [16].

ADA is an enzyme that increases in TB because of stimulation of T-cell lymphocytes by mycobacterial antigen. It is a sensitive marker of cell mediated delayed immune response. As it is well known that increase in the serum ADA levels in disease is as a result of lymphocytes and macrophages turnover [17].

According to Mishra OP [13] evaluated serum ADA levels in children with confirmed tuberculosis (pulmonary, peritoneal, meningeal and bone) and healthy controls showing significant increase in serum ADA in tuberculosis compared to controls (p < 0.001). Kuyucu [14] estimated ADA level in the serum of children with tuberculosis was significantly higher than that of healthy children and concluded that serum ADA activity is a useful diagnostic tool in childhood pulmonary tuberculosis and in the tuberculosis diagnosis the cut-off value of serum ADA level was declared as 53.76 IU/L.

The association between TB and malnutrition has been recognized for long time. The malnutrition may predispose to TB and in turn TB also causes malnutrition [18]. The nutritional indicator BMI is significantly deficit in patients. In the present study BMI was significantly lower in cases than compared to controls (p < 0.0001).

Serum acute phase reactants CRP and ESR significantly increase (p < 0.001) in patients with pulmonary TB than compared to healthy controls. Sukesh R [19] assessed the serum CRP in 100 pulmonary tuberculosis patients and reported that serum CRP levels were significantly higher in smear positive group compared with smear negative group.
The total leukocyte counts were significantly higher in cases compared to controls (p < 0.0001) these are in favor of other studies Zafer Kartaloglu [20] whereas total lymphocyte count in cases and controls not that much differ.

In our study all 36 pulmonary TB cases were followed after completion of six month of antitubercular therapy. There was a significant decrease (p < 0.0001) in the serum ADA levels. Our findings are in accordance with the study of Collazos [21], Altas [10] and Zafer kartaloglu [20]. Collazos [21] performed a prospective follow up study of 25 cases of pulmonary and/or pleural tuberculosis with a normal immune response for a period of six months after initiation of treatment. There was a significant decline in the serum ADA values during the first two months in the patients as a whole followed by stabilization of the serum ADA activity. Perhaps this decrease might reflect the normalization of the altered lymphocyte turnover induced by tuberculosis. This indicates the response to the therapy. Altas [10] emphasized the importance of serum ADA levels in diagnosis and follow up of pulmonary tuberculosis and monitoring the efficiency of therapy. Zafer Kartaloglu [20] studied 35 patients with smear positive pulmonary tuberculosis. The levels of serum ADA were significantly higher in patients than in controls. They have observed serum ADA levels in tuberculosis patients showed a slight elevation in the first month but decreased during treatment in parallel with the effectiveness.

Other parameters like BMI increases slightly with the improvement of general health after the completion of the treatment. The incidence of pulmonary TB decreased with increasing BMI. Studies reported a slight decreasing mortality of TB seen with increasing BMI [22]. Acute phase reactants like CRP, ESR decreased significantly (p<0.0001) after the completion of the treatment. Our findings are in statement with that of Immanuel C [23] and Bajaj G [24]. Fall in these levels correlated with clinical response to therapy. They concluded that CRP can serve as a sensitive indicator of activity of the disease. In 1966, Lotfali H [25] evaluated CRP in pulmonary tuberculosis patients and observed the increased CRP in pulmonary TB patients and concluded that it is a proper test for evaluation and prognosis of pulmonary TB. CRP returns to normal faster than an elevated erythrocyte sedimentation rate (ESR).

The peripheral smear abnormalities reverted to normal with antitubercular treatment [26]. There is significant decrease (p < 0.001) in total leukocyte count and total lymphocyte count significantly increased after the completion of treatment.

Limitations of the present study were the small sample size and the follow up of all the patients was not possible. To trace the transfer TB cases from one centre to another health centre. Further studies are required with large sample size so that the serum ADA cut-off value can be determined for this ethnic group.

Conclusion

Assessment of serum ADA levels help in the early diagnosis of pulmonary tuberculosis. Estimation of serum ADA activity is a simple, rapid, non-invasive and relatively less expensive method and particularly helps in the diagnosis of smear negative AFB cases. So it should find a place in routine laboratory investigation. Estimation of serum ADA before and after treatment helps to evaluate the prognosis and response to therapy and also aids in objective assessment of the efficiency of chemotherapy used in the treatment.

References


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Conflict of Interest : None Declared
Evaluation of Serum adenosine deaminase activity during the course of pulmonary tuberculosis treatment


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Abstract

Tuberculosis is an ancient human scourge that continues to be an important public health problem worldwide. The risk of increasing spread of tuberculosis and development of drug resistance make early diagnosis a matter of utmost concern. The significance of serum ADA levels in the diagnosis of tuberculosis is known. However, there is hardly any study literature that compares the serum ADA level during the treatment of the disease. The objective of the study was to evaluate the effectiveness of Serum ADA in prognosis of pulmonary tuberculosis at different durations of the therapy in relation with other diagnostic tests of pulmonary tuberculosis. Serum ADA was measured along with other diagnostic markers during the course of treatment. The serum ADA values in the study group was (mean ± SD) 41.48 ± 8.02 U/L and in controls (mean ± SD) of 17.60 ± 5.17 U/L. After the 1st month of treatment there was no significant change (p > 0.05) in serum ADA levels 39.60 ± 0.79 U/L, however significant difference was recorded after the second month of treatment in serum ADA level 29.66 ± 0.83 U/L and similarly at sixth month of treatment 22.12 ± 0.51 U/L difference in serum ADA was significantly high. The difference in serum ADA levels between the first month and second month & first month and sixth month were statistically significant. (p < 0.001, p < 0.001 respectively). The measurement of serum ADA could be of significant help to evaluate the response to the therapy, particularly during second and sixth month. This may have an association with lymphocytic activation.

Key words: Serum ADA, pulmonary Tuberculosis, prognostic markers

Introduction

India is the highest TB burden country accounting for one fifth of the global incidence (Global annual incidence estimate is 9.4 million cases out of which it is estimated that 1.98 million cases are from India) [1]. India is 17th among 22 High Burden Countries in terms of TB incidence rate. [2] In diagnosis of tuberculosis, microbiologic, genetic, immunologic and biochemical methods are used [3]. The measurement of adenosine deaminase (ADA) activity is one of the biochemical methods. ADA is an enzyme that converts adenosine to inosine, and deoxyadenosine to deoxyinosine in the pathway of purine catabolism, and by this way catalyses irreversible deamination [4]. ADA acts in proliferation and differentiation of lymphocyte and especially T lymphocyte [5]. However there is no study literature that compares the serum ADA level with tuberculosis categories or investigates the relation of the serum ADA levels with response to antituberculosis therapy. Therefore, this study was aimed to examine the level of serum ADA in patients undergoing anti tubercular treatment at different intervals.

Objectives:
To know the effectiveness of serum ADA in the prognosis of pulmonary tuberculosis at different durations of therapy in relation with known prognostic markers of pulmonary tuberculosis.

Material and Methods:
The Prospective study was carried out in Navodaya medical college hospital & research centre, Raichur between April 2009 & July 2010.

Locus of the study:
The study was conducted in the department of Biochemistry NMC, Raichur and Biochemistry section, Microbiology & Pathology sections of central clinical laboratory of
Navodaya Medical College Hospital and Research Centre, Raichur, Karnataka.

**Study Group**
A total of 44 clinically and laboratory diagnosed pulmonary tuberculosis patients, who have consented to participate in the study during treatment were selected. The study and data collection were carried out with the approval from institutional ethical committee and informed consent were taken from all the study subjects. Of 44 cases 32 were males (72.72%) and 12 were female (27.27%) cases, the mean age group was 42.96 ± 15.29.

**Control Group**
A total of fifteen normal healthy individuals (paramedical and medical staff of Navodaya medical college) aged between 20 years - 50 years [11 Males (73.3%) and 4 females (26.66%)] were selected as controls after getting their consent for participation.

**Inclusion and exclusion criteria followed in the present study are as follows:**

**Inclusion criteria for pulmonary TB**
Cases diagnosed as a “new case” of tuberculosis; Possessing at least two positive sputum smear test positive for Acid Fast Bacilli; Radiographic abnormalities consistent with pulmonary tuberculosis. Only those freshly diagnosed pulmonary tuberculosis cases, who were willing to participate during treatment were included in the study group.

**Exclusion criteria for pulmonary TB**
Patients with extra pulmonary TB and /or patients requiring surgical intervention. Chronic pulmonary TB (receiving at least two courses of anti-TB treatment for more than six months), and dropouts were excluded from the study group.

All selected cases under study group were analyzed for complete blood count, ESR, serum ADA and CRP and control group were analyzed for serum ADA levels. Patients were treated with standard anti tuberculosis treatment as per RNTCP (DOT) regimen under the supervision of I/C of pulmonary medicine dept. NMCH&RC.

Venous blood was drawn aseptically and collected in two sterile containers, one anticoagulated container and another plane container. The anticoagulated blood was used for total leukocyte count, lymphocyte count and ESR. Serum sample was collected from plane blood after centrifugation and used for ADA and CRP estimation. ESR by Westergren’s method [6], Quantitative determination of C-Reactive Protein (CRP) [7,8,9] by Turbidometric Immunoassay using ERBA Chem. 5 Plus semi auto analyzer. Leukocyte total count and differential count were measured in a semi automated cell counter ABX Micros 60.

ADA estimation was done by standard colorimetric method as described by Guisti G Galanti enzymatic analyses [10,11]. Adenosine deaminase activity was determined in serum by using ADA MTB diagnostic kit from Microxpress a division of Tulip diagnostics (P) Ltd.

**Statistical Analysis**
The results were expressed as mean ±SEM (Standard error of mean) taking 95% CI. Analysis of variance (ANOVA) followed by Bonferroni Multiple Comparison Test was used for statistical evaluation. P values less than 0.05 were considered as significance and \( p <0.01 \) as highly significant. Correlation matrix between normally distributed indices was determined using the Pearson correlation coefficient.

**Results**

**Control Group**
In this group the serum ADA activity level ranged from 12.43 to 22.73 U/L with a mean value (mean ± SD) of 17.60 ± 5.17 U/L.

**Study Group**
The serum ADA values in study group before treatment ranged between 33.46 U/L and 49.50 U/L with the mean ± SEM serum ADA activity of 40.22 ± 0.79 U/L. After first month of treatment values are 39.60± 0.79 U/L followed by second and sixth month with a mean of 29.66±0.83 U/L and 22.12±0.51 U/L respectively as shown in table-1.

After 1st month of treatment there was no significant \( (p>0.05) \) change in serum ADA 39.60±0.79U/L levels. However highly significant difference of 29.66 ± 0.83 \( (p<0.001) \) was recorded after the second month of treatment. Similarly at sixth month of treatment difference in serum ADA (22.12 ± 0.51) was significantly high \( (p<0.001) \). The difference in serum ADA levels between the first month and second month & first month and sixth month were statically significant.\( (p<0.001, p<0.001 \) respectively) as shown in Figure-1.

C-reactive protein values before treatment have shown decrease from 74.14 ± 3.86 to 48.40± 2.99 after first month, 21.24 ± 1.55 after second month and 6.32± 0.59 mg/l after sixth month. Similarly ESR values have shown significant decrease from 73.16± 1.97 before treatment to 54.50±1.97 after first month, 36.55 ±1.65 after second month and 14.38 ± 0.64 mm/hr after sixth month.

There was a significant decrease in erythrocyte sedimentation rates and C-reactive protein values after the 2nd and
Serum adenosine deaminase in pulmonary tuberculosis treatment

6th month of treatment corresponding with the serum ADA levels as shown in Figure 2.

Lymphocyte count showed 1331 ± 50.49 before treatment, 1625.5 ± 48.65 after first month, 1389.7 ± 44.7 after second month and 1719.7 ± 43.81 after sixth month of treatment. A positive correlation and trend between serum ADA and lymphocyte count was found after 1st, 2nd & 6th month of treatment as depicted in Figure -3, 4 & 5.

Table 1 Serum ADA and CRP levels, ESR & peripheral blood leukocyte and lymphocyte counts at before treatment and at one, two and six months after treatment (Mean ± SEM)

<table>
<thead>
<tr>
<th>B.T.</th>
<th>1 M</th>
<th>2 M</th>
<th>6 M</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>s-ADA U/L</td>
<td>40.22 ± 0.79</td>
<td>39.60 ± 0.79**</td>
<td>29.66 ± 0.83</td>
<td>22.12 ± 0.51**</td>
</tr>
<tr>
<td>ESR mm/hr</td>
<td>73.16 ± 1.97**</td>
<td>54.50 ± 1.97**</td>
<td>36.55 ± 1.65</td>
<td>14.38 ± 0.64**</td>
</tr>
<tr>
<td>CRP Mg/L</td>
<td>74.14 ± 3.86**</td>
<td>48.40 ± 2.99**</td>
<td>21.24 ± 1.55</td>
<td>6.32 ± 0.59**</td>
</tr>
<tr>
<td>Leukocyte count mm</td>
<td>9322.2 ± 230.62</td>
<td>9116.6 ± 238.83*</td>
<td>8168.4 ± 245.77</td>
<td>7087.9 ± 239.29**</td>
</tr>
<tr>
<td>Lymphocyte count</td>
<td>1331 ± 50.49**</td>
<td>1625.5 ± 48.65**</td>
<td>1389.7 ± 44.37</td>
<td>1719.7 ± 43.81</td>
</tr>
</tbody>
</table>

* p<0.05, **p<0.01, Bonferroni Multiple Comparisons Test. If the value of t is greater than 2.419 then the P value is less than 0.05.

SEM: Standard error of mean

\[ \text{Figure-1} \text{ The Course of serum ADA levels} \]

\[ \text{Figure 2. Trends of Serum ADA, ESR and CRP during the treatment of Pulmonary tuberculosis} \]

\[ \text{Figure 3. Correlation between s-ADA U/L and Lymphocyte count after 1st month} \]

Pearson correlation of s-ADA U/L and Lymphocyte count = 0.895 P-Value = 0.000
Lymphocyte count
s-ADA U/L
2000 1800 1600 1400 1200 1000
45  40  35  30  25  20

Correlation between S-ADA/L and Lymphocyte count at 2 month of treatment
Figure 4. Correlation between s-ADA U/L and Lymphocyte count after 2nd month
Pearson correlation of s-ADA U/L and Lymphocyte count = 0.832 P-Value = 0.000

Lymphocyte count
s-ADA U/L
2400 2200 2000 1800 1600 1400 1200
30.0 27.5 25.0 22.5 20.0

Correlation between S-ADA/L and Lymphocyte count at 6 month of treatment
Figure 5. Correlation between s-ADA U/L and Lymphocyte count after 6th month
Pearson correlation of s-ADA U/L and Lymphocyte count = 0.856 P-Value = 0.000

Discussion
The values of serum adenosine deaminase were significantly higher in the study group than in the control group. After the first month of treatment of pulmonary tuberculosis, there was no significant change (p > 0.05) in serum ADA when compared to initial reading, but after the second month & sixth month of treatment there was a significant decrease in the serum ADA values (p < 0.05). However serum CRP and ESR values also decreased immediately after the first month of treatment (Figure-2).

In our previous work on serum ADA activity in pulmonary tuberculosis patients, we found that serum ADA was elevated in 88% of study group compared to control group followed by chest x-ray (76%) and sputum AFB (63%). And also significantly high serum ADA was noted in pulmonary tuberculosis cases compared to non tuberculosis pulmonary diseases. [11]

The highest level of serum adenosine deaminase in patients with pulmonary tuberculosis corresponds to the severity of the disease, high bacterial isolation, the extent of destructive and infiltrative changes in lung tissue, and endotoxemia. This indicates that ADA measurement is an additional criterion for assessing the health status in pulmonary tuberculosis patients and the magnitude of destructive processes. Moreover, the measurement is of prognostic value as it provides an objective assessment of the course of an acute progressive process and the efficiency of chemotherapy used [12].

A study by Zafer Kartaloglu et al [13] showed a slight elevation of serum ADA in the first month, but it decreased during treatment in parallel with the effectiveness. A study by Meftun Unsal et al [14] revealed gradual decrease in serum ADA from 30.1 ± 11.7 U/L to 24.8 ± 15.6 after first month of treatment & after second month 22.0 ± 10.6 in limited lesion cases. And in extended lesion cases before treatment ADA values was 31.3 ± 18.3, first and second month values were 27.5 ± 13.0 and 27.1 ± 12.2 U/L respectively.

Suzuki K et al [15] reported that the serum acute phase reactants decreased to normal levels within three to five weeks after negative results for tubercle bacilli were obtained in the sputum cultures. In addition, hematological abnormalities were observed in patients, such as normocytic normochromic anemia, leukopenia, neutropenia, and lymphocytopenia. These abnormalities reverted to normal with antituberculosis treatment [16]. Studies on lymphocyte subgroups reported that especially CD4+ T cells showed an increase after the beginning of treatment [17, 18]. Callazos et al [19] was observed that a decrease in both the leukocyte and the lymphocyte counts paralleled the decrease in s-ADA levels after the first month of treatment. In our study the lymphocyte count and s-ADA levels showed a parallel course for two months. After the second month the lymphocyte count increased, while s-ADA levels continued to decrease. The levels of s-ADA showed no significant change (p>0.05) in the first month of the treatment and decreased after the second month. Perhaps this pattern is related to lymphocytic activation and/or a complex cytokine network regulating the lymphocytes. It is known that the course of the ADA level is associated not only with lymphocytes, but also some chemical mediators secreted by these cells [21]. The disease is under control after the second month, and the lymphocytes and cytokines normalize. Although lymphocyte numbers increased at the sixth month, s-ADA levels decreased. It could be thought that the patients who had lymphopenia in the beginning showed increase in lymphocyte count after the treatment and influences the change in s-ADA level. The lymphocyte counts and serum ADA levels demonstrated a parallel course in the
first two months and then they had different course. CRP & ESR reduced quickly after first month of treatment. The levels of serum ADA showed decrease after first month of treatment. This decrease in s-ADA after the first month might reflect the normalization of the altered lymphocyte turnover induced by tuberculosis. Therefore measurement of serum ADA could be of significant help to evaluate the response to therapy, particularly in those patients with increased value of the enzyme.

References

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ADENOSINE DEAMINASE (ADA) ANALYSIS AND ITS DIAGNOSTIC ROLE IN TUBERCULOUS PLEURAL EFFUSION

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Sinamangal and Duwakot, Kathmandu, Nepal
*Author for Correspondence

ABSTRACT
Background: Accurate and early diagnosis of tuberculous pleurisy is essential for its correct treatment and management in clinical practice. Pleural fluid ADA estimation had been proposed as a useful diagnostic biomarker in tuberculous pleurisy. Objective: The study was designed to investigate the diagnostic role of ADA in tuberculous pleurisy in adult Nepalese population. Methods: One hundred and twenty pleural fluid specimens were consecutively selected and divided into two groups: Group I - tuberculous exudates (n=70) and Group II - transudates (n=50), the control cases based on the standard diagnostic criteria. ADA was estimated in pleural fluid in both the groups. Results: The mean ± SD in the tuberculous pleurisy patient group was 78.83 ± 30.85 U/L and in the transudates, it was 21.59 ± 9.46 U/L (highly significant, P < 0.001). Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) in the tuberculous pleurisy patients were 100% (CI 94.80 - 100.00), 96.00% (CI 86.64 - 98.90), 97.22% (CI 90.42-99.23) and 100% (CI 92.58-100.00), respectively. Conclusion: The pleural fluid ADA levels were significantly higher in tuberculous pleural effusions as compared with transudates cases. Our findings thus, support the view that pleural fluid ADA estimation is a very useful biomarker in establishing an accurate and early diagnosis in the tuberculous pleurisy patients. In addition, it is a simple, rapid, an inexpensive procedure that could be a useful complimentary test in the diagnostic work-up of tuberculous pleural effusions in clinical practice.

Key Words: Adenosine Deaminase, Diagnostic Role, Tuberculous Pleural Effusion, Sensitivity and Specificity

INTRODUCTION
Definitive diagnosis of tuberculous pleural effusion (TPE) is critically essential for its early detection, management and an early institution of specific anti-tubercular therapy. Between 3% and 25% of patients with tuberculosis will have tuberculous pleuritis (Light, 2010). Conventional diagnostic methods had proved their utility for the diagnosis of pulmonary tuberculosis (TB) but have limited application in case of TPE due to its paucibacillary nature. Pleural fluid cultures are positive for Mycobacterium tuberculosis in less than 40% and smears are virtually always negative1. A pleural biopsy has been considered the gold standard in diagnosis of TPE but it is invasive (Ahmed et al., 2011) and pleural fluid sampling is more difficult than simple thoracocentesis. The diagnosis cannot be established in 10-20% of the patients with these methods even in the best conditions (Yildiz et al., 2011). Pleural fluid markers of TPE have been extensively evaluated as an attractive alternative to pleural biopsy (Garcia-Zamalloa and Taboada-Gomez, 2012; Krenke and Korczynski, 2010). ELISA and polymerase chain reaction (PCR) are expensive diagnostic tests (Choudhury and Patel, 2010). Variety of new biomarkers have been proposed, viz. interferon gamma (INF-γ), interleukin (IL)-12p40, IL-18, tumour necrosis factor alpha (TNF-α), soluble IL-2 receptors (Sil-2R) and pro-calcitonin (Ambade et al., 2011; Hiraki et al., 2003 and Malekmohammad et al., 2012). Trace elements analysis (such as copper, zinc, magnesium, manganese, selenium and iron) of urine and hair in tuberculous pleurisy have also been investigated and may provide an additional disease correlate for assessing tuberculous pleurisy risk (Liu et al., 2011). Other molecules such as pleural fluid levels of neopterin - a marker of Th1 immune activation, serum leptin - which cross regulates nutritional status and the immune system, lysozyme and products of complement activation such as C3a-desArg and
Adenosine deaminase (ADA) (EC.3.5.4.4) is an enzyme involved in the breakdown of adenosine to urea and ADA levels were found to be elevated in the pleural fluid of patients with TPE way back in 1978 by Piras et al., (1978). Since then, several studies had been carried out that have shown the usefulness of ADA estimation in pleural fluid for the rapid diagnosis of TPE (Ocana et al., 1983; Gupta et al., 1990; Burgess et al., 1995; Kaisemann et al., 2004; Verma et al., 2008; Gupta, 2010 and Haque, 2012). At present, ADA is the most cost-effective pleural fluid biomarker and is routinely employed as a screening tool, in particular in countries where TB is endemic (Yildiz et al., 2011 and Porcel, 2009). Limited studies had been reported from Nepal that had investigated ADA role in the diagnosis of TPE (Lamsal et al., 2007). The aim of this investigation was to assess the significance of ADA activity in pleural fluid for the diagnosis of TPE in Nepalese population.

MATERIALS AND METHODS

Setting

This prospective study was carried out on patients admitted in the medical ward of a centrally located tertiary care hospital in Kathmandu, Nepal from January 2009 to December 2010. The ethical review committee of the hospital permitted to carry out this study and informed consent was taken from the patients before inclusion in the study. Their results were dispatched immediately after the tests were performed, so that the patients get appropriate treatment.

Patients

120 consecutive cases of patients admitted in the medical ward of the hospital on account of pleural effusion were selected and a final diagnosis was made based on standard criteria. These were divided into two different patient groups. Group I - tuberculous exudates -70 cases - based on presence of acid-fast bacilli in pleural fluid or biopsy tissue and radiological findings consistent with TB, clinical presentation consistent with TB with exclusion of other clinical conditions, definite clinical and radiological improvement in two months of administration of anti-tubercular treatment. Group II - transudates - 50 cases - congestive heart failure (38 cases), end-stage liver disease (5 cases), nephritic syndrome (5 cases) and hypoproteinemia (2 cases) and were diagnosed by standard clinical and diagnostic procedures.

Laboratory Tests

Pleural tap was done in all the cases and blood samples were also taken at the same time. A battery of laboratory investigations was done i.e. pH, glucose, protein, lactate dehydrogenase (LDH), Ziehl - Neelsen staining and total ADA. Light’s criteria (Light et al., 1972) - plural fluid protein serum protein > 0.5; fluid LDH / serum LDH > 0.6 was used to ensure exudative pleural effusions in tuberculous cases.

ADA Estimation

ADA estimation was carried out by spectrophotometry method based on the principle of Guisti and Galanti method of enzymatic analysis (Guisti and Galanti, 1984). ADA MTB diagnostic kit from Microexpress - a division of Tulip Diagnostics Pvt. Ltd., India was used according to the manufacturer’s instructions. Briefly, ADA hydrolyzes adenosine to ammonia and inosine. The ammonia formed further reacts with phenol and hypochlorite in an alkaline medium to form a blue iodophenol complex with sodium nitroprusside acting as a catalyst. Intensity of the blue coloured iodophenol complex formed is directly proportional to the amount of ADA present in the specimen, and is measured with the aid of a colorimeter at a wavelength of 623 nm.

Adenosine + H2O $\xrightarrow{\text{ADA}}$ Ammonia + Inosine
Ammonia + Phenol + Hypochlorite $\xrightarrow{\text{Alkaline medium}}$ Blue iodophenol complex
Control for each specimen was performed alongside with the test specimen. The reading was taken by technicians who were blinded as to the origin of the pleural fluid specimens (from which group of patients). The readings were converted to U/L in order to make the statistical calculations. Reference values of ADA levels above 40 U/L in pleural fluid were taken as positive for calculating the diagnostic parameters of sensitivity and specificity.

Statistical Analysis
The results were expressed as mean ± SD. Statistical comparison was carried out by using the Student’s t test. A two-tailed P value of < 0.05 was taken as statistically significant. Diagnostic test 2 x 2 contingency tables were made. Sensitivity, specificity, positive and negative predictive value were calculated. All parameters were estimated with 95% confidence interval using the Stata 10.1 statistical software package (Stata Corp. College Station, Tx).

RESULTS
Table 1 shows age and sex ratio of the different population groups that were investigated in this study. It comprised a total of 120 consecutive pleural effusions in - patients in the age group of 17 to 80 years. TPE group included 70 cases with age in years, (Mean ± SD) of 39.50 ± 17.63 and male to female ratio of 4:1. The control group comprised a total of 50 cases with age in years, (Mean ± SD) of 45.40 ± 17.57 and a male to female ratio of 2.12:1.

Table 2 depicts pleural fluid ADA levels (Mean ± SD) in TPE cases as 78.83 ± 30.85 U/L and in transudates cases, it was 21.59 ± 9.46 (highly significant, P < 0.001)

Table 3 shows the different diagnostic parameters in TPE cases with a sensitivity, specificity, PPV and NPV of 100% (CI 94.80 - 100.00), 96.00% (CI 86.64 - 98.90), 97.22% (CI 90.42-99.23) and 100% (CI 92.58-100.00), respectively. None of the pleural fluid specimens in TPE cases showed an ADA level of < 40 U/L and only two pleural fluid specimens showed an ADA value of > 40 U/L in the transudates cases.

Table 1: Age and Sex ratio of the different study population groups

<table>
<thead>
<tr>
<th>Study Group</th>
<th>No. of patients</th>
<th>Age ( years)</th>
<th>Sex Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPE</td>
<td>70</td>
<td>39.50 ±17.63</td>
<td>4 : 1</td>
</tr>
<tr>
<td>Non-TPE (transudates)</td>
<td>50</td>
<td>45.40±17.57</td>
<td>2.12 : 1</td>
</tr>
</tbody>
</table>

Table 2: CSF ADA levels in different study populations groups

<table>
<thead>
<tr>
<th>Study Group</th>
<th>No. of patients</th>
<th>Mean ± S.D (U/L)</th>
<th>P value comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPE</td>
<td>70</td>
<td>78.83±30.85</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>Non-TPE (transudates)</td>
<td>35</td>
<td>21.59± 9.46</td>
<td></td>
</tr>
</tbody>
</table>

Table 3: Validity of CSF ADA as a diagnostic test in suspected cases of tuberculous pleurisy cases

<table>
<thead>
<tr>
<th>Study Group</th>
<th>Sensitivity % (CI )</th>
<th>Specificity % (CI )</th>
<th>PPV % (CI )</th>
<th>NPV % (CI )</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPE</td>
<td>100.00 (94.80 - 100.00)</td>
<td>96.00 (86.64-98.90 )</td>
<td>97.22 (90.42-99.23)</td>
<td>100.00 (92.58-100.00)</td>
</tr>
</tbody>
</table>

PPV= positive predictive value, NPV= negative predictive value, CI = 95% confidence interval

DISCUSSION
Since conventional diagnostic methods have their limitations in detecting TPE, several alternative diagnostic approaches have been extensively evaluated (Udwadia and Sen, 2010). However only ADA
Research Article

and IFN-γ have become reliable for diagnosing TPE. The long history of the successful use of the ADA test, its simplicity, low cost and quickly available results, makes it the preferred option 4. ADA is an essential enzyme in the metabolism of purine nucleosides (Garcia-Zamalloa and Taboada-Gomez, 2012). ADA acts in proliferation and differentiation of lymphocyte, especially T lymphocyte. It is a significant indicator of active cellular immunity. Furthermore, it has been proposed to be a useful surrogate marker for TB because it can be detected in body fluid, such as pleural, pericardial and peritoneal fluid (Dinnes et al., 2007). The levels of ADA increase in TB because of the stimulation of T cells by mycobacterial antigens (Boonyagars and Kiertiburanakul, 2010). Apart from TB, the main disease that causes an elevated ADA is parapneumonic pleural effusion, one-third of cases of UPE and two-thirds of those of CPE/empyema may have a high ADA level but both conditions are easily distinguished from TPE because they develop neutrophilic effusions (Krenke and Korczynski, 2010; Porcel, 2009). ADA has been found to be a reliable marker of TPE in HIV-positive patients, even in those with a low CD4 - cell count (Baba et al., 2008).

This research was carried out on a total of 120 cases of pleural effusions, in which 70 were of TPE and 50 were the transudates, the control group. The ADA level (Mean ± SD) in TPE cases was 78.83 ± 30.85 while in the transudates it was 21.59 ± 9.46 (highly significant, P < 0.001). With a cut off value for ADA of 40 U/L, the specificity and sensitivity for diagnosing TPE was 100% (CI 94.80 - 100) and 96% (CI 86.64 - 98.90) with PPV and NPV of 92.22% (CI 90.42 - 99.23) and 100% (CI 92.58 - 100) respectively in this study. Almost all research workers have shown sensitivity and specificity of 90% to 100% for the value of ADA in pleural fluid using different cut off levels (Mathur et al., 2006). The most widely accepted cut off level of ADA for the diagnosis of TPE is 40 U/L (Garcia-Zamalloa and Taboada-Gomez, 2012; Liang et al., 2008). However, Kaur et al., (1992) showed a poor diagnostic value of ADA in pleural, peritoneal and cerebrospinal fluid in tuberculosis. One limitation of this study is that all the cases in the TPE group were not confirmed by the pleural biopsy diagnostic method, which is considered to be the gold standard method for its confirmatory detection. This could have introduced some bias in the selection of this patient group. Though other standard diagnostic procedures were scrupulously followed up to confirm these cases were of tuberculous pleural effusions.

Krenke et al., (2008) studied 94 patients (28 cases of TPE and 66 cases of non-TPE group). The ADA activity was significantly higher in TPE than in non - TPE (614.1 ± 324.5 vs. 15.1 ± 36.0 pg/ml, P < 0.0001). The diagnostic sensitivity and specificity were 100% and 93.9% at the cut off value of 40.3 U/L. Patel and Choudhury (2011) analyzed 53 patients with TPE and 96.67% had pleural fluid ADA > 40 IU/L. Kalantri et al., (2011) assessed 204 cases - 50 were confirmed pleural TB, 104 were probable pleural TB and 50 formed the non - TB group. For confirmed and probable pleural TB cases, ADA showed a sensitivity and specificity of 92% and 73%, respectively. Agarwal (2012) investigated 30 cases of TPE and showed sensitivity, specificity, PPV and NPV of 92%, 80%, 95.8% and 66.66%, respectively. Elevated levels of ADA in TPE have been noted by several authors. These observations were reproduced and further confirmed in this study. This research clearly showed that ADA levels are significantly elevated in TPE cases as compared to transudates cases. The results showed a sensitivity of 100% and a specificity of 96% for the diagnosis of TPE with PPV and NPV of 97.22% and 100%, respectively. Its cost-effectiveness, rapidity (just 2 hours), ease of performance and a high diagnostic value makes it a useful complimentary test for the diagnosis of TPE. Further, the results validates that a simple test like ADA estimation should be included routinely in the diagnostic work - up for TPE in clinical practice.

ACKNOWLEDGEMENT

The authors sincerely thank the Director, Bir Hospital (National Academy of Medical Sciences), Kathmandu for his permission and full support in carrying out this investigation. We also gratefully appreciate the help of the technical personnel of Sankata Pathology Laboratory, New Road and Kathmandu in performing the laboratory investigations.
REFERENCES


Research Article


ABSTRACT

Serum adenosine deaminase enzyme and Serum ferroxidase and albumin levels were determined in 50 newly diagnosed patients of tuberculosis, as Case. Fifty age and sex matched healthy individuals were taken as controls. Mean ± SD of serum adenosine deaminase and serum ferroxidase in controls and case was found to be 22.90±3.87U/L, 873.82 ± 117.44 IU/L, and 56.73±14.43 U/L, 1708.74±283.57 IU/L, respectively. Serum adenosine deaminase enzyme and Serum ferroxidase in case was significantly higher as compared to controls (p<0.001). The decreased levels of serum albumin in case, as compared to control was statistically significant (p<0.001). Serum ferroxidase: albumin ratio (Ferroxidase in International Unit per gram of albumin) in case (60.05±14.46 IU/g) was significantly higher than controls (25.02±4.5 IU/g), (p < 0.001) Serum adenosine deaminase enzyme good marker for diagnosis of TB but when it combine with Serum ferroxidase: albumin ratio (IU/g) it can therefore be employed as a surrogate marker to lend a hand in diagnosis and prognosis of pulmonary tuberculosis.

Keywords: Serum adenosine deaminase enzyme (ADA), Albumin, Ferroxidase, Diagnosis, Tuberculosis.

INTRODUCTION

TB is a major disease burden in India which causing a very high morbidity and mortality, leading to 3 million death annually in India. Mycobacterium tuberculosis bacteria have infected 9.27 million people in year 2007, globally and about 2 million incident cases of TB were in India (1, 2).

In addition, difficulty in diagnose to TB early is one of the main barrier in limit the spread and early treatment of this disease. A positive AFB smear and /or culture of Mycobacterium tuberculosis is gold standard for diagnosis but it is time consuming.(3) Usually diagnosis is based on clinical presentation, radiologic finding and positive tuberculin and BCG tests. Under such circumstances Antitubercular therapy therapy is started empirically .It therefore becomes vital to find some rapid and useful tests for the early and accurate diagnosis of tuberculosis. Various workers have tried different biochemical tests from time to time, which may help confirm the diagnosis of pulmonary tuberculosis.

Adenosine deaminase (ADA), an enzyme essential for the propagation and the differentiation of lymphocytes, particularly T-cells, so that estimation of its activity has been used for observation numerous diseases of altered immunity (4). The serum activity of ADA altered in diseases that cause a cell-mediated immune reaction such as lung cancer, tuberculosis, rheumatoid arthritis and systemic lupus erythematosus. (5, 6).

ADA is an enzyme that catalyses the hydrolytic and irreversible deamination of adenosine to inosine and 2'-deoxyadenosine to 2'-deoxyinosine (7). This enzyme is mainly
distributed in the lymphoid tissues. It has been reflected on as a marker of T-cell activation (8). Serum ceruloplasmin is a blue α-2; copper transporting globulin synthesized in liver microsomes and possesses ferroxidase activity (9). It also contains an antioxidant property in serum by oxidizing ferrous iron which could otherwise act as a catalyst in generating toxic free radicals (10).

Increases in the level of serum ceruloplasmin were observed in new cases of pulmonary TB either sputum positive or negative which come down with antitubercular treatment in due course of time and shows that level of serum ceruloplasmin are related with the activity of the disease process (11). Albumin, a major plasma protein has been reported low in pulmonary TB (12).

This study aims to find an evaluation of serum Adenosine deaminase enzyme, serum ferroxidase, albumin and ferroxidase: albumin ratio in the diagnosis of pulmonary TB and a comparison of these two tests serum ADA and ferroxidase: albumin ratio which one is better in diagnosis of Tuberculosis.

MATERIALS AND METHODS
The present study was conducted in Department of Biochemistry, Geetanjali Medical College, Udaipur, in collaboration with Department of TB and chest, Geetanjali Medical College, Udaipur between March 2010 and January 2011. Fifty cases of pulmonary TB were taken for the present study whose were freshly diagnosed, sputum positive. Fifty age and sex matched healthy subjects without any history of pulmonary TB were also included in the study as controls. In all cases serum adiponectin deaminase, serum ferroxidase & serum albumin levels were studied. Permission was taken from the Institutional Ethics Committee. Informed consent has been taken in English or local language if applicable.

Inclusion criteria for pulmonary TB
Cases diagnosed as a “new case” of tuberculosis; Possessing at least two positive sputum smear test positive for Acid Fast Bacilli; Radiographic abnormalities consistent with pulmonary tuberculosis, A decision by physician to treat with a full course of anti-TB Chemotherapy, non tubercular pulmonary diseases: a decision by physician.

Exclusion criteria for pulmonary TB
Patients with extra pulmonary TB and/or patients requiring surgical intervention, chronic pulmonary TB (receiving at least two courses of anti-TB treatment for more than six months), Presence of secondary immunodeficiency states: HIV, organ transplantation, diabetes mellitus, treatment with corticosteroids.

A detailed clinical history was taken and thorough physical examination was carried out in every subject. A set of investigations including three consecutive (spot-early mornings-spot) sputum samples examination, Mantoux test, Postero-anterior chest x-ray and ESR by Westergren method were carried out on study group. Serum ADA estimation was done in healthy controls, pulmonary tuberculosis patients.

Principle
Adenosine deaminase hydrolyses adenosine to ammonia & inosine. The ammonia formed further reacts with a phenol as hypochloride in an alkaline medium to from a blue color 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.

\[
\text{Adenosine} + \text{H}_2\text{O} \rightarrow \text{Ammonia} + \text{Inosine}
\]

\[
\text{Ammonia} + \text{Phenol} + \text{Hypochloride} \rightarrow \text{Blue indophenol complex}
\]

This method of serum ADA estimation is based on the principle of Guisti G Galanti methods of enzymatic analyses 1974 (13). For the determination of Adenosine deaminase activity
in serum, plasma, and biological fluids, ADA MTB diagnostic kit from Micropex press a division of Tulip diagnostics (P) Ltd has been used. Serum albumin was estimated by Autopak kit from Roche, USA on “HITACHI 902” Clinical Chemistry analyzer. Serum ferroxidase was estimated by a Kinetic method of Somani & Ambade (14) on Spectrophotometer. Statistical analysis was carried out using the software program “SPSS”.

RESULTS

Control Group

Fifty normal individuals aged between 20 years - 55 years [32 Males (64.0%) and 18 females (36.0%)] were included as controls. In this group the serum ADA activity level ranged from 13.4 to 29.3 U/L with a mean value of 22.90±3.87 U/L as mentioned in Table 1. Serum ferroxidase levels in controls ranged from 690 IU/L to 1180 IU/L with a mean of 870± 117.44 IU/L units and the serum albumin were ranged from 3.0 g/dl to 4.7 g/dl with a mean of 3.5± 0.33.g/dl. The calculated ratio of ferroxidase and albumin were ranged from 17.89 IU/g to 34.70 IU/g with a mean of 25.02± 4.5 IU/g.

Case Group

Fifty TB patients aged between 19 years - 62 years [35 Males (70.0%) and 15 females (30.0%)] were included as case. Values of serum ADA were varying from 40.3U/L to 97.65 U/L and the mean ± SD were 56.73± 14.43 U/L. The mean ± SD of ferroxidase (IU)/Alb (g) ratio in case was 60.05 ±14.46 IU/L and albumin were ranged from 2.91 g/dl to 4.7 g/dl with a mean 2.91± 0.41 g/dl.

Table 1 Serum adenosine deaminase, ferroxidase (FOD), albumin (Alb) and ferroxidase: albumin ratio in controls and case

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CONTROLS</th>
<th>CASES</th>
<th>P VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADA (U/L)</td>
<td>22.90±3.87</td>
<td>56.73±14.43</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Ferroxidase (IU/L)</td>
<td>873.82±117.44</td>
<td>1708.74±283.57</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>3.53±0.33</td>
<td>2.91±0.41</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>FOD/Alb (IU)/(g)</td>
<td>25.02±4.5</td>
<td>60.05±14.46</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Values are mean ± SD;

Three groups of patients according to age 0-25 yrs, 26-50 yrs and ≥ 51 yrs was formed, as shown in Table 2. The diagnosis of pulmonary tuberculosis was based on ESR, Sputum AFB, Serum ADA, Mantoux test and chest x-ray.

Table 2: Showing different parameters with positive findings in the diagnosis of Pulmonary Tuberculosis

<table>
<thead>
<tr>
<th>AGE GROUP</th>
<th>ESR</th>
<th>AFB</th>
<th>ADA</th>
<th>FERROXIDASE</th>
<th>ALBUMIN</th>
<th>F/A RATIO</th>
<th>MT</th>
<th>X-RAY CHEST</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-25Yrs (n=11)</td>
<td>04(H)</td>
<td>10</td>
<td>11(H)</td>
<td>11(H)</td>
<td>10 (L)</td>
<td>11(H)</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>26-50Yrs (n=33)</td>
<td>21(H)</td>
<td>26</td>
<td>33(H)</td>
<td>33(H)</td>
<td>30 (L)</td>
<td>33(H)</td>
<td>29</td>
<td>28</td>
</tr>
<tr>
<td>≥51Yrs (n=6)</td>
<td>2(H )</td>
<td>3</td>
<td>6(H )</td>
<td>6(H )</td>
<td>5 (L)</td>
<td>6(H)</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>TOTAL=50</td>
<td>27</td>
<td>39</td>
<td>50</td>
<td>50</td>
<td>45</td>
<td>50</td>
<td>40</td>
<td>34</td>
</tr>
</tbody>
</table>
DISCUSSION

We evaluated 50 patients, the most prevalent complaint among the 50 subjects was cough as it was found in all (50) patients, then expectoration was found in 33(66%) patients, dyspnea in 15 (30%) patients, Hemoptysis in 9 (18%) patients, chest pain in 5(10%) patients, fever in patients 40 (80%) and toxemic manifestations in 13 (26%) patients.

Although mycobacterial culture is sensitive and standard for diagnosing tuberculosis, the time for diagnosis requires a minimum of 2-3 weeks, whereas a negative result is available after 8 to 12 weeks (15). Acid fast bacilli smear, the rapid screening method for the diagnosis of pulmonary tuberculosis, is insensitive for detecting mycobacteria among tuberculosis patients (16). The management of pulmonary TB becomes difficult due to this long incubation period. Patient are getting a delayed diagnosis or an unwanted administration of antituberculosis drugs if decisions taken by the clinician without culture results (15).

Recently Some molecular and accurate tests are available like in vitro nucleic acid direct amplification tests (DATs) and Polymerase Chain Reactions (17,18). Inspite of appearance of these a positive AFB smear and/ or culture of mycobacterium is still remain the “gold standard” for diagnosis and follow up (15,16). To overcome these difficulties, we necessitate different biochemical parameters to assist in early diagnosis of pulmonary TB.

Mishra et al. assessed serum ADA levels of 51 children with confirmed tuberculosis and 20 healthy controls showing significant increase in the first group with a p-value of <0.001 (19).

Khalid et al. assessed the role of adenosine deaminase level in serum and Broncho Alveolar Lavage (BAL) in the diagnosis of pulmonary tuberculosis. They found that patients with pulmonary tuberculosis had significantly higher ADA level in serum and BALF than patients with non- tuberculosis lung diseases as cancer, pneumonia and normal persons (P< 0.001) (20)

Saeed et. al. evaluated the level of serum adenosine deaminase in association of active pulmonary tuberculosis and other infectious diseases of lung. Mean serum adenosine deaminase level in pulmonary tuberculosis (42.4±21.5 IU/ml) and other infectious diseases (38.3±23.4 IU/ml) was significantly more than controls (26.6± 8.2 IU/ml), (P<0.0001 and p<0.03 respectively), but the difference between the pulmonary tuberculosis and other infectious diseases was not statistically significant, (21)

Our study, the mean ± SD of serum adenosine deaminase levels in controls and case, were found to be 22.90±3.87U/L and 56.73±14.43U/L, respectively. This showed that the serum adenosine deaminase increases significantly (p<0.0001) by about 100% as compared to controls This is in accord with the studies of Mishra et al. (19),Khalid et al (20) Saeed et. al. (21) who reported increased levels of serum adenosine deaminase in pulmonary TB before treatment.

Motiani (1983) analyzed Serum ceruloplasmin activity in 80 patients of pulmonary tuberculosis and in 30 healthy individuals. Serum ceruloplasmin in sputum positive patients was more than double of that in controls. Significant increase also occurred in patients with tubercular toxemia even though sputum negative. (22) Singhvi & Maitra (1977), found the levels of serum ceruloplasmin in untreated patients of pulmonary tuberculosis to be increased. Levels were reduced considerably after 6 months of chemotherapy. (23)

In this study, the mean ± SD of serum ferroxidase levels in controls and case, were found to be 873.82±117.44 and 1708.74±283.57 IU/L, respectively. This showed that the serum ferroxidase increases significantly (p<0.0001) compared to controls. This is in agreement with the studies of Motiyani P.O. (22), Singhvi & Maitra (23) and Sudha Rao (24), who also state increased
levels of serum ceruloplasmin in new case of pulmonary TB. Adebisi et.al. (25) and Batra et.al. (26) reported significantly decreased serum albumin levels in newly diagnosed pulmonary TB patients. Observation of above studies were in concord with our study, which showed the mean ± SD of serum albumin levels in control and case 3.53±0.33 and 2.91±0.41 g/dL, respectively indicating significant (p<0.0001) decrease in serum albumin levels in case as compared to controls.

Ferroxidase albumin ratio is very useful to assist in the diagnosis and therapy of pulmonary TB. The mean ± SD of ferroxidase (IU)/Alb (g) ratio in control was 25.02±4.5 while in case it was found to be 60.05±14.46. Highly significant increased ratio was found in case to the control levels (p<0.0001). The result of present study are in agreement with Batra et. al. study. (26)

Our study is unique in comparing serum ADA, ferroxidase and ferroxidase (IU)/Alb (g) ratio values in pulmonary tuberculosis, showing that serum ADA, ferroxidase and albumin estimation should be done routinely, particularly if the diagnosis of tuberculosis is in doubt. Serum ADA is better marker than ferroxidase in diagnosis of tuberculosis but ferroxidase (IU)/Alb (g) ratio can use adjunct marker in doubtful tuberculosis cases. Serum ADA, ferroxidase and albumin can be possibly included as an alternate marker to support in the diagnosis of pulmonary TB. Its utility in the prognosis of pulmonary tuberculosis could be evaluated with follow up studies.

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REFERENCES


Biochemical markers of tubercular ascites

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ABSTRACT

The diagnosis of abdominal tuberculosis requires a high index of suspicion due to its vague symptomatology. Early diagnosis of tubercular ascitis is crucial to prevent progression of disease to its advanced stages, thereby preventing the fatal complication like intestinal obstruction, fistulas and peritonitis. The objective of our research work is to evaluate the role of biochemical parameters such as Adenosine Deaminase (ADA), IgG, Lactate, Total protein and albumin, Glucose, Cholesterol and pH in diagnosis of abdominal tuberculosis. Ascitic fluid samples were taken from patients admitted in medicine wards of SSK Hospital after informed consent. A total of 100 patients meeting the selection criteria were enrolled in the study. The biochemical investigations performed for the ascitic fluid samples were Adenosine Deaminase (ADA), IgG against 38 kDa mycobacterial antigen, Lactate, Total protein, albumin, Glucose, Cholesterol and pH. In the 79 patients that had been followed up, with ATT response as a reference, a highly significant association was observed with ascitic fluid assays ADA, IgG, Serum Ascitic Albumin Gradient(SAAG), Cholesterol, Lactate and pH. Adenosine Deaminase (ADA) and IgG against 38 kDa mycobacterial antigen can be used as corroborative markers for diagnosis of extra pulmonary paucibacillary tubercular ascitis where conventional methods like smear microscopy and culture frequently fail to establish the diagnosis.

Keywords: Abdominal tuberculosis, Adenosine Deaminase, IgG against 38 kDa mycobacterial antigen.

*Corresponding author
INTRODUCTION

Abdominal tuberculosis can have a varied presentation, frequently mimicking other diseases. The diagnosis of abdominal tuberculosis requires a high index of suspicion due to its vague symptomatology [1]. The clinician must look for tuberculosis and confirm or exclude this treatable disease in any patient who present with symptoms related to gastrointestinal tract. Initial symptoms of abdominal tuberculosis like fever, pain, diarrhea, constipation, weight loss, anorexia and malaise are non-specific and non-alarming [2]. Thus the primary disease progresses to the advanced stages leading to complications like ascites, obstruction, fistulas and peritonitis. This increases the morbidity and worsens the prognosis. Ascites as a presenting complaint is seen in 21-30% of patients suffering from abdominal tuberculosis [3]. However, its multiple etiologies like cirrhosis, malignancies, cardiac and renal pathologies, present a diagnostic challenge to the clinicians. An early and accurate diagnosis is imperative to initiate early intervention and treatment. In the absence of any rapid and reliable method of diagnosis, most of the time, treatment is started on presumptive diagnosis.

Conventional diagnosis of tuberculosis employs the microscopic identification of Acid Fast Bacilli (AFB) in smears stained by Ziehl-Neelsen technique and culture in Lowenstein-Jensen medium. Culturing of bacilli has a specificity approaching 100% and is considered as the gold standard. It also permits susceptibility testing of isolates to various drugs [4, 5]. However diagnosis by these methods is difficult in paucibacillary samples like ascitic fluid besides the long period needed for growth in culture.

Hence there is a need for an early and reliable marker of diagnosis of abdominal tuberculosis.

The objective of our research work is to evaluate the role of biochemical parameters such as Adenosine Deaminase (ADA), Lactate, Total protein, albumin, Serum Ascitic Albumin Gradient (SAAG), Glucose, Cholesterol and pH in diagnosis of abdominal tuberculosis.

MATERIALS AND METHODS

The study was conducted jointly in the Departments of Biochemistry, Microbiology and Medicine, Lady Hardinge Medical College and Associated Hospitals, New Delhi, India after Institutional ethical clearance.

A total of 100 patients meeting the selection criteria were enrolled in the study. Ascitic fluid samples were taken from patients admitted in medicine wards of SSK Hospital after informed written consent.
Selection criteria-

Any patients presenting with unexplained ascites were selected for study initially. Ascitic fluid samples were collected under all aseptic conditions by abdominal paracentesis. The ascitic fluid samples were subjected to routine cytology (Inflammatory cell count like total leukocyte count, polymorphonuclear neutrophils and differential counts) and routine biochemical analysis for ascitic fluid (Total protein, albumin).

Of the above patients, all patients with exudative ascites (Total protein >2.5 gm/dl) were included in the study. Patients with cardiac and chronic liver disease were included in the study only if the ascitic fluid indicated an increased inflammatory cell count.

Diagnosed cases of cancer, which could present with ascites were excluded from the study.

The patients were subjected to detailed history and clinical examination routine and special investigations.

Venous blood samples were also collected under aseptic condition and are processed to get the desired serum. These serum samples were further subjected to routine biochemical investigations (Glucose, Liver function tests like total and direct bilirubin, Alanine aminotransferase, Aspartate aminotransferases, Alkaline phosphatase, Kidney function tests like urea, creatinine, uric acid, Electrolytes like sodium, potassium, calcium and phosphate, Total protein and albumin and lipid profile like cholesterol, triglyceride).

The biochemical investigations performed for the ascitic fluid samples were Adenosine Deaminase (ADA), Lactate, Total protein, albumin, Serum Ascitic Albumin Gradient (SAAG), Glucose, Cholesterol and pH. The pH of ascitic fluid was measured immediately by pH paper and then the ascitic fluid samples were stored at -20°C in aliquots for ELISA until batch analysis. Microbiological tests like smear microscopy by ZN staining for AFB and culture in LJ medium was also done.

Serological tests like IgG antibody against 38 kDa antigen of Mycobacterium tuberculosis complex was done.

Ascitic fluid analysis

1. Adenosine Deaminase (ADA)-

Principle - Adenosine Deaminase hydrolyses adenosine to ammonia and inosine. The ammonia formed further reacts with phenol and hypochlorite in an alkaline medium to form a blue
indophenols complex with sodium nitroprusside acting as a catalyst. Intensity of the blue
coloured indophenols complex formed is directly proportional to the amount of ADA present in
the sample.
The test was performed by using Microexpress ADA-MTB kit with the instructions provided by
the manufacturer.
Interpretation - Normal < 30 U/L, Positive > 30 U/L
Ascitic fluid lactate, total protein, albumin, glucose and cholesterol were measured using
Beckman CX 9 Autoanalyzer.

2. Lactate - Measured by lactate oxidase method.

Lactate reacts with oxygen in presence of the enzyme lactate oxidase to form pyruvate
and hydrogen peroxide. This hydrogen peroxide then reacts with p-aminophenazone and p-
chlorphenol in the presence of peroxidase to form a red colored chromogen. The increase in
absorption at 546 nm is proportional to the lactate concentration.
Lactate > 25 mg/dl indicates infected fluid.

3. Total protein – Measured by Biuret method.

Cupric ions, in alkaline medium, interact with protein peptide bonds resulting in
formation of a colored complex. The increase in absorbance is measured at 546nm.
Total protein >2.5 gm/dl indicates exudates and <2.5 gm/dl indicates transduate.


Albumin is positively charged at pH lower than its isoelectric point, and has affinity for
anionic dyes. BCG with pH range 3.8-5.4 binds to albumin. Reaction between albumin and dye
BCG produces a colour change from yellow to blue green changing absorption at 578nm which
is proportional to the albumin concentration. Yellow is the monovalent anion and blue is
divalent anion that changes the absorption reading with albumin.
Serum Ascitic Albumin Gradient (SAAG) >1.1 gm/dl indicates portal hypertension and SAAG <
1.1 gm/dl indicates non-portal hypertension.

5. Cholesterol - Determined after enzymatic hydrolysis and oxidation.

The indicator quinoneimine is formed from hydrogen peroxide and 4-aminoantipyrine in the
presence of phenol and peroxidase. Change in absorbance is measured at 500nm.
Ascitic fluid cholesterol is < 55 mg/dl in cirrhosis.

Glucose is determined after enzymatic reaction in the presence of glucose oxidase. The hydrogen peroxide formed reacts under catalysis of peroxidase, with phenol and 4-amino phenazone to red violet quinoneimine dye as indicator and absorbance is measured at 540 nm.

7. Quantitative assay of antibodies (IgG) against 38-kda antigen of *Mycobacterium tuberculosis* complex

Estimation of IgG antibodies was carried out using Pathozyme-Myco kit. Pathozyme-Myco kit is Enzyme Linked immunoassays for the detection of antibodies against *Mycobacterium* species.

**Principle:** A recombinant 38-kDa protein and a highly purified antigen derived from *Mycobacterium tuberculosis* are bound to the surface of microtitration wells. Test sera diluted 1/100 are applied. Specific antibodies to *Mycobacterium* species bind to the antigens in the wells. Unbound material is washed away and anti-human IgG antibody conjugated to Horseradish Peroxidase is applied. The conjugate binds to the human antibodies which are bound to the antigen. Unbound material is again washed away. On addition of the substrate, stabilized 3, 3', 5, 5', Tetamethyl Benzidine (TMB), a colour will develop, the intensity of which is determined by the amount of the antimycobacterial antibody in the sample. The enzyme reaction is stopped by the addition of dilute sulphuric acid and the absorbance is then measured at 450nm.

Interpretation: ≥ 400 U/ml considered as positive and < 400 U/ml as negative. The data were analyzed by using SPSS version 19 and p value < 0.05 was considered as significant.

**Observations and Results**

In our study, we observed the following ascitic fluid investigations results.

<table>
<thead>
<tr>
<th>Ascitic fluid Investigations (n=100)</th>
<th>Number of patients</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenosine Deaminase(ADA) (&gt;30 U/L)</td>
<td>83</td>
<td>83</td>
</tr>
<tr>
<td>IgG (&gt; 400 U/ml)</td>
<td>82</td>
<td>82</td>
</tr>
<tr>
<td>SAAG (&lt; 1.1 gm/dl)</td>
<td>29</td>
<td>29</td>
</tr>
<tr>
<td>Cholesterol (&gt;55 mg/dl)</td>
<td>61</td>
<td>61</td>
</tr>
<tr>
<td>Lactate (&lt;25 mg/dl)</td>
<td>62</td>
<td>62</td>
</tr>
<tr>
<td>pH (&gt;7)</td>
<td>73</td>
<td>73</td>
</tr>
</tbody>
</table>

A significant number of patients in our study had raised levels of ADA and IgG antibodies to 38 kDa tubercular antigen.

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Follow-up of patients

100 patients were enrolled in our study on the basis of clinical suspicion of tubercular ascites and biochemical investigation like total protein. All were put on therapeutic trial (category I / 2(HRZE)+ 4 HR as per Revised National tuberculosis Control Programme (RNTCP), India) of anti-tubercular therapy (ATT). Patients were followed up after 3 months to see the response to therapy. 21 patients were lost to follow up. 79 patients were left in the study to see the response to therapy. Out of these, only 35 patients responded to therapy.

### Comparative analysis of ascitic fluid parameters in ATT responders (n=35)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Positive</th>
<th>Percentage (%)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADA (&gt;30 U/L)</td>
<td>34</td>
<td>97.1</td>
<td>0.0001</td>
</tr>
<tr>
<td>IgG (&gt;400 U/ml)</td>
<td>32</td>
<td>91.4</td>
<td>0.001</td>
</tr>
<tr>
<td>SAAG (&lt;1.1 gm/dl)</td>
<td>14</td>
<td>40</td>
<td>0.007</td>
</tr>
<tr>
<td>Ascitic fluid / serum Glucose ratio (&lt;0.96)</td>
<td>22</td>
<td>62.9</td>
<td>0.0475</td>
</tr>
<tr>
<td>Cholesterol (&gt;55 mg/dl)</td>
<td>24</td>
<td>68.6</td>
<td>0.004</td>
</tr>
<tr>
<td>Lactate (&gt;25 mg/dl)</td>
<td>31</td>
<td>88.6</td>
<td>0.0001</td>
</tr>
<tr>
<td>pH (&gt;7)</td>
<td>22</td>
<td>62.9</td>
<td>0.0001</td>
</tr>
<tr>
<td>Smear microscopy (ZN stain)</td>
<td>1</td>
<td>2.9</td>
<td>0.870</td>
</tr>
<tr>
<td>Culture (LJ medium)</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

In the 79 patients that had been followed up, with ATT response as a reference, a highly significant association was observed with ascitic fluid assays ADA, IgG, SAAG, Cholesterol, Lactate and pH.

### DISCUSSION

In our study we found only one patient showed positivity for AFB smear while culture was negative. This correlates with work done by Singh et al\(^5\) in tubercular ascites in which smear positivity was less than 3%.

A cut off level of ascitic fluid pH < 7.35 was used to exclude other bacterial causes of ascitis [6]. As reported a significant association of SAAG (<1.1 gm/dl) with tubercular ascitis [7]. Cholesterol levels of ascitic fluid were analyzed to exclude out cirrhosis again showed a significant association with tubercular ascitis [7]. A value of ascitic fluid lactate >25 mg/dl indicates other bacterial etiology [6] and in our study 89.6 % of the patients had values < 25 mg/dl.

The ascitic fluid analysis of our study shows a highly significant association with ADA, IgG, lactate and pH. The levels of ADA observed in our studies are in accordance with earlier studies [8, 9]. A cut off level of 60 U/L increases the specificity and sensitivity to 90 % for tubercular infection\(^10\). A good concordance was also seen for the levels of IgG against 38kDa antigen with the studies, sensitivity and specificity of 81 % and 88% respectively with a
diagnostic accuracy of 84% [9]. The other parameters such as ascitic fluid pH, cholesterol and lactate levels showed a significant association in tubercular ascitis and they can be used as corroborative diagnostic aids [6, 7].

Thus, Adenosine Deaminase (ADA) and IgG against 38 kDa mycobacterial antigen can be used as corroborative markers for diagnosis of extra pulmonary paucibacillary tubercular ascitis where conventional methods like smear microscopy and culture often fail to establish the diagnosis. The significance of our study is that Adenosine Deaminase (ADA) and IgG against 38 kDa mycobacterial antigen can be used for early diagnosis as it is time saving and for early treatment as abdominal paracentesis is a simple safe procedure with immense diagnostic potential.

REFERENCES

Diagnostic role and estimation of adenosine deaminase in serosal effusions

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Abstract

Objective: The objective of the study was to estimate the minimum value of adenosine deaminase (ADA) in tubercular (TB) and non-tubercularserosal effusions.

Methods: The study was conducted on 336 subjects attending to OPD & IPD of TB chest & respiratory disease, Medicine and Orthopedics departments of Subharti Medical College and its associated hospital, Meerut, U.P. India. Out of these 155 subjects were tubercular (tubercular pleural effusion 45, peritoneal 34, synovial 34 and cerebrospinal fluid 42) and 181 were non tubercular (pleural 42, peritoneal 48, synovial 37 and cerebrospinal fluid 54). ADA levels of different serosal fluids were estimated by Microexpresskit, based on Guisti&Galantimethod. Data was analyzed by one way Anova.

Results: In our study, we found the following cut off values of ADA in different types of serosal effusions. In tubercular pleural effusion it was 65Unit/Litre (U/L), peritoneal 75U/L, synovial 42U/L and cerebrospinal fluid 13U/L respectively. In non-tubercular pleural effusion it was 6U/L, peritoneal 4U/L, synovial 13U/Land cerebrospinal fluid 2U/L respectively. Sensitivity & specificity of tubercular effusion were 100% and 0% respectively. While for non-tubercular pleural effusion it was 93% & 53%, for peritoneal 90% & 67%, for synovial & CSF it was 0%& 100% respectively.

Conclusion: ADA estimation will be helpful as an alternative diagnostic method for early detection of TB and to differentiate between pulmonary and extra pulmonary tuberculosis.

Keywords: ADA, Pleural effusion, Peritoneal effusion, Synovial effusion, TB

1. Introduction

Tuberculosis is one of the oldest and commonest infections in India affecting not only lungs but also extra pulmonary sites. Diagnosis of TB is confirmed by sputum examination of acid fast bacilli (AFB) and its culture which is positive in only one third of serosal fluid sample and has low sensitivity.¹-³ Other tests are X-ray and tuberculin test which can be negative and non-specific.⁴-⁵ Adenosine deaminase (ADA)is an enzyme (E.C. no 3.5.4.4)for the catabolism of purine bases, capable of catalyzing the deamination of adenosine forming inosine in the process.⁶ADA is encoded by 12 exon, 32 kb gene located on chromosome no 20q13.11. ADA catalyze the replacement of 6-amino group of adenosine (Ado) and 2’-
deoxyadenosine (dAdo) with oxygen producing inosine (Ino) and 2'-deoxyinosine (dIno). These ADA products as well as guanosine and 2'-deoxyguanosine, which are also 6-oxypurines, undergo purine nucleoside phosphorylase catalyzed phosphoryl cleavage to yield component base, hypoxanthine or guanine and ribose or 2'-deoxyribose-1-PO$_4$.

ADA is expressed at high levels in lymphoid cells. Its activity is greatest in cortical thymocytes and decreases with maturation of B-cells. In tissues like activated T-cells ADA is complex of >200 kD bound to cell membrane associated glycoprotein. Its principle biological activity is related to proliferation and differentiation of lymphocytes. The enzyme activity is greater in T-lymphocytes then in B-cells.$^7$-$^9$

Tubercular effusion is the result of a cell mediated immune response to the presence of Mycobacterium tuberculosis and is characterized by the accumulation of activated T-lymphocytes and macrophages. Cellular immune response and in particular activation of T-lymphocytes is reflected by the presence of ADA in pleural fluid. Thus ADA activity, a marker of T cell activation and cell mediated immune response can help differentiate tubercular etiology from non-tubercular. ADA was introduced in 1978 for diagnosing of tubercular effusions. It is simple and inexpensive colorimetric test. Studies have confirmed high sensitivity and specificity of ADA for early diagnosis of extra pulmonary TB.$^{10-19}$ As very few studies have considered all serosal effusions (pleural, peritoneal, synovial and cerebrospinal fluid), so we took this study to estimate cut off values of ADA in different serosal effusions due to tubercular and non-tubercular etiology.

2. Materials and Methods

The research protocol for the present study was approved by the ethical committee of our institution and informed consent was obtained from each subject prior to inclusion in the study. The study was conducted from 2009 to 2012. Subjects were selected from OPD & IPD of TB chest & respiratory disease, medicine and orthopedics department of Subharti Medical College hospital and its associated rural and urban health centre at Sarawani, Mahalwala, Khajoori and Multanagar coming under District Meerut of India. Only those, who were having serosal effusion due to tubercular or non-tubercular etiology, were selected. Total no of patients included in our study were 336, out of which 155 were tubercular and 181 were non tubercular. There were 186 males and 150 females, ranging from 15-65 years of age. Patients on anti-tubercular therapy were excluded from the study. After taking written consent from the patients, detailed clinical history was taken and investigation like Hb, TLC, DLC, GBP, CBC, AFB culture, sputum for AFB and X-ray were done. Cases of TB were diagnosed by – clinical presentation of TB, AFB staining of sputum and radiological findings. Five ml of serosal fluid was collected in plain vial. After centrifuging it, ADA was estimated in supernatant by colorimetric method using Microexpress readymade kit from Tulip Diagnostic India Pvt Ltd, based on Guisti & Galanti method.$^{20}$ Absorbance was read at 580 nm. Data was analyzed by one way Anova.

3. Results

Total no of patients in our study were 336, out of which 155 were tubercular (pleural 45, peritoneal 34, synovial 34 and CSF 42) and 181 were non tubercular (pleural 42, peritoneal 48, synovial 37 and CSF 54). Following were the results of our study – the range of ADA value in tubercular pleural effusion was 65-162U/L with mean ± standard deviation (SD) of 109.6±28.9, peritoneal was 75-176U/L with mean ± SD of 119.3 ± 36.4, synovial was 42-92U/L with mean ± SD of 56.6 ± 8.6 and CSF was 13-112U/L with mean ± SD of 32.8 ± 23.4 respectively (Table 1).

The range of ADA value in non-tubercular pleural effusion was 6-60U/L with mean ± SD of 37.3 ± 21.7, peritoneal was 4-38U/L with mean ± SD of 20.3 ± 14.8, synovial was 13-39U/L with mean ± SD of 20.5 ± 5.5 and CSF was 2-22 U/L with mean ± SD of 11.5 ± 7.2 respectively (Table I). From our observation, we found the following minimum (cut off) value of ADA in different types of effusion. In the tubercular pleural effusion it was 65U/L, peritoneal 75U/L, synovial 42U/L and CSF was 13U/L respectively. In the non-tubercular pleural effusion it was 6U/L, peritoneal 4U/L, synovial 13U/L and CSF it was 2U/L respectively. The minimum values of ADA in effusion due to tubercular etiology were higher as compared to non-tubercular ones.

Sensitivity and specificity of tubercular effusion (pleural, peritoneal, synovial & CSF) were 100 percent and 0 percent respectively. While for non-tubercular pleural effusion it was 93% and 53%, for peritoneal it was 90% and 67%, for synovial and CSF both, it was 0% and 100% respectively (Table II).
### Table I – Comparison of ADA values in Tubercular and Non Tubercular serosal effusions.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Pleural effusion</th>
<th>Peritoneal effusion</th>
<th>Synovial effusion</th>
<th>CSF</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tubercular</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range(U/L)</td>
<td>65-162</td>
<td>75-176</td>
<td>42-92</td>
<td>13-112</td>
</tr>
<tr>
<td>Cut off value</td>
<td>65</td>
<td>75</td>
<td>42</td>
<td>13</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>109.6 ± 28.9</td>
<td>119.3 ± 36.4</td>
<td>56.6 ± 8.6</td>
<td>32.8 ± 23.4</td>
</tr>
<tr>
<td><strong>Non tubercular</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range (U/L)</td>
<td>6-60</td>
<td>4-38</td>
<td>13-39</td>
<td>2-22</td>
</tr>
<tr>
<td>Cut off value</td>
<td>6</td>
<td>4</td>
<td>13</td>
<td>2</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>37.3 ± 21.7</td>
<td>20.3 ± 14.8</td>
<td>20.5 ± 5.5</td>
<td>11.5 ± 7.2</td>
</tr>
</tbody>
</table>

### Table II – Sensitivity and Specificity of Tubercular and Non Tubercular serosal effusions.

<table>
<thead>
<tr>
<th>Variables</th>
<th>No of true positive</th>
<th>sensitivity</th>
<th>specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pleural</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tubercular</td>
<td></td>
<td>45</td>
<td>100%</td>
</tr>
<tr>
<td>Non tubercular</td>
<td>39</td>
<td>*(TN 5, FN 3, FP 4)</td>
<td>93%</td>
</tr>
<tr>
<td><strong>Peritoneal</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tubercular</td>
<td></td>
<td>34</td>
<td>100%</td>
</tr>
<tr>
<td>Non tubercular</td>
<td>43</td>
<td>(TN 2, FN 5, FP 1)</td>
<td>90%</td>
</tr>
<tr>
<td><strong>Synovial</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tubercular</td>
<td></td>
<td>34</td>
<td>100%</td>
</tr>
<tr>
<td>Non tubercular</td>
<td>(FP 37)</td>
<td></td>
<td>0%</td>
</tr>
<tr>
<td><strong>CSF</strong></td>
<td></td>
<td>42</td>
<td>100%</td>
</tr>
<tr>
<td>Tubercular</td>
<td></td>
<td>(FP 54)</td>
<td></td>
</tr>
<tr>
<td>Non tubercular</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*TN – true negative, FN – false negative, FP – false positive

### 4. Discussion

TB is a common cause of serosal effusions. In our study the cut off values of ADA were in agreement with following other studies. Piras et al\textsuperscript{11} reported high ADA value in tubercular effusion (more than 40U/L). Ocaña et al\textsuperscript{12} in 1983 & Valdes et al\textsuperscript{13} in 1993 reported 100 percent sensitivity & specificity, positive predictive value and negative predictive value in larger sample size study. Meta-analysis of studies\textsuperscript{21} between 1966 & 1999 concluded that the test performance was reasonably good (sensitivity range 47.1-100 percent and specificity range 0-100 percent) in diagnosing etiology in pleural effusion. Voight et al\textsuperscript{22} studied 41 cases with bacteriologically confirmed tuberculosis and 41 cases with other cause and found that mean ADA level for tubercular etiology were 99.8U/L with sensitivity of 95 percent and specificity of 98 percent. Shrish et al\textsuperscript{23} reported value of ADA as 12.2±3.13U/L for TB meningitis group and it was significantly higher (P value > 0.001) than the partially treated pyogenic meningitis (5.39±2.7U/L) and aseptic meningitis (1.92±0.56U/L). Burgess et al\textsuperscript{24} showed ADA activity in tuberculous effusion to be higher than in any other diagnostic group. At a level of 50U/L the sensitivity and the specificity for the identification of tuberculosis was 98 and 89 percent respectively. Mathur P.C. et al\textsuperscript{25} found in their study that ADA level in the tubercular pleural effusion ranged from 45-160U/L with a mean level of 100U/L, while in non-tubercular group it ranged from 5-33U/L with the mean of 18U/L (P value < 0.001, highly significant). ADA level in tubercular ascites was 13-135U/L with a mean level of 92U/L while in the non-tubercular group it was 1-28U/L with a mean of 12U/L. (P value < 0.001, highly significant). Different researchers have found different minimum values of ADA in serosal effusions, so more studies are required to come to a final minimum value which can be set as standard for diagnosing tuberculosis in tubercular and non-tubercularserosal effusions.
5. Conclusion

The method of ADA estimation is easy, simple and does not require expensive equipments. It can be estimated by simple colorimeter. It is economical and less time consuming (takes only two hours). This test may find a place in routine investigation for early detection of TB in coming days and for differentiating tubercular from non-tubercular etiology in pulmonary and extra pulmonary TB.

References

Study of Adenosine Deaminase Levels in Patients of Pulmonary Tuberculosis with and Without Pleural Effusion

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Abstract: Tuberculosis (TB) is one of the oldest and commonest infectious diseases also known as “master of death” or “Captain of death”. Pulmonary TB (PTB) and TB with pleural effusion remains a diagnostic challenge. Adenosine deaminase (ADA) is an enzyme of purine catabolism which is an inexpensive and easy test in early routine evaluation of patients with pleural effusion and helps to avoid invasive test like biopsy. Therefore, this study was aimed to determine the exact role of ADA in TB patients with and without pleural effusion and in non-tuberculosis pleural effusion and to provide a clear picture of ADA for early diagnosis & management of TB.

Materials and method: Study comprised of 132 subjects of which 33 are healthy controls and 33 are confirmed cases of pulmonary TB without effusion, 33 are PTB with effusion and 33 are non-PTB effusion patients. Age group range was from 20-70 years. Estimation of serum and pleural fluid Adenosine deaminase by Guisti and Galanti method of enzymatic analysis.

Results: Serum ADA levels in pulmonary tuberculosis (55.09 ± 11.02) patients and pulmonary tuberculosis with pleural effusion (44.01 ± 7.82) were significantly higher (p <0.001) when compared with healthy controls (18.11 ± 6.13). Pleural fluid ADA levels were significantly higher (p <0.0001) in pulmonary tuberculosis with pleural effusion (82.61± 12.05) than in non tuberculosis pleural effusion (27.72±7.80).

In the present study, the mean pleural fluid ADA were significantly higher as compared to mean serum ADA in pulmonary tuberculosis with pleural effusion (p <0.0001) and in non tuberculosis pleural effusion (p <0.0008).

Conclusion: ADA level in serum as well as in pleural fluid in the diagnosis of pulmonary TB with or without pleural effusion is a very sensitive, specific, inexpensive, rapid, easily available & reliable investigation.

Keywords: Adenosine deaminase, pulmonary TB, pulmonary TB with pleural effusion, non-tuberculosis pleural effusion.

I. INTRODUCTION

TB is one of the oldest and commonest infectious diseases also known as “master of death” or “Captain of death” (1). It is still a global burning problem and now the world’s seventh leading cause of death (2). The severity of the disease can be judged by the fact that it affects all ages, irrespective of the sex. No other disease has so much socio-economic health significance as TB in a country like India. TB, a bacterial disease is chronic granulomatous infection, caused by Mycobacterium tuberculosis and occasionally by Mycobacterium africanum. TB is categorised as pulmonary tuberculosis and extra-pulmonary tuberculosis. The main symptoms of TB are chronic cough, low grade fever – evening rise of temperature, haemoptysis, chest pain, dyspnoea, loss of weight, unresolved pneumonia (3). Its complications being massive haemoptysis, cor pulmonale, fibrosis/emphysema, calcification, obstructive airway disease, bronchiectasis, bronchopleural fistula. Pleural TB is one of the most common extra-pulmonary manifestations of the disease and may represent up to 10% of all cases (4). PTB and TB with pleural effusion remains a diagnostic challenge. TB can be diagnosed by Mantoux, Acid fast bacilli (AFB) staining, sputum culture, X-ray chest and newer advances like polymerase chain reaction (PCR). Directly observed treatment (DOTS) has helped in curing tuberculosis to some extent but unless and until a proper diagnosis is made, TB will remain a major health problem. The above mentioned diagnostic methods are very useful for the diagnosis of TB but have a low yield. Direct analysis of pleural fluid...
for detection of acid-fast bacilli (AFB) by the Ziehl-Neelsen or similar method is positive in less than 5% of cases, and the culture on Lowenstein-Jensen medium takes more than four weeks and does not surpass a 40% positivity rate (5).

Adenosine deaminase (EC 3.5.4.4), called ADA by Spencer et al (6), is an enzyme of purine catabolism which catalyses the pathway from adenosine to inosine (7). Its distribution in the human organism is ubiquitous (8), but its physiologic role is especially important in lymphoid tissue. Its level is ten times higher in lymphocytes than in erythrocytes (9), and particularly in T-lymphocytes with variations according to cellular differentiation (10). Estimation of serum ADA activity is a simple, rapid, non-invasive and relatively less expensive method, so it should find a place in routine laboratory investigation (11, 12). It also helps in early diagnosis and treatment of the patient and prevents the spread of disease in the community (13).

Therefore, this study was planned to determine the exact role of ADA in TB patients with and without pleural effusion. The results of this study will help to provide a clear picture of ADA for diagnosis & prognosis of TB, & hence future plans for TB can be better executed.

II. AIM OF THE STUDY

To determine the mean values of serum ADA in the patients of PTB with and without pleural effusion and in non-tuberculosis pleural effusion

III. OBJECTIVES OF THE STUDY

1. To assess & compare the serum Adenosine deaminase (ADA) level in pulmonary TB, pulmonary TB with pleural effusion and non-tuberculosis pleural effusion patients and compare with the healthy controls.

2. To compare value of Adenosine deaminase in serum and pleural fluid in patients of pulmonary TB with pleural effusion and with those in patients with non TB pleural effusion

IV. MATERIALS AND METHODS

1. Study design: This is a prospective, non-randomised, single centric, non-interventional, open labelled study.

2. Study duration: It was carried out in a span of 1 year from June 2012 to May 2013.

3. Study population with sample size: Study comprised of 132 subjects of which 33 are clinically, radiologically and microscopically confirmed cases of pulmonary TB without effusion (group A), 33 are PTB with effusion (group B), 33 are non-TB effusion patients (group C) and 33 are healthy controls (group D). Age group range was from 20-70 years. The controls and patients voluntarily participated in the study. Informed consent was taken from controls and cases before collecting the blood and pleural fluid samples.

4. Study site: Patients coming to pulmonary medicine department were taken for the study and samples were analysed in central laboratory of biochemistry department of the J.J hospital, Mumbai.

5. Ethical committee approval - The study was approved by the ethical committee of the institute.

6. Inclusion criteria: Healthy controls.
   - Participants of either gender with age >18 years.
   - Microbiologically and radiologically confirmed cases of pulmonary TB, pulmonary TB with pleural effusion and nontubercular effusion
   - Participants willing to sign informed consent form.

7. Exclusion criteria:
   - Participants who were suffering from other chronic illness in which ADA levels are also affected like – Extra-pulmonary TB, Enteric fever, Viral hepatitis, Diabetes mellitus, Nephrotic syndrome, Leprosy, Infectious mononucleosis, HIV, Chronic malnutrition.
   - Pregnant and lactating women.
   - Participants on Drugs which affect ADA values like interferon alpha, deoxycoformycin, ribavirin and viramidine.
   - Participants not ready to give written consent.


9. Collection of blood sample: Under all aseptic precautions about 2 ml of venous blood was drawn. Serum was separated immediately by centrifugation (2500 rpm for 10 mins) and was kept in deep freezer (-70°C) till further assay and maximum 2 freezing and thawing were allowed.

10. Collection of pleural fluid: Under all aseptic precautions, tapping was done. The recommended location varied depending upon the source. The preferred site was the mid-axillary line, in the sixth, seventh, or eighth intercostals spaces. In case of little effusion procedure was performed under ultrasound guidance.
Study Of Adenosine Deaminase Levels In Patients Of Pulmonary Tuberculosis With And Without


12. Principle: Adenosine deaminase hydrolyses adenosine to ammonia and inosine. The ammonia 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 coloured indophenols complex formed is directly proportional to the amount of ADA present in the sample.(1)

   - Normal - < 30 U/L
   - Suspect – 30 - 40 U/L
   - Strong suspect - > 40 - 60 U/L
   - Positive - > 60 U/L

14. Linearity: The procedure was linear up to 150 U/L if values exceed, the sample was diluted with distilled water and the assay was repeated.

V. STATISTICAL ANALYSIS

Descriptive statistical analysis was carried out in the present study. Results on continuous measurements were presented on Mean ± SD and results on categorical measurements in Number. Unpaired t test was used to find the significance of study parameters on continuous scale between two groups (Inter group analysis), one way ANOVA test was applied to compare different parameters in between different groups along with post hoc Tuckey’s test to compare parameters within group. Diagnostic statistics viz. Sensitivity, Specificity, Positive predictive value and Negative predictive value was computed to find the correlation of ADA for diagnosis with tuberculosis patients. For statistical analysis the “Graphpad instat 3, San Diego, California” software was used. Microsoft word and excel have been used to generate tables and graphs.

VI. RESULTS AND OBSERVATIONS

Comparison of mean values of ADA (u/l) in serum in different groups:
TABLE 2.

Fig 1.
Serum ADA levels in group A (55.09 ± 11.02) patients were significantly higher (p <0.001) when compared with group D (18.11 ± 6.13). At the same time significant difference was found when compared with groups B and C (p <0.001).
Serum ADA levels in group B (44.01 ± 7.82) patients were significantly higher (p value <0.001) when compared with group D (18.11 ± 6.13). Also, significant difference was found when compared with group A and C (p < 0.001).
Serum ADA levels in group C (21.92 ± 5.33) patients were higher but not statistically significant (p >0.05) when compared with group D (18.11 ± 6.13).
The sensitivity, specificity, positive predictive value and negative predictive value of the serum ADA in pulmonary tuberculosis group by taking cut off value of 33.3 U/L came to be 96.69%, 96.69%, 96.69% and 96.69% respectively. Similarly, the sensitivity, specificity, positive predictive value and negative predictive value of the serum ADA in pulmonary tuberculosis with effusion group by taking the same cut off value of 33.3 U/L were 93.93%, 96.69%, 96.87% and 94.11%

Mean value of ADA (u/l) in pleural fluid in groups b &c:
TABLE 3.

Fig 2.
In the present study, the mean pleural fluid ADA (82.61 ±12.03) was higher as compared to mean serum ADA (44.01± 7.82) in group B. This was highly significant (p <0.0001).
Similarly, the mean pleural fluid ADA (27.72 ±7.80) was higher than mean serum ADA (21.92 ± 5.33) in group C and was highly significant p-value <0.0008.
But, the values of ADA in serum and pleural fluid in group B samples were higher as compared to group C samples

Comparison of mean serum ADA and pleural fluid ada within groups b and c:
TABLE 4.

Fig 3.
VII. Discussion

In this context, the exact role of ADA in TB patients with and without pleural effusion and its usefulness in diagnosing tuberculosis is tried to justify.

Adenosine deaminase is an enzyme in the purine salvage pathway required for converting adenosine to inosine. Its levels are ten times higher in lymphocytes than in erythrocytes (16) and particularly so in T-lymphocytes. The enzyme activity increases during mitogenic and antigenic responses of lymphocytes and T-lymphocyte blastogenesis can be inhibited by inhibitors of ADA (17,18). Likewise, a deficiency of ADA is associated with severe defects in the cell mediated and the humoral arms of the immune system, predisposing the patient to opportunistic infections like pulmonary tuberculosis.

In the present study, there was statistically significant increase (p<0.001) in mean serum ADA levels in pulmonary TB (55.09 ± 11.02), pulmonary TB with effusion cases (44.01 ± 7.82) as compared to healthy controls (18.11 ± 6.13) and non-TB pleural effusion patients (21.92 ± 5.33). These findings are in accordance with the studies made by -

Lakshmi et al, in 1992 found the average serum ADA values in 61 active pulmonary tuberculosis patients and 25 healthy controls. In their study, they stated that serum ADA level was more in TB patients than controls which helps for diagnosis (19). Saeed Amniasfhar et al, in 2004 evaluated 51 cases of active pulmonary tuberculosis (21 females and 30 males aged 47.7 ± 19 years), 50 (14 female and 36 male aged 48.4 ± 11 years) of healthy controls. Mean serum ADA level in pulmonary tuberculosis (42.4 ± 21.5 IU/L) and was significantly more than controls (26.6 ± 8.21 IU/L) (p< 0.0001) (20). Meena Verma et al, in 2004 studied 100 patients of which 53 were suffering from pulmonary tuberculosis and 35 normal healthy control subjects. The mean serum ADA activity in pulmonary TB patients was 35.5 ± 6.93 IU/L as 52 compared to 16.20 ± 2.85 IU/L in control group, showing highly significant (P<0.001) difference. ADA activity was highest in tuberculosis than compared to controls (21). Jhamaria JP et al, in 1988 estimated serum ADA level in 20 healthy controls, 102 cases of pulmonary tuberculosis, 20 cases of suppurative lung diseases (lung abscess and bronchiectasis) and 18 cases of lung malignancy. The mean serum ADA in healthy controls was 10.09 ± 2.99 IU/L, in pulmonary TB 42.47 ± 3.34 IU/L were observed. They concluded that ADA activity is highest in pulmonary tuberculosis patients than compared to controls (22). K.Srinivasa Rao et al in 2010 found that in diagnosis of pulmonary TB, serum ADA showed high percent positivity of 88% followed by chest X-ray 76%, ESR 72%, Sputum AFB 63%, Mantoux 61%. He also reported high serum ADA levels in pulmonary TB as compared to non tubercular pulmonary diseases and sputum AFB negative pulmonary tuberculosis cases showed elevated level of serum ADA at par with sputum AFB positive cases (23).

Significantly increased ADA activity (p<0.001) in the serum of pulmonary TB and tubercular pleural effusion patients compared to healthy controls and non-TB pleural effusion is due to activation of cell mediated immunity. In tuberculosis there are increased numbers of T-lymphocytes and macrophages in pleural fluid which may be associated with highly elevated ADA activity in such patients. The ADA activity is greater in lymphocytes and is related to differentiation of lymphocytes. In pathological conditions, the clearance capacity of lungs is decreased leading to increased numbers of cells in pleural fluid and the recirculation of activated T-cells may cause a high serum ADA activity in patients with pulmonary disease (24).

Also, in the present study serum ADA levels in non tubercular pleural effusion were higher as compared to healthy controls but not statistically significant (p>0.05).

In this study, findings seem to confirm that ADA activity is a useful parameter for the diagnosis of tuberculosis and tubercular effusion. The mean levels of pleural fluid ADA in tubercular pleural effusion (82.61 ± 12.03) were higher significantly (p<0.0001) as compared to pleural fluid ADA levels in non tubercular pleural effusion (27.72 ± 7.80). This is in accordance with the studies of Y.C.Gary Lee et al, who in 2001 studied 106 cases of lymphocytic pleural effusion origin of different etiologies and concluded saying that ADA levels in TB pleural fluid exceeds than that in other non tuberculosis lymphocytic pleural fluid (25). Morays Casagrande Kaisemann et al, in 2004 concluded that ADA determination in pleural fluid is a sensitive and specific method for diagnosis of pleural TB and its use can preclude need for pleural biopsy in initial workup of pleural effusion of patients (4). Bharat Kumar Gupta et al, in 2010 determined ADA activity in 96 pleural fluid samples comprising of tubercular and non tubercular pleural fluid samples and found that pleural fluid ADA levels were significantly higher in TB pleural fluid as compared with non TB pleural fluid (26).

So, pleural fluid ADA level is very helpful test to rule out a tubercular aetiology of pleural effusion. Cell-mediated response is the predominant form of immune response to tubercular infection, while both cell-mediated and humoral immune responses are elicited by most non-tubercular infections in human body. Thus ADA activity, a maker of T-cell activation and cell-mediated immune response can help differentiate tubercular aetiology from non-tubercular. Piras et al were first to report high ADA in tubercular pleural effusion (27).

Present study showed that levels of pleural fluid ADA were significantly higher than serum ADA levels in both tuberculous (p< 0.0001) and non-tuberculous (p<0.0008) pleural effusions, suggesting a localized intra-pleural production of ADA. This is in accordance with the study of S K Sharma et al, in 2001 who stated...
that using 100 IU/L as the cut-off, it is possible to avoid pleural biopsy in as much as 40% of patients as certain diagnosis of TB. And also, concluded that ADA estimation is a useful test for the diagnosis of TB with pleural effusion which is adequately sensitive and specific and at same time inexpensive and easy to perform (28).

Most of the authors suggest ADA assay as the routine screening test, a sensitive marker and an inexpensive test. The present study evaluates the usefulness of ADA with a cut-off value of 33.3 U/L in serum to diagnose pulmonary tuberculosis patients efficiently. Statistically analysed data shows significance of ADA in diagnosis of PTB without effusion with sensitivity, specificity of 96.69% and 96.69 % respectively, with positive predictive and negative predictive values of 96.69 % and 96.69 % respectively. The usefulness of ADA was evaluated with a cut-off value of 33.3 U/L in serum to diagnose pulmonary tuberculosis with pleural effusion patients. The sensitivity, specificity, positive predictive value and negative predictive value of 93.93%, 96.69%, 96.87% and 94.11% was found respectively. Thus, ADA estimation in serum of pulmonary tuberculosis without pleural effusion may be of more diagnostic importance than ADA estimation in serum of pulmonary tuberculosis with pleural effusion patients.

In the present study, a few cases with pulmonary tuberculosis showed elevated ADA activity in spite of sputum negative for tuberculosis. This suggests that ADA activity in pleural fluid samples from patients suffering from the same is a sensitive marker for the diagnosis and patients can be started on Empirical treatment while waiting for other test reports to be positive. This is in accordance with the study made by Mukesh Kumar Agarwal et al, in 1991. They studied serum ADA levels in 38 healthy controls, 36 cases of smear negative, culture positive patients of pulmonary TB, 34 cases of non-TB respiratory diseases. They found that mean serum ADA levels compared with healthy controls and non TB cases were higher in smear negative, culture positive patients of pulmonary TB (28).

Thus, from present study results, it is observed that serum & pleural fluid ADA measurement plays a very important role in diagnosis of PTB without pleural effusion & with effusion respectively but, current study has certain limitations like small sample size which may be because of the knowledge or attitude of the patients with their condition. Major limiting factor is about the cut off value of ADA which was taken in the study, as it may vary at different places but considering the values given on ADA kit different values were tried & decided for this value which may or may not be accurate but in given circumstances with the patients values this cut off value gave a significant result. Therefore, it is recommended that a large sample size study in which different groups of PTB patients with their different categories like new cases, defaulters, relapse or failure, MDR should be taken & ADA values should be assessed which will give the exact role of ADA not just only in diagnosis of PTB but also in the prognosis of the disease. Measurement of ADA values is definitely having a very important role in the diagnosis of PTB patients with and without pleural effusion if other factors which may also affect ADA levels are excluded thus giving a sensitive, specific, reliable, inexpensive, rapid, non invasive and easily available investigation in a disease like TB for which whole nation is deeply concerned.

VIII. INDENTATIONS AND EQUATIONS

\[
\text{Adenosine} + \ H_2\text{O} \xrightarrow{\text{ADA}} \text{Ammonia} + \text{Inosine} \\
\text{Ammonia} + \text{Phenol} + \text{Hypochlorite} \xrightarrow{\text{Alk medium}} \text{Blue Indophenol complex}
\]

IX. FIGURES AND TABLES

Fig 1 COMPARISON OF MEAN VALUES OF ADA (U/L) IN SERUM IN DIFFERENT GROUPS:

![Mean ADA values in serum in different groups](image)

Fig 2. MEAN VALUE OF ADA (U/L) IN PLEURAL FLUID IN GROUPS B & C:
Table 1: Categorisation of Study Population in Different Groups (N = 132).

<table>
<thead>
<tr>
<th>Study group</th>
<th>No. of patients</th>
<th>Nature of group</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>33</td>
<td>Pulmonary TB</td>
</tr>
<tr>
<td>B</td>
<td>33</td>
<td>Pulmonary TB with pleural effusion</td>
</tr>
<tr>
<td>C</td>
<td>33</td>
<td>Non TB with pleural effusion</td>
</tr>
<tr>
<td>D</td>
<td>33</td>
<td>Healthy controls</td>
</tr>
</tbody>
</table>

Table 2: Comparison of Mean Values of ADA (U/L) in Serum in Different Groups:

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>MEAN ADA ± SD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>55.09 ± 11.02</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>44.01 ± 7.82</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>21.92 ± 5.33</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>D</td>
<td>18.11 ± 6.13</td>
<td></td>
</tr>
</tbody>
</table>

Footnote – all values are expressed as mean ± SD.
For comparison of P value in all groups one way ANOVA test was applied.
*P <0.05 was considered to be significant.
TABLE 3: MEAN VALUE OF ADA (U/L) IN PLEURAL FLUID IN GROUPS B & C:

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>MEAN ADA ± SD</th>
<th>P-VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>82.61 ± 12.03</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>C</td>
<td>27.72 ± 7.80</td>
<td></td>
</tr>
</tbody>
</table>

Footnote: Unpaired t test, *p-value <0.0001, highly significant.

TABLE 4: COMPARISON OF MEAN SERUM ADA AND PLEURAL FLUID ADA WITHIN GROUPS B AND C:

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>PARAMETER</th>
<th>MEAN ADA ± SD</th>
<th>P – VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>ADA serum</td>
<td>44.01 ± 7.82</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>ADA pleural fluid</td>
<td>82.61 ± 12.03</td>
<td>&lt; 0.0001*</td>
</tr>
<tr>
<td>C</td>
<td>ADA serum</td>
<td>21.92 ± 5.33</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>ADA pleural fluid</td>
<td>27.72 ± 7.80</td>
<td>&lt; 0.0008*</td>
</tr>
</tbody>
</table>

Footnote: All values are expressed as mean ± SD *p value < 0.05 considered significant

X. CONCLUSION

Serum ADA was significantly higher in pulmonary TB patients and pulmonary TB with pleural effusion patients compared to healthy controls and non TB pleural effusion. Pleural fluid ADA was significantly higher in pulmonary TB with pleural effusion patients compared to non-TB with pleural effusion patients. Pleural fluid ADA was significantly higher than serum ADA levels in pulmonary TB with pleural effusion and in non-TB with pleural effusion patients.

Thus serum ADA and pleural fluid ADA plays a very important role as an investigation in the diagnosis of PTB with or without effusion. Serum and pleural fluid ADA level measurement in the diagnosis of pulmonary TB without pleural effusion and pulmonary TB with pleural effusion respectively is a very sensitive, specific, inexpensive, rapid, easily available & reliable investigation.

REFERENCES

Study Of Adenosine Deaminase Levels In Patients Of Pulmonary Tuberculosis With And Without


Diagnostic significance of ascites adenosine deaminase levels in suspected tuberculous peritonitis in adults

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ABSTRACT

Objective: There are contradictory reports about the use of adenosine deaminase (ADA) as a diagnostic marker in tuberculous peritonitis patients. Reports evaluating significance of ADA activity in the diagnosis of tuberculous peritonitis in adults are lacking in Nepal. We thus set out to investigate the ascitic fluid ADA levels in suspected tuberculous peritonitis patients and to determine the diagnostic significance of the test statistically.

Methods: This study population comprised of two different adult patients groups. Group I - 35 suspected cases of tuberculous peritonitis and Group II - 35 cases of transudative ascites - the control group (patients with biochemically proved transudates or hypoproteinaemia) and peritoneal tap was done. ADA estimation was carried out by spectrophotometry.

Results: ADA levels (Mean ± SD) in suspected tuberculous peritonitis and transudative ascites cases were 48.5±17.9 U/L and 19.8±7.7 U/L respectively (P<0.001). In the receiver operating characteristic (ROC) curve for ascites, ADA cut-off level of 41.5 U/L was found to yield the best results of differential diagnosis; sensitivity, specificity, positive predictive value, negative predictive value and accuracy of the test in tuberculous peritonitis cases were 80.0%, 97.2 %, 96.6%, 82.9%, 88.6% respectively.

Conclusion: ADA levels are elevated in suspected tuberculous peritonitis cases and it is a simple, rapid, inexpensive and the least invasive test. It is thus a useful biochemical marker for the early diagnosis of tuberculous peritonitis while waiting for the results of mycobacterial cultures or biopsies.

Key words: adenosine deaminase, sensitivity, specificity, diagnostic significance, tuberculous peritonitis

Yetişkinlerde şüpheli tüberküloz peritonitte asit adenosin deaminaz düzeylerinin tanısal önemi

ÖZET


Yöntemler: Çalışmaya alınan hastalar iki farklı gruba ayrılmıştır. Grup I; 35 şüpheli tüberküloz peritonit hastaları, ve Grup II; transüdatif asitli 35 hastanın oluşturduğu kontrol grubu (transüda olduğu biyokimyasal olarak kanıtlanmış veya hipoproteinemisi olan hastalar) çalışmaya dahil edildi ve periton sıvıları alınmıştı. ADA ölçümleri spektrofotometri ile yapıldı.

Bulgular: ADA düzeyleri (ortalama ± SS) şüpheli tüberküloz peritonit ve transudatif asit olgularında, sırasıyla, 48.5 ± 17.9 U/L ve 19.8 ± 7.7 U/L idi (P<0.001). Asit değerleri için “alıcı işletim karakteristik” (ROC) eğrisinde, ADA kesme seviyesi 41.5 U/L altında en iyi ayırtıcı tanı değerleri bulundu ve tüberküloz peritonit olgularında testin duyarlılık, özgüllük, pozitif prediktif değer, negatif prediktif değer ve doğruluk düzeyleri, sırasıyla, % 80.0, % 97.2, % 96.6, % 82.9, % 88.6 idi.

Sonuç: Şüpheli tüberküloz peritonit olgularında ADA düzeyleri yükselmiştir ve bu basit, hızlı, ucuz ve en az invaziv testtir. Mikobakteri kültürleri veya biyopsi sonuçlarını beklenen tüberküloz peritonit erken tanısı için bu en kullanışlı bir biyokimyasal belirteçtir.

Anahtar kelimeler: adenosin deaminaz, duyarlılık, özgüllük, tanısal değer; peritonit tüberküloz
INTRODUCTION

Adenosine deaminase (adenosine amino hydrolase, EC 3.5.4.4. ADA, isoenzymes ADA1 and ADA2) an enzyme required for purine degradation is widely distributed in human tissues.1 ADA helps in proliferation and differentiation of lymphocytes especially T lymphocytes. ADA is a significant indicator of active cellular immunity.2 Thus, ADA has been proposed to be a useful surrogate marker for the diagnosis of tuberculosis (TB) because it can be detected in body fluids such as pleural, pericardial, cerebrospinal fluid and peritoneal fluid and elevated ADA levels have been reported in these cases.3-4

By estimation of ADA levels for the diagnosis of extra pulmonary tuberculosis,5-11 sensitivities and specificities of greater than 90 percent have been reported. ADA predicts disease probability by 99% in countries with high prevalence of TB12 but there are other studies which do not show such good results.13-15

The prevalence of TB in Nepal is high. The annual incidence rate of TB in Nepal is quite high and is reported to be 163 per 100,000 population.16 This has been attributed to difficulties in providing health care and treatment facilities in the remote mountainous terrain of the country. Literature survey revealed no studies being reported from Nepal that evaluates the significance of ADA levels in the diagnosis of suspected tuberculous peritonitis. Hence, this study was designed and conducted to assess the role of ascitic fluid ADA in the early laboratory diagnosis of tuberculous peritonitis in adults in Nepalese population.

METHODS

Setting

This prospective study was carried out on patients admitted in the medical ward of a centrally located tertiary care hospital in Kathmandu, Nepal from July 2008 to July 2010. Patients from all parts of the country and especially the poor sections of the society come for treatment in this hospital as most of the services provided are free, including all investigations. The hospital receives about 60 sputum specimens per day for performing Ziehl-Neelsen staining for diagnosis of TB and eight to ten peritoneal fluid specimens for various ascites investigations every month. The ethical review committee of the hospital permitted to carry out this study and informed consent was taken from the patients before inclusion in the study. Their results were dispatched immediately after the tests were performed, so that the patients get appropriate treatment.

Patients

Abdominal paracentesis was performed on 70 consecutive patients with ascites and these were divided into two different patients groups. Group I - clinically suspected cases of tuberculous peritonitis - 35 cases, on the ground of clinical findings and lymphocytic exudates (less than 3 g of protein per 100 ml of fluid) with no response to one week of broad spectrum antibiotics treatment and / or radiologic findings consistent with lung TB and sputum positive for acid fast bacilli (AFB). Group II - control group - transudative ascites cases (less than 2 g of protein per 100 ml of fluid, patients with biochemically proved transudates or malnutrition with hypoproteinaemia) and with no evidence of TB clinically and sputum smear negative for acid fast stain. Ascitic fluid samples (2-3 ml) were collected with aseptic precautions by abdominal paracentesis from both the study population groups. Patients with any malignancies (or hemorrhagic ascites) and less than 15 years of age were excluded in the study. All subjects selected in the study were tested for HIV and only those found negative for HIV were included in the study.

Laboratory tests

ADA estimation was carried out by spectrophotometry method based on the principle of Guisti and Galanti method of enzymatic analysis which is based on indirectly measuring the formation of ammonia produced when adenosine deaminase acts in an excess of adenosine. ADA levels were calculated and expressed in unit per litre (U/L). ADA MTB diagnostic kit from Microexpress - a division of Tulip Diagnostics Pvt. Ltd., India was used according to the manufacturer’s instructions.

Statistical analysis

The continuous variables were presented as range, mean ± SD or quartiles and the categorical variables were calculated by percentages and ratio (sex ratio). The continuous variables were compared by using the Student t test or Mann-Whitney U test. A receiver -operating characteristic (ROC) curve was plotted and the area under the curve (AUC) was calculated and an optimum cut-off value was established. For all analyses, a P value of <0.05 (two-tailed) was taken as statistically significant. Diagnostic test 2x2 contingency tables were used. Sensitivity, specificity, positive and negative predictive values and accuracy of the test were calculated All evaluations were performed with SPSS version 17.0.
RESULTS

All patients in the suspected tuberculous peritonitis group were sputum smear positive for AFB, had some abnormality on CXRs (upper lobe lesion, lateral or bilateral or with cavities) and had lymphocytic exudates with total protein > 3 g/dl. In contrast, in the transudative ascites (control) group, all patients were sputum smear negative for AFB, had no abnormality on CXRs and had ascites total protein < 2 g/dl. The age (mean ± SD) and the ratio of male / female in suspected tuberculous peritonitis was 43.80 ± 16.31 and 3/1 and in transudative ascites, the control group it was 44.03 ± 14.64 and 2 / 1 respectively. The mean ADA levels (mean ± SD) in suspected tuberculous peritonitis and in transudative ascites cases, were 48.51 ± 17.91 U/L and 19.28 ± 7.69 U/L, respectively. The difference between the ADA values in the two groups was found to be highly significant (P< 0.001). Box plots of the ADA activity in the suspected tuberculous peritonitis (case) and transudative ascites (control) groups are shown in Figure 1, together with the 90th percentile range, 75th and 25th percentiles. The usefulness of ascites ADA level as a biomarker for diagnosis of tuberculous peritonitis was evaluated using ROC curve analysis and the optimal cut-off value was determined to be 41.5 U/L (Figure 2). The area under the curve (AUC) for suspected tuberculous peritonitis group was 0.928 and standard error (SE) was 0.032 (95% confidence interval (CI) =86.4% - 99.1%, P <0.001). Seven patients in suspected tuberculous peritonitis group showed ADA values of less than 41.5 U/L and one patient in transudative ascites, the control group showed ADA value of greater than 41.5 U/L. Based on the cut-off value of 41.5 U/L, the ascites ADA sensitivity and specificity were 80.00% and 97.14% respectively. Positive predictive value was 96.55% and negative predictive value was 82.6%. The accuracy of the test in suspected tuberculous peritonitis cases was 88.6% (Table 1).

Table 1. Validity of Ascites ADA as a diagnostic test in suspected cases of tuberculous peritonitis

<table>
<thead>
<tr>
<th>Study Group</th>
<th>Sensitivity % (CI)</th>
<th>Specificity % (CI)</th>
<th>PPV % (CI)</th>
<th>NPV % (CI)</th>
<th>Accuracy %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tuberculous peritonitis</td>
<td>80.00 (62.5-90.9)</td>
<td>97.14 (83.5-99.9)</td>
<td>96.55 (80.4-99.8)</td>
<td>82.92 (67.4-92.3)</td>
<td>88.57</td>
</tr>
</tbody>
</table>

PPV= positive predictive value, NPV= negative predictive value, CI=95% confidence interval

Figure 1. Box plots for ADA (U/L) levels in tuberculous peritonitis (case) and transudative ascites (control) groups. The plots show the 90th percentile (bars), 75th and 25th percentile (box) and median (bar in box).

Figure 2. ROC curve of ascitic fluid ADA activity. Diagonal line indicates the line of no discrimination. AUC of this ROC curve is 0.928. SE = 0.032, 95% CI = 86.4% - 99.1%, P < 0.001. AUC: Area Under the Curve, SE: Standard Error, CI: Confidence Interval.
DISCUSSION

With the lack of specific clinical and laboratory markers, extrapulmonary manifestations of Mycobacterium tuberculosis in general and tuberculous peritonitis in particular have posed complex diagnostic challenges for centuries. Tuberculous peritonitis is usually paucibacillary and the classical method of Ziehl-Neelsen stain for TB bacilli is not always definitive and requires an invasive diagnostic approach. Computed tomography of the abdomen is the most useful radiographic study. Even though rapid diagnostic tests, such as polymerase chain reaction (PCR) for tuberculous peritonitis are promising, the role of ascitic fluid PCR is not firmly established. In areas with a high prevalence of TB, there is an urgent need for an alternate highly sensitive and a highly specific test for the early and accurate diagnosis of tuberculous peritonitis.

ADA is a helpful diagnostic tool in tuberculous ascites, specificity and sensitivity as high as 97 and 100% respectively, when the level is above 33 U/L. In this study, ADA level (mean ± SD) in suspected tuberculous peritonitis was 48.51 ± 17.91 U/L while in the transudative ascites, the control group it was 19.28 ± 7.69 U/L (highly significant, P < 0.001). The ascitic fluid ADA ROC analysis revealed the best cut-off value of 41.5 U/L yielding a good sensitivity of 80.0% and a high specificity of 97.14% to validate its use as a reliable diagnostic marker in these cases.

Kaur A et al., showed that ADA is not of sufficient discriminative value for diagnosing TB in peritoneal fluid with a sensitivity, specificity, positive and negative predictive values of 89%, 81%, 25% and 99% respectively (ADA >15 U/L). Dwivedi M et al., studied 49 patients with ascites of which were of tuberculous etiology where at an ADA level of >33 U/L, the sensitivity, specificity, positive and negative predictive values were 100%, 96.6%, 95% and 100% respectively. Gupta V K et al., analysed ascitic fluids samples, of which seven were due to tubercular etiology and with an ADA level of >30 U/L, the sensitivity and specificity were 100% and 94%. The sensitivity and specificity for tubercular ascites on the basis of ADA levels were 100% and 97% respectively, as per the study of Bhargava et al.

Agarwal studied 30 cases of tuberculous ascites and using a cut-off value of 40 U/L reported sensitivity, specificity, positive and negative predictive values of 96%, 80%, 96% and 80% respectively. Thus ADA activity is a practical and useful approach to take therapeutic decisions in patients with suspected peritoneal TB. The beginning of empirical treatment when a patient has a high ADA value in ascitic fluid seems to be a good approach while waiting for the results of mycobacterial cultures and biopsies. The results of the study clearly showed that ADA levels are significantly elevated in suspected tuberculous peritonitis as against non-tuberculous ascites causes. Estimating ascites ADA levels has a particularly significant role in areas where either the facilities for culture of TB bacilli or tissue biopsies are not available. A high ascites ADA value is strongly suggestive of it being of tubercular origin. A low ascites ADA value not necessarily eliminate it of not being of tubercular origin, but strongly suggestive of non-tubercular origin.

The study had a few limitations. The major one was that the investigation was carried out on the suspected cases of TBP and definite diagnostic tests such as culture or biopsies were not performed. Isoenzymes ADA1 and ADA2 levels were not determined individually in the ascitic fluid specimens. Further studies with a larger numbers of proven cases of tuberculous peritonitis are needed before definitive conclusions can be drawn. In addition to clinical findings and radiologic characteristics, ascites ADA estimation should also be considered in cases of tuberculous peritonitis especially in cases where the conventional methods like smear microscopy and culture often fail to establish an early diagnosis. Estimating ascites ADA levels in tuberculous peritonitis cases is a simple, rapid, inexpensive and the least invasive, highly specific and fairly sensitive method and significantly elevated ascites ADA levels are highly suggestive of tuberculous etiology and of diagnostic relevance.

REFERENCES


CSF-ADA As Diagnostic Tool For Tuberculous Meningitis Patients

Dr. Margeyi Mehta*, Dr. Jigish Shah**, Dr. A.T. Leuva***.

Abstract

Introduction: TB is widely prevalent in India and a common form of TB is Tuberculous Meningitis (TBM), with great mortality, mainly in childhood. Available methods of TBM diagnosis are time-consuming and expensive. ADA is being recognized as a marker of T-cell-mediated immunity. There is a need for cost-effective and relatively rapid method at tertiary-healthcare-settings. The study was carried out to assess the role of CSF-ADA for TBM diagnosis. Materials and Methods: In this cross-sectional study at SSG Hospital, Baroda between June-October 2011, 50 patients and 20 controls were selected. ADA in CSF and other biochemical markers were assessed after due informed consent. Microxpress ADA-MTB reagent was used for CSF-ADA estimation.

Results: Out of 50, 22 children were male and 28 were female. 17 TBM (34%), 19 PM (38%) and 14 AM (28%) cases were detected. 72% of cases had CSF-ADA levels less than 10 IU/L (mean 4.37±1.32), CSF-ADA levels were less than 10 in all controls (mean 2.8±0.8). Comparative CSF-ADA estimation in TBM, PM and AM showed higher values for TBM (p<0.001). Sensitivity and specificity of CSF-ADS for TBM diagnosis were 78% and 98% respectively at a cut-off value of 11 IU/L. Discussion: Difference in the CSF-ADA levels of meningitis due to tuberculous and non-tuberculous etiology is statistically highly significant. CSF-ADA level at 11 IU/L differentiate tuberculous from non-tuberculous meningitis with reasonable sensitivity and great specificity. A country with extremely high TB prevalence requires a robust healthcare delivery and accurate therapy to prevent subsequent emergence of MDR and XDR-TB forms. High specificity of CSF-ADA test can prove immensely useful. ADA estimation in CSF is simple, inexpensive, rapid and highly specific method for making a diagnosis of tuberculous etiology in TBM. [Mehta M et al. NJIRM 2012; 3(3): 160-164]

Key words: Adenosine Deaminase, Tuberculous meningitis diagnosis, CSF-ADA
A study of Cerebrospinal Fluid Adenosine deaminase and C-reactive protein in Bacterial, Tubercular and Viral meningitis.

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Cerebrospinal Fluid Adenosine deaminase (ADA) activity and C-reactive protein (CRP) measurement were done in 30 patients of tubercular meningitis (TBM), 36 patients of bacterial meningitis (BM) and 34 patients of Viral meningitis (VM), to evaluate, whether CSF CRP and ADA levels could be used to differentiate the bacterial, tubercular & viral meningitis. The mean CSF ADA activity was significantly raised in TBM as compared to BM and VM. (p< 0.001) while mean CSF CRP activity was significantly raised in BM as compared to TBM and VM. (p< 0.001). At cut of level 10 IU/L, the sensitivity and specificity of ADA for TBM was 90% and 97.14% respectively while at cut of level 15 mg/L, the sensitivity and specificity of the CRP for BM was 86.11% and 98.43% respectively. Since both the tests are simple and take lesser time to perform, they can be used to differentiate BM, TBM and VM.

ABSTRACT:
Cerebrospinal Fluid Adenosine deaminase (ADA) activity and C-reactive protein (CRP) measurement were done in 30 patients of tubercular meningitis (TBM), 36 patients of bacterial meningitis (BM) and 34 patients of Viral meningitis (VM), to evaluate, whether CSF CRP and ADA levels could be used to differentiate the bacterial, tubercular & viral meningitis. The mean CSF ADA activity was significantly raised in TBM as compared to BM and VM. (p< 0.001) while mean CSF CRP activity was significantly raised in BM as compared to TBM and VM. (p< 0.001). At cut of level 10 IU/L, the sensitivity and specificity of ADA for TBM was 90% and 97.14% respectively while at cut of level 15 mg/L, the sensitivity and specificity of the CRP for BM was 86.11% and 98.43% respectively. Since both the tests are simple and take lesser time to perform, they can be used to differentiate BM, TBM and VM.

INTRODUCTION:
Infectious diseases remain a major cause of death and disability for millions of people around the world, despite decades of dramatic progress in their treatment and prevention. As vital tissues are involved, CNS infection can cause devastating sequelae and in some cases may result in both neurological and medical emergencies.¹

Meningitis is an inflammation of the membranes that surround the brain and spinal cord. It is a common clinical problem during infancy and childhood. Delay in distinguishing between bacterial, tubercular & viral meningitis & treatment may have irrevocable consequences that lead to significant morbidity & mortality. The initiation of proper medication in meningitis patients can often be delayed because of a lack of confidence in the presently available laboratory tests.²⁻³ Most of the tests developed for the early diagnosis of meningitis are not sensitive ⁴ and although some other tests are useful, they may not be affordable for routine use.⁵⁻⁶

So it is necessary to introduce simple, reliable and cost effective method for rapid diagnosis and differentiation of various types of meningitis. In view of such observations, the present study was

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Conflict of interest: Authors reported none
conducted to find out the usefulness of these two tests, CSF-ADA & CSF-CRP for the rapid diagnosis & differentiation of bacterial, tubercular & viral meningitis.

METHODS
A present observational study was conducted in Department of Biochemistry, Dr. Shankarrao Chavan Government medical college, Nanded, on 100 meningitis children of age between 1 to 12 year. Depending on clinical features, examination, investigations like CSF Biochemistry, Cytology, Culture & others investigation, these cases were further divided in to 3 groups.

**Group I- Bacterial meningitis:**
This group included 36 Patients with clinical and CSF laboratory findings consistent with BM. Clinical features being the acute onset of symptoms of meningitis, may be associated with sinusitis, otitis media, and signs of meningeal irritation. CSF analysis showing Pleocytosis of > 250 cells/mm3 predominantly neutrophils, Proteins > 50mg/dl, Sugar < 40mg/dl. Gram stains and culture positivity. Neuroimaging showing evidence of diffuse meningeal enhancement, abscesses or parameningeal focus.

**Group II- Tubercular meningitis:**
This group included 30 Patients with clinical and CSF laboratory findings consistent with TBM. Clinical features being the insidious in onset, may be associated with tuberculosis of other organs, signs of meningeal irritation. CSF analysis showing Pleocytosis of > 60 cells/mm3 predominantly lymphocytes, Proteins > 40mg/dl, Sugar < 40mg/dl. ZN, culture or manotux positive. Neuroimaging showing evidence of Meningeal enhancement, basal exudates and/or tuberculoma.

**Group III-Viral meningitis:**
This group included 34 Patients with clinical and CSF laboratory findings consistent with VM. Clinical features being the Usually acute in onset with signs of meningeal irritation. CSF analysis showing Pleocytosis of > 25 cells/mm3 predominantly lymphocytes, Proteins > 45mg/dl, Sugar normal.

While patients of Febrile seizures, patients with Non infectious conditions of CNS such as epilepsy, drug or vaccine induced, patients with acute infections at sites other than the central nervous system, those in whom lumbar puncture was contraindicated, and those with severe hepatic dysfunction were excluded from the study. Similarly Those cases after examination & investigation were not diagnosed as above three meningitis or diagnosed other than meningitis like febrile convulsion, cerebral malaria, subarachnoid hemorrhage were withdrawn from the study.

CSF CRP was measured by using CRP turbilatex kit of agapee diagnostics in accustar semi-autoanalyzer based on agglutination of the latex particles coated with anti-human CRP.

CSF ADA was estimated by using ADA-MTB kit of Microxpress- A Division of Tulip Diagnostics [P] Ltd. Here ADA hydrolyzes adenosine to ammonia and inosine. The ammonia formed further reacts with phenol and hypochlorite in an alkaline medium to form blue indophenol complex with sodium nitroprusside acting as a catalyst. Intensity of the blue is proportional to the activity of ADA.

**Statistical Methods:**
Descriptive statistical analysis has been carried out in the present study. Significance is assessed at 5 % level of significance. ANOVA test has been used to find significance of association of CRP & ADA with type of meningitis. Sensitivity, Specificity and Accuracy were calculated to know the diagnostic performance of CRP and ADA levels in relation to type of meningitis.

**RESULTS:**

<table>
<thead>
<tr>
<th></th>
<th>TBM</th>
<th>BM</th>
<th>VM</th>
<th>ANOVA p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP</td>
<td>4.85 ± 3.53</td>
<td>25.22 ± 10.38*</td>
<td>2.24 ± 1.63</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>ADA</td>
<td>22.50 ± 11.43*</td>
<td>4.55 ± 3.21</td>
<td>2.37 ± 1.41</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Table 1: Mean Levels of CRP & ADA in different types of meningitis.

The comparison of CSF CRP & ADA in different types of meningitis are presented in Table 1. In TBM group mean CRP level was 4.85 ± 3.53 and mean ADA level was 22.50 ± 11.43. In BM group mean CRP level was 25.22 ± 10.38 and mean ADA level was 4.55 ± 3.21. While in VM group mean CRP level was 2.24 ± 1.63 and mean ADA level was 2.37 ± 1.41, in which mean level of CRP was significantly increased in BM group as compared TBM and VM, while mean level of ADA was significantly increased in TBM group as compared BM and VM.

At cut of level 15 mg/L, the sensitivity and specificity of the CRP for BM among these 100 patients was 86.11% and 98.43% respectively with an accuracy of 94%. While at cut of level 10 IU/L, the sensitivity and specificity of ADA for TBM among these 100 patients was 90% and 97.14% respectively with an accuracy of 95%.

**DISCUSSION**

**CRP and meningitis.**
The results of the present study show that CRP level was significantly increased in bacterial meningitis as
compared to tubercular & viral meningitis. This increase in CRP level might be due to entry of CRP into CSF by passive diffusion across the highly inflamed meninges or de-novo synthesis in central nervous system.\textsuperscript{7} Present study was consistent with the findings of various studies.

In a study conducted by Vaishnavi C \textit{et al}, CRP in CSF was significantly higher in patients with pyogenic meningitis compared to tubercular meningitis. Authors concluded that the estimation of CRP in the CSF was significantly higher in patients with pyogenic meningitis compared to tubercular meningitis.\textsuperscript{19.0} Riberio MH \textit{et al} estimated the levels of CRP in CSF from 33 patients with bacterial meningitis, 21 patients with lymphocytic meningitis and 54 controls. 100\% of these patients with bacterial meningitis were correctly classified on the basis of measurement of CRP levels in CSF. In conclusion authors recommend the estimation of CRP in CSF in the differentiation of bacterial meningitis.

A meta-analysis by Gerdes LU \textit{et al} suggested that a negative CRP test in either CSF or serum can be used with a very high probability to rule out bacterial meningitis.\textsuperscript{10,11}

\textbf{ADA and meningitis.}

Present study found that ADA level was significantly increased in tubercular meningitis as compared to bacterial & viral meningitis.

ADA is released by T cells during cell mediated immune response (CMI) to the tubercle bacilli. ADA is now being recognized as a marker of cell mediated immunity particularly as a marker of T lymphocyte activation. Adenosine deaminase levels (ADA) have also been considered by several researchers to differentiate tubercular disease from non-tubercular meningitis.\textsuperscript{12-15} The ADA2 isoenzyme is the major contributor to increased ADA activity in the CSF of patients with tuberculous meningitis, probably reflecting the monocyte–macrophage origin of the ADA.

The source of raised ADA in CSF of TBM patients may be the damaged blood brain barrier permitting ADA to enter into CSF from the blood or adjacent cerebral tissue and/or as a result of lymphocyte-macrophage proliferation indicating local immune response.\textsuperscript{12} Sang-Ho Choi \textit{et al} studied ADA activity in CSF of 182 patients with meningitis. The mean ADA level in the tuberculous meningitis group was 12.7±7.5 U/L and it was significantly higher than the other groups (3.10±2.9U/l; p<0.001). The sensitivity and specificity was 0.83 and 0.95 respectively when a cut-off value of 7U/L was used.\textsuperscript{16} Pettersson \textit{et al} reports sensitivity of 1.0 and specificity of 0.99 when a cut-off value of 20 U/L was used, but in that study there were only 3 enrolled tuberculous meningitis patients.\textsuperscript{17} Chotmongkol V \textit{et al} identified a CSF ADA level of 15.5 U/l as the best cut-off value to differentiate tuberculous meningitis and non-tuberculous meningitis, with a sensitivity of 75\% and specificity of 93\%. When tuberculous meningitis was compared with aseptic and carcinomatous meningitis, a CSF ADA level of 19.0 U/l was the best cut-off value for differentiation, with a sensitivity of 69\% and a specificity of 94\%.\textsuperscript{18} Some studies have reported a lower efficacy of this test in differentiating tuberculous meningitis and bacterial meningitis.\textsuperscript{19}

Malan C \textit{et al} showed that in both bacterial and TBM groups, the mean ADA level in the CSF was significantly higher than in aseptic meningitis (p<0.001), but a significant difference was not shown between bacterial meningitis and TBM groups.\textsuperscript{12} Gambhir IS \textit{et al} found that the mean CSF ADA levels in TBM patients was 9.61±4.10 U/l and was significantly elevated as compared to viral encephalitis and enteric encephalopathy cases; but the difference was insignificant in comparison to pyogenic meningitis and cerebral malaria.\textsuperscript{20} From above discussion, elevated CRP level in meningitis patient highly suggest Bacterial meningitis, while elevated ADA level in meningitis patient highly suggest Tubercular meningitis.

But either test done alone would still cause confusion in the probable diagnosis and differentiation of these three meningitis, since some studies shows overlap of ADA level between tuberculous meningitis and bacterial meningitis\textsuperscript{19} like Malan C \textit{et al}\textsuperscript{15}, Gambhir IS \textit{et al}\textsuperscript{20}, in such situation Differentiation of BM from TBM by ADA alone is difficult. Also the cell type in tubercular meningitis initially can predominantly be neutrophilic leucocytosis, which favours diagnosis of Bacterial meningitis falsely, in which case the diagnosis of tubercular meningitis is never entertained until the patient shows no response to the antibiotics. Patients with partially treated meningitis can have lymphocytic predominance when tubercular meningitis is wrongly considered. To overcome this fallacy, it is essential to do CRP as well ADA simultaneously in order to increase the specificity of the test.

Even elevated ADA level in meningitis patient highly suggest Tubercular meningitis, it’s low level in meningitis patient unable to differentiate BM from VM, as both cases shows low level of ADA which are not statistically different.

Similarly Even elevated CRP level in meningitis patient highly suggest Bacterial meningitis, it’s low level in meningitis patient unable to differentiate TBM...
from VM, as both cases shows low level of CRP which are not statistically different. Thus, it is clearly evident that neither CSF ADA level nor CRP level alone could differentiate these three types of meningitis completely. But their simultaneous use along with other tests of meningitis may helpful in probable diagnosis and differentiation of these three meningitis. In which elevated CRP levels in meningitis are highly suggestive of Bacterial meningitis. High ADA and normal CRP suggest the diagnosis of TBM. On the other hand having both ADA and CRP negative can strengthen the diagnosis of viral meningitis. A high CRP in cases with high ADA would again favour the diagnosis of pyogenic meningitis thereby overcoming the false positive ADA.

**CONCLUSION:**

From above discussion, study concludes that combine use of these two tests i.e. CSF CRP and ADA can be used for early differentiation of Bacterial, Tubercular, and Viral meningitis. This is necessary when gold standard test for meningitis like Smear and/or culture for AFB, smear and/or culture for bacteria, is not available or negative or time consuming. These tests for ADA and CRP in CSF are simple and can be carried out in a central laboratory with a rapid diagnosis, thus reducing unwarranted or harmful therapy for patients. Further studies may need in this regard considering appropriate sample size.

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**Cite this article as**

ADENOSINE DEAMINASE ACTIVITY IN PULMONARY TUBERCULOSIS

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ABSTRACT:
Adenosine deaminase (ADA, adenosine aminohydrolase, EC 3.5.4.4), an enzyme involved in purine metabolism, catalyses the hydrolytic cleavage of adenosine and 2'deoxyadenosine, irreversible converting them into inosine and 2'deoxyinosine respectively. ADA activity increases during cellular activation for energy demand to detoxify the toxic metabolites.

Studies have suggested the sensitivity and specificity of the assay of serum and pleural fluid ADA in serum and pleural effusions due to pleural TB, malignancy, and other pulmonary diseases. The assay of serum/pleural fluid ADA is simple, easy, cost-effective and reliable criteria that are considered important in the routine evaluation of the patients with symptoms suggestive of PTB particularly where the prevalence of tuberculosis is still high. This is the situation in our country, hence it should be recommended to include serum and pleural fluid ADA activity in the battery of routine investigations for the diagnosis and prognosis of PTB.

INTRODUCTION
Pulmonary tuberculosis (PTB) remains one of the leading causes of morbidity and mortality worldwide accounting for approximately 8 million new cases and 2 million deaths annually. Definitive diagnosis of PTB depends on sputum smear examination and culture. Only up to 50% of pulmonary and extra-pulmonary tuberculosis cases can be diagnosed by smear examination. Traditional culture methods take around 2 to 3 weeks before the diagnosis can be established. If the diagnosis of PTB is delayed it leads to increased morbidity and mortality. Early diagnosis, initiation of optimal treatment and response of therapy would not only enable the cure of an individual patient but also curb the transmission of infection as well as the chances of emergence of drug-resistant strains.

Adenosine deaminase (ADA, adenosine aminohydrolase, EC 3.5.4.4), an enzyme involved in purine metabolism, catalyses the hydrolytic cleavage of adenosine and 2'deoxyadenosine, irreversible converting them into inosine and 2'deoxyinosine respectively. ADA activity increases during cellular activation for energy demand to detoxify the toxic metabolites. It plays an important role in lymphocyte and monocyte maturation and activity. Increased serum level of ADA has been reported in several diseases characterized by an enhanced cell mediated immune (CMI) response, such as typhoid fever, bacterial pneumonia, infectious mononucleosis and tuberculosis. There is limited data on the use of serum ADA levels to diagnose active PTB in adults. Further, the prognostic role of serum ADA in active PTB has not been studied so far. The purpose of this study was to evaluate the role of serum and pleural fluid ADA activity in the diagnosis and prognosis of PTB.

MATERIAL AND METHODS
405 subjects(indoor and outpatient clinic of either sex) aged 10 to 80 years, suffering from PTB, were taken from Kamla Nehru TB and Chest hospital, attached to Dr.S.N.Medical College, Jodhpur. 54 persons served as controls. All the patients were examined clinically and
investigated. Sputum examination for acid fast bacilli and chest skiagram were carried out. Routine hematological examinations were also performed in peripheral blood of controls as well as patients before and after 3 months of treatment. Pleural fluid analysis was also carried out in 142 patients suffering from PTB. The exudates were distinguished from transudates by Pleural fluid protein cut-off level of 3 g/dl or more. Pleural fluid was subjected to routine microscopic and biochemical analysis.

The subjects were divided into three groups-Group I, healthy controls (n=54); Group II, (n=405) untreated and recently diagnosed, clinically as well as radiologically established patients suffering from active PTB; and Group III (n=124) included followed up cases of PTB who were receiving effective antituberculosis therapy for a period of three months.

The ADA assay was performed simultaneously in serum and pleural fluid, using the commercially available ADA-MTB kits, supplied by Microxpress, Tulip diagnostic (P) Ltd., Goa (India) based on the method of Guisti. The results were statistically analyzed by Student t-test and by calculating Pearson’s correlation coefficient (r).

RESULTS AND DISCUSSION

Among 54 healthy controls there were 27 males and 27 females, most of them in the age group 31-60 years. The mean serum ADA activity in control was 5.7±1.3 U/L. There was no significant difference in serum ADA activity in control subjects with respect to age and sex. The serum and pleural fluid ADA activity in untreated PTB subjects was observed to be 72.2±16.3 U/L and 100.0±19.48 U/L respectively. A positive correlation between serum and pleural fluid ADA, and percentage of lymphocytes in peripheral blood and pleural fluid was observed. This may be suggestive of their association with events in CMI.

Though it is difficult to explain the exact cause for higher ADA activity in patients with PTB, it is known that ADA is predominant enzyme of lymphocytes and its serum levels remain high in diseases where cellular immunity is stimulated, e.g. PTB. In pathological conditions, the clearance capacity of lungs is decreased leading to increased number of cells in the pleural fluid and the recirculation of the activated lymphocytes may cause a high serum ADA activity in patients with pulmonary diseases for detoxification of toxic metabolites. Ishii and Green reported that adenosine is toxic to the cultured mammalian cells and that it interferes with pyrimidine biosynthesis.

In the followed up subjects, the serum ADA activity was 30.9±11.7 U/L. In the untreated phase, the serum ADA activity in PTB patients was significantly higher than the healthy controls and followed up subjects (p<0.001). a significant difference in the mean serum ADA activity was also observed among controls and followed up subjects. There were neither any significant difference in serum ADA activity after the first three months of treatment. This could be due to repeated thoracocentesis, improvement in the clearance capacity of lungs and normalization of the altered lymphocytes turnover. It seems that the activity of the enzyme is correlated more to the maturity stage of lymphocytes than to their number.

CONCLUSION

Previous reports have suggested the sensitivity and specificity of the assay of serum and pleural fluid ADA in serum and pleural effusions due to pleural TB, malignancy, and other pulmonary diseases. The assay of serum/pleural fluid ADA is simple, easy, cost-effective and reliable criteria that are considered important in the routine evaluation of the patients with symptoms suggestive of PTB particularly where the prevalence of tuberculosis is still high. This is the situation in our country, hence it should be recommended to include serum and pleural fluid ADA activity in the battery of routine investigations for the diagnosis and prognosis of PTB.

REFERENCES


Performance Evaluations

AS A MARKER FOR DIABETES

ADA-MTB

For the determination of ADA activity in serum, plasma and biological fluids
STUDY OF SERUM ADENOSINE DEAMINASE ACTIVITY (ADA) IN DIABETES MELLITUS WITH COMPlications

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Department of Biochemistry, Deccan College of Medical Sciences
Hyderabad, A.P.
*Author for Correspondence

ABSTRACT
Diabetes mellitus is a complex syndrome characterized by hyperglycemia, leading to vascular complications such as retinopathy, neuropathy and macrovascular disease like atherosclerosis. Patients with diabetes with complications and without complications are taken up for the study. The parameter serum Adenosine Deaminase, Activity (ADA) is considered, which is helpful in understanding the predisposing factors and the assessment of these patients to develop complications. The present study reveals that serum ADA is found to be raised in diabetic patients without complications while it is similar to control group with complications. This indicates that increased susceptibility of diabetes to develop a variety of bacterial and fungal infection may not be due to immune deficiency.

Key Words: Serum Adenosine Deaminase Activity (ADA), Diabetic

INTRODUCTION
Adenosine deaminase in lymphoid tissue might efficiently deaminase deoxy adenosine and prevents phosphorylation.
Galanti and Giusti Altivita (1968) and Gold Berg and Elis (1976) observed that Human serum ADA increases in acute viral hepatitis and active cirrhosis and only to a much lesser extent in other hepatic diseases. Goldblum et al., (1978) demonstrates that increased serum ADA has been found in leukaemic patients and lymphocyte ADA levels can be considered a parameter of immune response Lymphocyte ADA activity is decreased in erythrocytes from patients with severe combined immunodeficiency, while heterozygous carrier of this autosomal recessive defect have half the normal enzyme activity.
Alan Taylor (1986) observed an increase in serum ADA activity in 18 untreated patients with active sarcoidosis and suggested with some reservation that its measurements might be useful for diagnosis of sarcoidosis. Singh et al., (1981) reveals that the estimation of ADA activity will be of value in the diagnosis of tuberculous effusions. The high ADA activity in tuberculous effusions could be attributed to cell mediated immune reactions or to increased demands for energy. This high level may be useful in the pleural differential diagnosis of tuberculous from other pleural effusions. Delias et al., (1987) studied ADA activity in acquired Immunodeficiency syndrome and reveals high ADA activity in these subjects. ADA activity in lymphocytes and erythrocytes as well as in serum, is absent in about 20 – 30% of children affected by a severe inherited T cell immune deficiency. Yasuhera and Nakamera (1987) determined the activity of serum ADA in patients who had various types of pneumonia or pulmonary tuberculosis. ADA activity in children with bacterial pneumonia showed a higher value than those of viral and mycoplasma pneumonia but a lower value than that of tuberculosis. The peak ADA activity was found on 5th or 6th disease day in bacterial pneumonia. The number of lymphocytes is predominant over that of neutrophils at this period Serum ADA in tuberculosis showed highest concentration than that of pneumonia. Increased serum ADA in tuberculosis seems to be influenced by activated T lymphocytes.

MATERIALS AND METHODS
The study was carried out in 25 normal adult patients between the age group of 30-55 years from outpatient & inpatient department of OHRC & Princess Esra Hospital, Hyderabad, these patients show no family history of diabetes they did not suffer from any complication.
Research Article

20 patients with NIDDM of 5-7 years duration is studied there was no renal impairment as shown by urine examination, blood urea and serum creatinine.

18 patients is studied suffering from NIDDM of more than 10 years duration with complications like retinopathy or nephropathy or neuropathy blood samples were collected in dry bottles using EDTA as auticoagulent for estimation of blood sugar and serum creatinine adenosine deaminase are also estimated.

The following parameters are studied:

- Fasting plasma glucose
- Serum adenosine deaminase activity
- Serum creatinine

Estimation of Glucose

Method – Glucose Oxide – Peroxidase Method:

Principle

This is an enzymatic method employed in the clinical laboratory for the estimate of glucose. Glucose is oxidized by glucose oxidase to gluconic acid and \( \text{H}_2\text{O}_2 \) is liberated. The colorimetric indicator, quinonemine is generated from 4 – amino antipyrene and phenol by \( \text{H}_2\text{O}_2 \) under the catalytic action of peroxidase intensity of colour generated is directly proportional to glucose concentration.

\[
\text{Glucose } + \text{O}_2 + \text{H}_2\text{O} \\
\text{Gluconic acid } + 2 \text{H}_2\text{O}_2 \\
2 \text{H}_2\text{O}_2 + 4 \text{Aminoantipyrine} + \text{Phenol Quinonemine} + 4 \text{H}_2\text{O}
\]

Reagent 1: Phosphate buffer PH 7.0 = 100 mm 01/1

Phenol ......................= 5mm 01/1

4 Aminoantipyrine.........=0.5mm 01/1

Glucose oxidase ..........> 15 ku/1

Peroxidase ...................> 1 ku/1

Reagent 2: Glucose standard 100 mg/1

Sample Material

Serum, Heparin, Plasma or Flouride – Plasma

The stability in serum and plasma is 1 day at 2-8 degree centigrade, serum or plasma must be separated from erythrocyte within 60 minutes of collection.

Assay Procedure

Wave length Hg 546mm 500-540mm

Light path 1 cm

Temperature 37 degree centigrade

Measurement Against regent block

Reference Range

Serum / Plasma 70 – 110 mg/dl

- Raaboe, Terkildsen Tc on the enzymatic determination of blood glucose, scand and clin lab invest.

Serum Adenosine Deaminase Activity

Principle

Adenosine deaminase hydrolyses adenosine to ammonia and inosine. The ammonia formed further reacts with a phenol and hypochlorite in an alkaline medium to form blue indophenols complex with sodium nitroprusside acting as a catalyst. Intensity of the blue coloured indo phenol complex formed is directly proportional to the amount of ADA present in the sample.

\[
\text{Adenosine } + \text{ADA ammonia } + \text{inosine} \\
\text{Ammonia } + \text{Pheno } + \text{Hypo chlorite} \\
\text{Alkaline } \rightarrow \text{Blue indophenols complexes}
\]
Reference Values

Serum Plasma Pleural, Pericardial and ascetic Fluids Normal suspect strong <30 u/l 30 u – 40 u/l.
Suspect positive >40 u/l – 60 ul.
>60 u/l.

CSF Normal Positive < 10 u/l
> 10 u/l

Reagents

• L₁ – ADA – MTB Reagent – Buffer Reagent, ready to use.
• L₂ – ADA – MTB Reagent – Adenosine Reagent, ready to use.
• L₃ – ADA – MTB Reagent – Phenol Reagent.
• L₄ – ADA – MTB Reagent – Hypochlorite Reagent.
• S – ADA – MTB Standard ADA standard – ready to use.

Reagent Preparation

Reagents L₁ L₂ and standard are ready to use adenosine reagent (L₂) may be form crystals at 2-8°C dissolve the same by gently warming (35°C–50°C) the reagent for some time before use both the phenol reagent (L₃) AND THE Hypochlorite reagent (L₄) need to be diluted 1:5 distilled water before use (1) part of reagent + 4 parts of distilled water).

Test Procedure

• Bring all reagent and samples to room temperature before use.
• Prepare the working phenol regent and working hypochlorite reagent.
• Set the spectrophotometer filter at 570 – 630 (Hg 578 to 623nm) at 37°C
• Pipette into clean dry test tubes labeled blank (B) standard (S) sample.

Blank B (SB) and test (T) as follows:

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

5. Mix well and incubate at 37°C for exactly 60 minutes and then add the following:

<table>
<thead>
<tr>
<th>B</th>
<th>S</th>
<th>SB</th>
<th>T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Working phenol reagent</td>
<td>0.20</td>
<td>0.20</td>
<td>-</td>
</tr>
<tr>
<td>Sample</td>
<td>-</td>
<td>-</td>
<td>0.20</td>
</tr>
<tr>
<td>Working hypochlorite reagent</td>
<td>0.20</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

6. Mix well and incubate at 37°C for 15 minutes at RT for 30 minutes.
7. Measure the absorbance of the blank (Abs B) standard (Abs S) sample blank (Abs SB) and test (Abs T) against distilled water.

Calculations

Total ADA activity in u/l = \[
\frac{\text{Abs } t - \text{Abs SB}}{\text{Abs } S - \text{Abs B}} \times 50
\]
Research Article

The procedure is linear up to 150 u/l. If values exceed his limit dilute the sample with deionised water and repeat the assay.

• Imma ocana et al., (1986) thorax 41:888-889.
• Diagnostic value of ADA and its isoenzymes in tuberculosis effusions, Dept. of internal medicine.
• Date of file Tulip Diagnostic (P) Ltd.

Estimation of Serum Creatinine

Determination of creatinine based on Jafae’s kinetic method without deproteinization.

Principle

Creatinine forms a yellow orange compound in alkaline solution with picric acid. At a low concentration of picric acid as used in this method, precipitation of protein does not take place. As a result of rapid reaction between creatinine and picric acid, the secondary reactions do not cause interference.

Reagents

Reagents 1 Buffer solution
Reagents 2 picric Acid
Reagents 3 standard solution

Storage and Reagent Stability

The reagent stable till the date of expiry if stored at 15 – 25°C.

Reagent Preparation

Pre-warm the reagents as well as sample Serum or Plasma and Urine Sample.

Reagent Start

Mix reagent 1 and reagent 2 in the ration of 1 + 1 (eg: 1 ml of buffer solution and 1 ml of picric acid solution) the mixing ration should be observed exactly.

Leave the monoreagent for at least 10 min. at room temperature before using. The stability of the reaction solution is 5 hours at 15-20°C.

Test Concentrations

Reagent 1 NaoH 313 mmol/l
Phosphate 12.5 mmol/l
Reagent 2 Picric acid 8.73 mmol/l
Reagent 3 Creatinine standard 1.0 mg/dl standard

Sample Material

Serum – Heparin – Plasma

Dilute Urine 1 + 99 with distilled water. The stability in serum and plasma is 7 days at 4 – 25°C and at least 3 months at – 20°C the stability in Urine in 2 days at 20 – 25°C 6 days at 4-8°C and 6 months at 20°C.

Assay Procedure

Wave Length: Hg 492 mm (490 – 510nm)
Light Path: 1 cm
Temperature: 20°C – 25 / 37°C

Substrate Start: Sample / Std.

Sample / Standard 100 ul
Reagent 1 500 ul
Mix and incubate for 0 – 5 min, then add
Reagent 2 500 ul
Mix and read absorbance A1, after 60 sec, read absorbance A2 after further 120 sec.

Sample Start: Sample / Standard
Sample / Standard 100 ul
Monoreagent 1000 ul
Mix and read absorbance A1 after 60 sec, read absorbance A2 after further 120 sec.
Calculations
Serum / Plasma
Creatinine (mg/dl) = A Sample x Conc. Std. (mg/dl)
A Std.

Urine
Women = 7.3 – 21.4 mg/kg/d
Men = 8.7 – 24.6 mg/kg/d

RESULTS AND DISCUSSION
Results of study indicate that predisposition of diabetics to develop complications such as retinopathy nephropathy and the predisposition to infections in multifactorial.

Table 1: Serum Creatinine Levels in Various Study Groups

<table>
<thead>
<tr>
<th>S. No</th>
<th>Group – I Normal Control</th>
<th>Group – II NIDDM without Complications</th>
<th>Group – III NIDDM with complications</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>1</td>
<td>1.7</td>
<td>1.5</td>
</tr>
<tr>
<td>2.</td>
<td>0.6</td>
<td>1.7</td>
<td>1.6</td>
</tr>
<tr>
<td>3.</td>
<td>0.8</td>
<td>1.2</td>
<td>1.5</td>
</tr>
<tr>
<td>4.</td>
<td>0.6</td>
<td>1.6</td>
<td>6.5</td>
</tr>
<tr>
<td>5.</td>
<td>0.8</td>
<td>1.3</td>
<td>1.1</td>
</tr>
<tr>
<td>6.</td>
<td>0.7</td>
<td>1.3</td>
<td>3.6</td>
</tr>
<tr>
<td>7.</td>
<td>0.8</td>
<td>1.2</td>
<td>3.5</td>
</tr>
<tr>
<td>8.</td>
<td>0.7</td>
<td>1.5</td>
<td>2.6</td>
</tr>
<tr>
<td>9.</td>
<td>0.8</td>
<td>1.6</td>
<td>2.5</td>
</tr>
<tr>
<td>10.</td>
<td>0.9</td>
<td>1.5</td>
<td>2.2</td>
</tr>
<tr>
<td>11.</td>
<td>0.6</td>
<td>1.5</td>
<td>2.2</td>
</tr>
<tr>
<td>12.</td>
<td>1.0</td>
<td>1.1</td>
<td>2.3</td>
</tr>
<tr>
<td>13.</td>
<td>0.8</td>
<td>1.2</td>
<td>1.5</td>
</tr>
<tr>
<td>14.</td>
<td>0.6</td>
<td>1.1</td>
<td>1.9</td>
</tr>
<tr>
<td>15.</td>
<td>0.6</td>
<td>1.3</td>
<td>4.2</td>
</tr>
<tr>
<td>16.</td>
<td>0.8</td>
<td>1.2</td>
<td>1.4</td>
</tr>
<tr>
<td>17.</td>
<td>0.7</td>
<td>1.4</td>
<td>1.8</td>
</tr>
<tr>
<td>18.</td>
<td>0.753</td>
<td>1.318</td>
<td>2.818</td>
</tr>
<tr>
<td>19.</td>
<td>0.1328</td>
<td>0.3661</td>
<td>1.9340</td>
</tr>
<tr>
<td>20.</td>
<td>0.0322</td>
<td>0.0888</td>
<td>0.4691</td>
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Serum Creatinine

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Sum of Squares</th>
<th>Mean of Square</th>
<th>F-Ration</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between group</td>
<td>2</td>
<td>42.18</td>
<td>21.09</td>
<td>15.74</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Within group</td>
<td>48</td>
<td>64.62</td>
<td>1.34</td>
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<td></td>
</tr>
</tbody>
</table>

Fasting blood sugar levels are found to be raised in all patients who have already developed micro vascular complications control (72.45 ± 8.662) NIDDM without complications (159.00±43.865) NIDDM with complications (212.64±74.938) (Table 3).
Serum creatinine is raised in chronic diabetic patients who already developed nephropathy (Table 1). In these patients the blood glucose is also raised (Table 3). Different clinical and biochemical studies also show that occurrence of diabetic complications is more in patients with poor glycemic control.

Serum ADA activity is normal in diabetic with retinopathy and nephropathy however the enzyme activity was found to be slightly higher in diabetics without these complications (Table 2). The present study suggest that degree of hyperglycemia related to ADA increased adenosine deaminase level reflecting increased ‘T’ cell function in diabetics without complications as retinopathy or nephropathy may be due to autoimmune reaction against modification glycated proteins. Serum ADA levels is found to be raised in diabetic patients without complications while it was similar to control group with above complications this indicate that increased susceptibility of diabetics to develop a variety of bacterial and fungal infection may not be due to immune deficiency (Table 2). It has been conclusively shown that strict control of blood sugar levels reduces the risk of developing complications like neuropathy retinopathy and prevention of CAD.

**Table 2: Adenosine Deaminase Levels in Various Study Groups**

<table>
<thead>
<tr>
<th>S. No</th>
<th>Group – I Normal Control</th>
<th>Group – II NIDDM without Complications</th>
<th>Group – III NIDDM with complications</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>16</td>
<td>17.8</td>
<td>12.12</td>
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<tr>
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<td>13</td>
<td>13</td>
<td>12</td>
</tr>
<tr>
<td>3.</td>
<td>12</td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td>4.</td>
<td>15</td>
<td>14</td>
<td>16</td>
</tr>
<tr>
<td>5.</td>
<td>12</td>
<td>15</td>
<td>13</td>
</tr>
<tr>
<td>6.</td>
<td>13</td>
<td>20</td>
<td>15</td>
</tr>
<tr>
<td>7.</td>
<td>14</td>
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<td>10</td>
</tr>
<tr>
<td>8.</td>
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<td>21</td>
<td>14</td>
</tr>
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<td>15</td>
<td>15</td>
<td>15</td>
</tr>
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<tr>
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<td>16</td>
<td>16</td>
</tr>
<tr>
<td>20.</td>
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<td>13</td>
<td>11</td>
</tr>
<tr>
<td>MEAN</td>
<td>13.800</td>
<td>14.850</td>
<td>13.600</td>
</tr>
<tr>
<td>SD</td>
<td>1.5424</td>
<td>2.7198</td>
<td>2.1126</td>
</tr>
<tr>
<td>SE</td>
<td>0.3449</td>
<td>0.6082</td>
<td>0.4724</td>
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</table>
Serum Creatinine

<table>
<thead>
<tr>
<th>Source</th>
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<th>Sum of Squares</th>
<th>Mean of Square</th>
<th>F-Ration</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between group</td>
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<td>17.73</td>
<td>8.865</td>
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<td>ns</td>
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<tr>
<td>Within group</td>
<td>57</td>
<td>-171.7</td>
<td>3.01</td>
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<td></td>
</tr>
<tr>
<td>Total</td>
<td>59</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

Table 3: Fasting Plasma Glucose (mg/dI)

<table>
<thead>
<tr>
<th>S. No</th>
<th>Group – I Normal Control</th>
<th>Group – II NIDDM without Complications</th>
<th>Group – III NIDDM with complications</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>76</td>
<td>150</td>
<td>280</td>
</tr>
<tr>
<td>2.</td>
<td>60</td>
<td>170</td>
<td>132</td>
</tr>
<tr>
<td>3.</td>
<td>76</td>
<td>180</td>
<td>230</td>
</tr>
<tr>
<td>4.</td>
<td>60</td>
<td>180</td>
<td>285</td>
</tr>
<tr>
<td>5.</td>
<td>68</td>
<td>170</td>
<td>192</td>
</tr>
<tr>
<td>6.</td>
<td>64</td>
<td>150</td>
<td>286</td>
</tr>
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<td>7.</td>
<td>78</td>
<td>250</td>
<td>260</td>
</tr>
<tr>
<td>8.</td>
<td>72</td>
<td>140</td>
<td>78</td>
</tr>
<tr>
<td>9.</td>
<td>90</td>
<td>80</td>
<td>286</td>
</tr>
<tr>
<td>10.</td>
<td>80</td>
<td>90</td>
<td>118</td>
</tr>
<tr>
<td>11.</td>
<td>92</td>
<td>80</td>
<td>187</td>
</tr>
<tr>
<td>12.</td>
<td>76</td>
<td>180</td>
<td>250</td>
</tr>
<tr>
<td>13.</td>
<td>64</td>
<td>148</td>
<td>364</td>
</tr>
<tr>
<td>14.</td>
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<td>75</td>
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<td>60</td>
<td>176</td>
<td>275</td>
</tr>
<tr>
<td>16.</td>
<td>76</td>
<td>180</td>
<td>240</td>
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<td>17.</td>
<td>72</td>
<td>132</td>
<td>118</td>
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<td>18.</td>
<td>68</td>
<td>172</td>
<td>180</td>
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<tr>
<td>19.</td>
<td>68</td>
<td>140</td>
<td>185</td>
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<tr>
<td>20.</td>
<td>74</td>
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<td>238</td>
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<td>21.</td>
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<tr>
<td>22.</td>
<td>80</td>
<td>200</td>
<td>230</td>
</tr>
<tr>
<td>MEAN</td>
<td>72.45</td>
<td>159.00</td>
<td>212.64</td>
</tr>
<tr>
<td>SD</td>
<td>8.662</td>
<td>43.865</td>
<td>74.938</td>
</tr>
<tr>
<td>SE</td>
<td>1.847</td>
<td>9.352</td>
<td>15.977</td>
</tr>
</tbody>
</table>

Serum Creatinine

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Sum of Squares</th>
<th>Mean of Square</th>
<th>F-Ration</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between group</td>
<td>2</td>
<td>127015.23</td>
<td>6250761.50</td>
<td>51.43</td>
<td>P&lt;0.01</td>
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<tr>
<td>Within group</td>
<td>57</td>
<td>70382.00</td>
<td>123477.20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
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REFERENCES
A STUDY OF SERUM ADENOSINE DEAMINASE LEVEL IN PATIENTS WITH TYPE 2 DIABETES MELLITUS AND ITS CORRELATION WITH GLYCEMIC CONTROL

Gohe1 MG*, Sirajwala HB, Kalaria TR, Kamariya CP

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2Professor, Department of Biochemistry, Govt Medical College, Baroda (India)
3Fellow Diabetologist, Dr. Mohan’s Diabetes Specialties Center, Chennai (India)
4Assistant Professor, Department of Biochemistry, P.D.U. Medical College, Rajkot (India)

ABSTRACT
Diabetes mellitus (DM) comprises a group of common metabolic disorders that share common phenotype of hyperglycemia. Adenosine deaminase (ADA) is a cytosolic enzyme of purine metabolism, which has been the object of considerable interest. Present study was undertaken to assess and compare level of serum ADA activity in type 2 DM patients with good and poor glycemic control. FBS, PP 2 BS and HbA1c were estimated as a measure of glycemic control. Further correlation between serum ADA level with FBS, PP 2 BS and HbA1c (markers of short and long term glycemic control) was studied. A cross sectional study consists of 150 patients out of them 50 patients having type 2 DM with good control (Group II), 50 patients with type 2 DM with poor control (Group III) and 50 normal healthy control (Group-I) were selected. Statistically significant increase in serum ADA level in group II and group III cases compare to Group I. There was a statistically significant positive correlation between ADA and FBS, PP 2 BS and HbA1c (markers of glycemic control) in group II and group II (more strong in group III). ADA was also found to be a marker of T cell activation and a producer of reactive oxygen species (ROS). A positive correlation between ADA level with short and long term glycemic control suggest its important role in glucose and lipid metabolic derangements seen in type 2 DM patients. Thus, ADA plays an important role in the pathophysiology of type 2 DM and its complications.

Keywords: Adenosine deaminase, Glycemic control, Type 2 diabetes mellitus

INTRODUCTION
Diabetes mellitus (DM) is a chronic, incurable, costly, and increasing but largely preventable non communicable disease which is responsible for millions of deaths annually, debilitating complications, and incalculable human misery. Thus, understanding the pathogenesis and preventing and/or ameliorating these long-term complications have been major goals of research in diabetes mellitus. Type 2 DM is a heterogeneous group of disorders characterized by variable degrees of insulin resistance, impaired insulin secretion, and increased glucose production. Distinct genetic and metabolic defects in insulin action and/or secretion give rise to the common phenotype of hyperglycemia in type 2 DM.

Hyperglycemia not only defines the disease but is the cause of its most characteristic symptoms and long-term
complications. Assessing glycemia in diabetes has always been a challenge. The monitoring of glycemia is an essential component of diabetic care [3]. Optimal monitoring of glycemic control involves plasma glucose measurements and measurement of glycated hemoglobin. These measurements are complementary: the patient’s glucose measurements provide a picture of short-term glycemic control, whereas HbA1c reflects average glycemic control over the previous 3 months [2]. Because the development of complications is linked to the accumulation of glycation adducts in tissue proteins, any analytical method that serves as an index of the extent of glycation should clearly be used to guide therapy in diabetes. The core of the issue is glycemic control. Amongst the various markers of glycemic control, glycated hemoglobin has now been established as the most reliable [4].

Adenosine deaminase (ADA) (Adenosine Aminohydrolase, EC 3.5.4.4) is an enzyme of purine metabolism. It acts on adenosine and other adenosine nucleoside analogues and catalyze its hydrolytic cleavage into inosine and ammonia. It is a cytosolic enzyme, which has been the object of considerable interest. Adenosine mimics the action of insulin on glucose and lipid metabolism in adipose tissue and the myocardium, while it inhibits the effect of insulin on total hepatic glucose output, which suggests that adenosine, causes local insulin resistance in the liver. Adenosine modulates the action of insulin on various tissues differently and its concentration in tissues is affected by ADA level [5].

Immunological disturbances in type 2 diabetic individuals have an association with cell mediated responses. Adenosine deaminase, an enzyme distributed in human tissues, was considered as good marker of cell mediated immunity [6]. The importance of ADA in the immune system and the role of its interaction with an ADA-binding cell membrane protein dipeptidyl peptidase IV (DPPIV) have attracted the interest of researchers for many years. Inhibitors of the regulatory protease DPPIV are currently under development in preclinical and clinical studies as potential drugs for the treatment of type 2 DM [7].

Present study was undertaken to assess and compare level of serum adenosine deaminase activity in type 2 DM patients with good and poor glycemic control. FBS, PP2BS and HbA1c were estimated as a measure of glycemic control in patients. Further correlation between serum ADA level with FBS, PP2BS and HbA1c (markers of short and long term glycemic control) was studied.

MATERIALS AND METHODS

Study design and subjects

A cross sectional study consists of 150 subjects out of them 50 patients having type 2 DM with good glycemic control (Group II), 50 patients with type 2 DM with poor glycemic control (Group III) and 50 normal healthy control (Group-I) were selected. Subjects were recruited according to simple random sampling method meeting the selection criteria. This study was conducted at SSG hospital and medical college, Vadodara (India).

Selection criteria

Inclusion Criteria:
Group I – Control group (n=50)
This group consisted of age and sex matched healthy subjects. They were free from any ailment which could affect the parameters under study. They were taken from general population.
Group II – Type 2 DM with good glycemic control (n=50)
This group consisted of patients with type 2 DM with duration less than 8 years. They were on life style modifications and oral hypoglycemic drugs and free from clinical evidence of any microvascular complication. Glycated hemoglobin (HbA1C) level was less than 7%. Group III – Type 2 DM with poor glycemic control (n=50)
This group consisted of patients with type 2 DM with duration less than 8 years. They were on life style modifications and oral hypoglycemic drugs and free from clinical evidence of any microvascular complication. Glycated hemoglobin (HbA1C) level was less than 7%.
Group III – Type 2 DM with poor glycemic control (n=50)
This group consisted of patients with type 2 DM with duration more than 8 years.
years. They were on life style modifications, oral hypoglycemic drugs, insulin or combination of all three and associated with one or more microvascular complication(e.g. diabetic nephropathy, diabetic retinopathy, heart disease, diabetic neuropathy). Glycated hemoglobin (HbA1C) level was more than 7%.

**Exclusion Criteria:** The patients with type 1 DM, hemolytic anaemia, hemoglobin variants, pregnancy, hepatic disease and infectious diseases like tuberculosis, sarcoidosis.

**Ethical Considerations**

The Institution’s Ethical Committee approval was obtained prior to the enrolment of subjects. The objectives of study were explained to all eligible subjects for this study. Informed consent of all subjects included in the study was obtained for involvement in study groups and for venipuncture. Emphasis was given that participation in this study was voluntary.

**Questionnaire and blood Sample collection**

The questions mainly focused on age of patients, duration, type, mode of anti-diabetic therapy and any complication of diabetes. A 5 ml of venous blood was drawn from each volunteer using a disposable vacutainer system in fasting condition (plain, EDTA and fluoride). Post prandial (2 hour) sample collected in fluoride vacutainer for PP2 BS estimation. Serum or plasma separated within half an hour and stored at 2-8° C till analysis was done.

**Analysis of Sample**

Fasting and Post prandial (2 hour) blood sugar (FBS & PP2 BS) estimated by Glucose Oxidase-Peroxidase (GOD-POD) enzymatic end point method. (Kit: Quantitative determination by glucose oxidase peroxidase method, Mfg by Spinreact)\(^8\). Glycated hemoglobin (HbA1C) concentration was measured by Immuno-turbidimetric method (Kit: Quantitative determination of glycated hemoglobin in human blood by latex turbidimetry Mfg by Spinreact)\(^9\). Serum ADA activity was measured by end point colorimetric method. (Kit: Quantitative determination of serum ADA activity by colorimetric end point method Mfg by Tulip diagnostics)\(^10\). Other biochemical parameters like total cholesterol, triglycerides, serum creatinine and ALT were also estimated. All biochemical investigation performed on fully automatic analyzer I.S.E. srl MIURA. Fundoscopy and Electrocardiogram were done in respective department.

**Statistical Analysis**

The data collected during the current study were recorded and analyzed statistically to determine the significance of different parameters by using SPSS package for windows version 16.0. Statistical analysis was done by using t-test to find out significance of difference between two groups and correlation coefficient to find out statistical correlation between two variables and its significance. Interpretation was done according to p-value as follows:

- \(p < 0.05\) was considered significant
- \(p \geq 0.05\) was considered not significant

**RESULTS**

Results of the study shows mean FBS, PP2 BS and HbA1c concentration was significantly higher in group III compared to group II and group I (table 1). Further the difference between mean serum ADA, FBS, PP2 BS, HbA1c value between groups is statistically significant (with p value <0.0001) (table 2). Among Group II and Group III cases, the prevalence of increased serum ADA value above the upper reference limit was 80% and 92% respectively compared to 4% in group I (healthy control) (table 3). Also serum ADA is significantly and positively correlated with FBS, PP2 BS and HbA1c concentration in group II and group III. This correlation was specifically strong in group III (table 4, figure 1,2,3).
Table 1: Comparison of FBS, PP2BS, HbA1c and serum ADA level between study groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting blood sugar (FBS) (mg/dl)</td>
<td>90.68±14.58</td>
<td>126.86±26.14</td>
<td>194.26±56.59</td>
</tr>
<tr>
<td>Post prandial blood sugar (PP2BS) (mg/dl)</td>
<td>113.18±14.18</td>
<td>156.98±26.65</td>
<td>274.08±63.00</td>
</tr>
<tr>
<td>Glycated Hemoglobin (HbA1c) (%)</td>
<td>5.48±0.48</td>
<td>6.37±0.43</td>
<td>8.19±1.02</td>
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<tr>
<td>Adenosine deaminase (ADA) level (U/l)</td>
<td>20.64±8.56</td>
<td>54.82±19.71</td>
<td>89.08±34.51</td>
</tr>
</tbody>
</table>

Results are expressed as mean±SD.

Table 2: Independent samples t-test study: Serum ADA, HbA1c, FBS and PP2BS between study groups

<table>
<thead>
<tr>
<th>Study groups</th>
<th>Serum ADA</th>
<th>HbA1c</th>
<th>FBS</th>
<th>PP2BS</th>
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<tbody>
<tr>
<td></td>
<td>t value</td>
<td>p value</td>
<td>t value</td>
<td>p value</td>
</tr>
<tr>
<td>I and II</td>
<td>11.93</td>
<td>&lt;0.0001</td>
<td>9.745</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>II and III</td>
<td>6.09</td>
<td>&lt;0.0001</td>
<td>11.62</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>I and III</td>
<td>13.61</td>
<td>&lt;0.0001</td>
<td>16.94</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

p value is two tailed probability value and t value is test statistic t

Table 3: Prevalence of increased Serum ADA level in study groups (in %)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal ADA (%)</td>
<td>96</td>
<td>20</td>
<td>08</td>
</tr>
<tr>
<td>High ADA (%)</td>
<td>04</td>
<td>80</td>
<td>92</td>
</tr>
</tbody>
</table>

Table 4: Correlation of ADA with glycemic control (HbA1c, FBS and PP2BS)

<table>
<thead>
<tr>
<th>Correlation between</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum ADA (U/l) and FBS (mg/dl)</td>
<td>Correlation coefficient r</td>
<td>0.0612</td>
<td>0.3365</td>
</tr>
<tr>
<td></td>
<td>Significance (p value)</td>
<td>0.6728</td>
<td>0.0169</td>
</tr>
<tr>
<td>Serum ADA (U/l) and PP2BS (mg/dl)</td>
<td>Correlation coefficient r</td>
<td>0.1788</td>
<td>0.2581</td>
</tr>
<tr>
<td></td>
<td>Significance (p value)</td>
<td>0.2141</td>
<td>0.0382</td>
</tr>
<tr>
<td>Serum ADA (U/l) and HbA1c (%)</td>
<td>Correlation coefficient r</td>
<td>0.0812</td>
<td>0.3687</td>
</tr>
<tr>
<td></td>
<td>Significance (p value)</td>
<td>0.5239</td>
<td>0.0084</td>
</tr>
</tbody>
</table>
Table 5: Comparison of other variables between study groups

<table>
<thead>
<tr>
<th></th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Sex (M/F) %</td>
<td>60/40</td>
<td>44/56</td>
<td>56/44</td>
</tr>
<tr>
<td>Average age (years)</td>
<td>56±7.12</td>
<td>55±10.7</td>
<td>59±8.6</td>
</tr>
<tr>
<td>Average duration of DM (in year)</td>
<td>-</td>
<td>5.11±1.7</td>
<td>12.28±3.4</td>
</tr>
<tr>
<td>Prevalence of Hypertension (in %)</td>
<td>-</td>
<td>62</td>
<td>82</td>
</tr>
<tr>
<td>Prevalence of smoking (in %)</td>
<td>-</td>
<td>26</td>
<td>50</td>
</tr>
<tr>
<td>Average Height (cm)</td>
<td>155.9±8.03</td>
<td>154.3±4.22</td>
<td>157.4±4.41</td>
</tr>
<tr>
<td>Average weight (kg)</td>
<td>57.96±4.535</td>
<td>63.6±5.67</td>
<td>66.6±5.78</td>
</tr>
<tr>
<td>Average BMI (kg/m²)</td>
<td>24.16±5.136</td>
<td>26.77±2.877</td>
<td>26.86±2.476</td>
</tr>
<tr>
<td>Mean Serum total cholesterol (mg/dl)</td>
<td>148±25.9</td>
<td>193.32±51.5</td>
<td>201.76±58.2</td>
</tr>
<tr>
<td>Mean Serum triglycerides (mg/dl)</td>
<td>103.36±23.2</td>
<td>130.98±32.1</td>
<td>140.56±65.2</td>
</tr>
<tr>
<td>Mean Serum Creatinine (mg/dl)</td>
<td>0.73±0.12</td>
<td>0.87±0.1</td>
<td>1.92±1.5</td>
</tr>
<tr>
<td>Mean Serum ALT (U/L)</td>
<td>19±6</td>
<td>21.46±7.95</td>
<td>43.11±74.8</td>
</tr>
</tbody>
</table>

Figure 1: Correlation between serum ADA level and HbA1c concentration in patients

![Correlation between serum ADA level and HbA1c concentration in patients](image-url)
DISCUSSION
This study shows increased level of serum ADA level in patient with type 2 DM and it further increased in patient with type 2 DM with poor glycemic control. Also serum ADA level is positively correlated with FBS, PP2BS and HbA1c concentration in diabetes mellitus patients. This correlation was specifically strong in diabetes patients with poor glycemic control. Poor glycemic control in group III
patients was evidenced by mean FBS, PP2BS and HbA1c values.

Adenosine deaminase is an enzyme of purine metabolism. It acts on adenosine and several other adenosine nucleoside analogues. It was concluded that increased adenosine activity mimics the activity of insulin on glucose and lipid metabolism in adipose tissue. Also, it was found to be a marker of T cell activation and a producer of reactive oxygen species (ROS). Immunological disturbances in type 2 diabetic individuals have an association with cell mediated responses and inappropriate T-lymphocyte function, which is vital in this pathogenic condition, has a link with insulin defect. Adenosine deaminase was considered as good marker of cell mediated immunity. Because ADA is associated with T-lymphocyte activity; its altered blood levels may help in predicting immunological dysfunction in diabetic individuals and might be one of the important biomarkers in predicting diabetes mellitus[11].

ADA was also found to increase lipid peroxidation. This indicated increased oxidative stress with increased levels of ADA. The proposed mechanisms for increased oxidative stress can be,

a. Role of ADA as a marker of T cell activation
b. Its relation to the production of ROS with the production of NO, O2, H2O2 and OH.
c. Plasma ADA amplifies the release of toxic oxygen radicals from neutrophils through a down regulation of the inhibitory adenosine-c-AMP system.
d. Increase in ADA activity is also known to increase superoxide production through xanthine oxidase.
e. Oxygen free radicals formed by glucose over and above the detoxifying capacity of erythrocytes can cause peroxidative breakdown of phospholipid fatty acids and accumulation of malonyldialdehyde. This results in an increased movement of phosphatidylserine and phosphatidylethanolamine from the inner bilayer to the outer bilayer[5-6].

Various studies show altered Adenosine deaminase level in type 2 DM. The values are varies but many of them shows increased adenosine deaminase activity in type 2 diabetes mellitus patients. Kurtul N et al have shown increased level of serum ADA activity in type 2 DM patients and its correlation with HbA1c and suggested that ADA is an important enzyme for modulating the bioactivity of insulin. Also suggest that ADA play important role in insulin effect and glycemic control. Increased activity of ADA might be marker for insulin. However further studies are required for the pathogenic role of elevated ADA activity in type 2 DM[12]. Hoshino T. et al also suggested that mean serum level of ADA1 and ADA2 level is high in NIDDM (noninsulin dependent diabetes mellitus) and IDDM (Insulin dependent diabetes mellitus) than healthy donor (higher in NIDDM than IDDM). ADA2 activity in the poorly controlled NIDDM patients directly correlated with the HbA1c level[13].

ISI Ogbu et al. has studied adenosine deaminase activities and fasting blood glucose in obesity and shown rise in ADA activities in obesity which may be due to insulin resistance or increased secretion of adenosine[14]. M Shivaprakash et al observed significant increase in adenosine deaminase activity in diabetic subjects and hypothesizes that increased ADA activity may be due to altered immunity. Therefore, ADA may serve as an immunoenzyme marker in the aetio-pathology of type 2 DM[11]. Anjali C. Warrier et al has shown increased ADA activity and its correlation with hyperglycemia (glycated hemoglobin) and lipid peroxidation in DM patients. They suggested that decreased tissue adenosine levels is due to increase in ADA activity, is related to the severity of hyperglycemia and lipid peroxidation in diabetes mellitus[5]. Gitanjali G, et al reported elevated level of serum ADA activity in DM type 2 patient and correlated it with
markers of lipid peroxidation. They concluded that hyperglycemia aggravates oxidative stress, as well as increased levels of adenosine deaminase in diabetes, which plays an important role in DM, which may be because of local insulin resistance in the target organs and also because of the increased production of free radicals and oxidative stress\[6\].

Physiological roles of ADA can be seen in connection with adenosine whose concentration can be modulated by enzymatic action of ADA. Adenosine is both a metabolic precursor for nucleic acids and a significant signaling molecule involved in regulation of various physiological processes which linked to its localized release (extracellular adenosine)\[15\]. Adenosine modulates the action of insulin on various tissues differently and its concentration in tissues is affected by ADA levels. It mimics the action of insulin on glucose and lipid metabolism in adipose tissue and the myocardium, while it inhibits the effect of insulin on total hepatic glucose output, which suggests that adenosine, causes local insulin resistance in the liver. In liver, increased ADA level will decrease the glucose output. Adenosine potentiated insulin and contraction stimulated glucose transport in skeletal muscles by enhancing the increase in GLUT-4 at the cell surface and raised the possibility that decreased adenosine production or action could play a causative role in insulin resistance. Increased ADA activity has also been observed in white adipocytes isolated from diabetic rats\[5\]. Adenosine deaminase has also been shown to impair the insulin sensitivity for glucose transport and antilipolysis by inactivating extracellular adenosine, which adipocytes release spontaneously\[15\].

In the present study, the values of ADA were significantly higher in cases compared to controls. It suggests that ADA plays a role in the pathophysiology of type 2 DM and its complications through biochemical mechanisms mentioned above. A positive correlation between ADA level with short and long term glycemic control suggest its important role in glucose and lipid metabolic derangements seen in type 2 DM patients. The limitation of this study is that the exact role of adenosine deaminase in the pathogenesis of diabetes mellitus is still not clear. Also large extended prospective study will be required which includes ADA, insulin, immunological markers and marker of oxidative stress. Further studies at molecular level are required to know the role of ADA levels in modifying the effect of insulin and oxidative stress in DM.

ACKNOWLEDGEMENTS
Authors gratefully acknowledge all participants, staff of diabetic OPD and clinical chemistry laboratory for technical help and cooperation.

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Original Article

Role of Adenosine Deaminase to Predict Glycemic Status in Type 2 Diabetes Mellitus

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Sri Devaraj Urs Medical College, Kolar

ABSTRACT

Introduction: Diabetes mellitus (DM) is a heterogeneous disease characterized by an abnormal or relative deficiency of insulin and insulin resistance. Adenosine Deaminase (ADA) is an enzyme involved in purine metabolism. Literature demonstrates that in the patients with type 2 DM, the level of ADA is higher than that of non-diabetics.

Objectives: 1. To estimate the serum ADA in patients with type 2 DM.
2. To correlate ADA levels with HbA1c (for glycemic status) in patients with type 2 DM.

Methods: The present study is a case control study. It includes 101 subjects, including 51 cases of type 2 diabetes mellitus and 50 age and sex matched healthy controls. Adenosine Deaminase, HbA1c, fasting and postprandial blood glucose levels were measured and the results were compared with controls.

Results: An elevation of serum ADA was found in diabetic subjects as compared to controls. Mean and Standard deviation of serum ADA in cases and controls was 32.06 ± 17.09 and 19.28 ± 5.59 respectively. Serum ADA is significantly higher in cases as compared to controls (p<0.001). ADA activity is correlated with fasting blood glucose (r =0.694, p < 0.001), postprandial blood glucose (r = 0.652, p<0.001) and HbA1c (r =0.290, p<0.05). Correlation is positive and significant.

Conclusion: Serum ADA is elevated in the patients with diabetes as compared to controls. This reflects that serum ADA can be considered as a marker of glycemic status in the diabetic population.

Keywords: Adenosine, Adenosine Deaminase, Blood glucose, HbA1c, Type 2 diabetes mellitus.

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INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from abnormal or relative deficiency of insulin and insulin resistance. The latest WHO Global Burden of Disease estimates the worldwide burden of diabetes in adults to be around 173 million in the year 2002. Around two thirds of these live in developing countries.
Diabetes is no longer a condition of developed, 'industrialized' or 'Western' countries.\(^1\)

Type 2 diabetes is characterized by insulin resistance where there is impaired ability of the hormone to suppress hepatic glucose output and to promote peripheral glucose disposal and compromised function of pancreatic \(\beta\) cells such that insulin secretion is insufficient to match the degree of insulin resistance.\(^2\)

The prevalence of obesity and type 2 DM is increasing rapidly. Obesity and type 2 diabetes are associated with metabolic alterations, such as elevated plasma fatty acids and a reduced ability of insulin to suppress lipolysis, which lead to the accumulation of intramyocellular lipid. This accumulation of lipid within muscle cells has been linked to the development of insulin resistance.\(^2\)

ADA (Adenosine Deaminase) is an enzyme involved in purine metabolism. ADA is an enzyme that converts adenosine into inosinethrough an irreversible deamination reaction.\(^3,4\)

Literature suggests that the levels of adenosine are reduced by ADA. Adenosine is called as a retaliatory metabolite. It has an anti-lipolytic property and through this effect it reduces free fatty acid level. Adenosine Deaminase increases basal and noradrenaline stimulated lipolysis in adipocytes.\(^5-7\)

Studies have shown that in the patients with type 2 DM, the level of ADA is higher than that of non-diabetics.\(^3,8,9,10\) In patients with type 2 diabetes, insulin administration has been shown to reduce the elevated ADA levels.\(^11\)

The half life of serum ADA is about 30 minutes.\(^12\) Adenosine metabolism also exhibit significant diurnal variations in human blood. In humans adenine nucleotides undergo important changes during the dark period, mainly decrease in ATP and enhancement of ADP and AMP levels. This observation might represent a local metabolic adjustment in the energy status of blood cells. Blood adenosinedeaminase shows a major peak at 08:00 hr, with almost no changes throughout the rest of the 24 hr. Adenosine-metabolizing enzymes functions as a chemical messenger or a metabolic modulator. Thus there are temporal variations in metabolites and enzymatic activities related to adenosine metabolism in the blood of human volunteers.\(^13\)

In addition to its association with diabetes, serum ADA activity is also increased in patients with liver cirrhosis as well as in patient with infectious diseases such as hepatitis, tuberculosis, brucellosis, and typhoid fever. The adipocytes produce large amounts of inflammatory cytokines than normal. Immune cells are already present in the close proximity of adipocytes and macrophages easily infiltrate the adipose tissues. This inflammation is also associated with insulin resistance. Adenosine is an endogenous regulator of many different functions of immune system. Adenosine receptors can also be drug targets in adipose tissue to suppress the underlying inflammation in obesity and increase insulin sensitivity.\(^10,5\)

The concentration of intracellular and extracellular adenosine is regulated by Adenosine Deaminase. There has been increase in the expression of ADA in the conditions like hypoxia which lead to elevated adenosine formation and release.\(^14\)

Adenosine exerts potent anti-lipolytic effects through the A1 receptor which is the only adenosine receptor expressed in the adipose
tissue. A1 receptor agonists, through its anti lipolytic property decrease free fatty acid levels hence increase insulin sensitivity.\cite{6}

The elevation of serum ADA in type 2 DM is explained through extracellular cyclic AMP adenosine pathway. The present study aims to estimate the serum ADA in patients with type 2 DM and to correlate ADA levels with HbA1c (for glycemic status) in patients with type 2 DM.

**MATERIALS AND METHODS**

The present study is a hospital based case control study. The study group and controls are selected from patients and healthy individuals visiting the outpatients/clinical lab and Inpatients of RL Jalappa Hospital and Research Centre, Kolar, India. This study includes 101 subjects of which 51 are cases and 50 are controls.

**Inclusion Criteria:** Clinically diagnosed cases of type 2 diabetes mellitus are included in the study and this is based on 2010 American Diabetic Association criteria.\cite{15} Cases are in the age group of 35-75 yrs and are on oral hypoglycemic drugs. Any patient with history of type 2 DM for more than 5 years are included in the study. Age and sex matched physically healthy volunteers with no history of diabetes mellitus or any other chronic diseases are selected as controls. A thorough clinical examination and appropriate investigations were done before selecting the cases and controls for the study.

**Exclusion Criteria:**

1. Non diabetic cases.
2. Patients with type 2 diabetes mellitus with any other concurrent chronic disease such as Cardiac diseases, thrombotic stroke, Tuberculosis, Rheumatoid Arthritis, Sarcoidosis, Gout, Renal failure or any other condition which alters the ADA levels in the serum.

Institutional ethical committee clearance was taken before the start of the study. Informed consent was taken from all the subjects. Relevant investigations (FBS, PPBS and HbA1c) were done before selection of the subjects for the study. BMI was calculated using the formulae weight (kg)/height (m^2).\cite{16} The estimations of ADA were done using the ADA-MTB kit from Micro express, a division of Tulip Diagnostics (P) Ltd by Colorimetric method described by Giusti and Galanti.\cite{17} Estimation of blood glucose was done by glucose oxidase/peroxidase method.\cite{18} Estimation of HbA1c was done by weakly binding cation exchange resin method.\cite{19}

**STATISTICAL ANALYSIS**

Statistical Analysis was done by student 't' test using SPSS windows version 10.0 software and results were expressed as mean ± SD. Pearson's bivariate correlation analysis was used to correlate each variable with ADA activity. p values less than 0.05 was considered statistically significant.

**RESULTS**

As depicted in the figure 1, the mean ± SD of Age (yrs.) in cases and controls is 49.12 ± 9.177 and 48.4 ± 8.822 respectively and they are age matched. As shown in figure 2, the
Fig. 1: Age distribution in cases and controls

Fig. 2: Gender distribution in cases and controls

Fig. 3: Comparison of ADA with HbA1c
Fig. 4: Pearson's correlation of ADA with HbA_{1c} in study group

Fig. 5: Pearson's correlation of blood glucose parameters with ADA in study group
Fig. 5: Pearson's correlation of blood glucose parameters with ADA in study group

Fig. 6: Adipocyte dysfunction in diabetes and obesity

[^27]:
cases and controls are gender matched. Among the cases 45.1% are females and 54.9% are males and among the controls 46% are females and 54% are males. Table 1 shows mean and SD of FBS is 192.92 ± 102.75 (cases) and 98.36 ± 17.90 (controls), of PPBS is 261.24 ± 109.05 (cases) and 142 ± 35.16 (controls), of HbA1c is 8.38 ± 4.00 (cases) and 5.24 ± 0.62 (controls), of ADA is 32.06 ± 17.09 (cases) and 19.28 ± 5.59 (controls) respectively. FBS, PPBS, HbA1c and ADA is significantly higher in cases as compared to controls (p<0.001). As depicted in figure 3, patients with type 2 DM are divided into two groups, those with HbA1c < 10% and those with HbA1c > 10%. For HbA1c < 10%, the Mean and SD of ADA is 22.41 ± 9.7 and for HbA1c > 10%, the Mean and SD of ADA is 41.32 ± 16.74 with p < 0.05. Figure 4 shows that ADA activity was correlated with HbA1c (r = 0.290, p < 0.05) and the correlation is positive and significant. Figure 5 shows that ADA activity was correlated with FBS (r = 0.694, p < 0.001) and PPBS (r = 0.652, p < 0.001) and a large positive correlation is found between blood glucose values and ADA and it is significant.

**DISCUSSION**

Reports from the previous studies done by Reddy M et al showed that as compared to the control group, ADA activity in type 2DM patients was higher. When glycemic control was relatively good, ADA activity was low. In line with previous reports done by other researchers, ADA activity in type 2DM patients in the present study was significantly higher than that in the control group( mean ± SD of 32.06 ± 17.09 vs. 19.28 ± 5.59, p < 0.001) as depicted in table 1. Moreover, compared to that of type 2 DM patients with relatively good glycemic control (HbA1c< 10%), the ADA activity in type 2DM patients with poor glycemic control (HbA1c> 10%) was significantly lower. These results are depicted in the figure 3 and they are consistent with those reported by Lee JG et al. Previously, in a study conducted by Shivaprakash M et al on a group of thirty-six adult patients of either sex who had history of not
less than six years of diabetes mellitus and equal number of healthy non-diabetics as controls, showed a significant (p < 0.001) increase in adenosine deaminase activity with a mean ± SD of 37.2 ± 9.29 U/l in diabetic subjects when compared to controls who had normal mean ± SD values of 18.2 ± 5.6 U/l.\[9\]

As shown in figure 5, a large positive and significant correlation exists between the blood glucose levels and serum ADA level with r = 0.6 and p < 0.001. A positive correlation is also found between long term index of glycemic control i.e. glycated hemoglobin (HbA1c) and serum ADA as depicted in figure 4. This finding is in agreement with some of the previous studies which show significant correlation between HbA1c and ADA levels.\[3, 20, 21, 22]\]

In the analysis of all patients, ADA activity did not have a significant correlation with age, gender or duration of diabetes.\[23, 24]\]

In addition, in a study conducted by Pawelczyk T et al found that the serum ADA levels return to normal following insulin infusion.\[11]\] However in the present study such intervention of the effect of insulin treatment on serum ADA levels could not be tested because of the inclusion criteria as defined in the selection of subjects.

As shown in figure 6, excess postprandial lipids and glucose circulate through the blood stream and are taken up by the pancreas, the liver, and adipose tissue. The adipocyte stores triglycerides in the lipid droplet, leading to adipocyte hypertrophy. These exposures in excess lead to cellular dysfunction increased circulating free fatty acids and a proinflammatory state. Exposing the hepatocytes to excess fats and carbohydrates leads to steatohepatitis and insulin resistance. Thus, there is elevation of free fatty acid levels in diabetes due to increased lipolysis which leads to worsening insulin resistance and beta cell failure.\[25, 26, 27]\]

Adenosine is an endogenous purine nucleotide that modulates many physiological processes. Cellular signaling by adenosine occurs through four known adenosine receptor subtypes (A1, A2A, A2B, and A3).\[6, 28]\]

Endogenous adenosine is supposed to be an important regulator of adipose tissue metabolism by increasing the sensitivity of adipocyte glucose transport and oxidation and by inhibiting lipolysis potently. Pharmacological studies have suggested that the antilipolytic effect of adenosine is mediated via the A1 receptor. In addition, it has also been shown that the basal levels of endogenous adenosine are sufficient to cause inhibition of lipolysis. Stimulation of inhibitory Gi protein-coupled receptors leads to inhibition of adenylyl cyclase and decreased cAMP levels and lipolysis. Increased lipolysis is associated with increased levels of cyclic AMP (cAMP).\[6, 29]\]

It has been shown that in the adipose tissue most of the adenosine is formed extracellularly. Extracellular adenosine is metabolized by adenosine deaminase. Thus, increased formation of adenosine leads to increased adenosine deaminase. The source of extracellular formation of adenosine is via an enzymatic cascade for the breakdown of ATP, ADP and AMP which appears to be the major mechanism that leads to elevated extracellular
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adenosine. Also there is accumulating evidence that cAMP, a well-known intracellular second messenger may also be adenosine precursor. Extracellular conversion of cAMP to adenosine is achieved by sequential activities of ectophosphodiesterase and CD73. Thus CD73 appears to be an important point of convergence for the extracellular formation of adenosine derived from ATP or cAMP. The concept of extracellular cAMP-adenosine pathway has been studied in many cell types but was explored in most detail in kidney. The effect of extracellular conversion of cAMP to adenosine would there by depend on the type of adenosine receptors expressed on the target and neighboring cells, where A1/A3 receptors attenuate cAMP levels and A2A and A2B receptors would increase cAMP levels.\(^{[30,31]}\)

The presence of extracellular cAMP adenosine pathway is suggested by three findings. First cAMP exists in isolated adipocytes in response to beta adrenoceptor and adenylylatecyclase stimulation. Second cAMP is metabolized to AMP and adenosine in adipocyte plasma membranes by ecto-phosphodiesterase and 5′nucleotidaserespectively. Third, cAMP and adenosine appear in the extracellular fluid of adiposetissue. Studies have shown, when increasing doses of cAMP were perfused into adiposetissue through micro dialysis, corresponding levels of AMP and adenosine increased.\(^{[32,33]}\)

Adenosine exerts its protective effects by inhibiting lipolysis through A1 receptors. Adenosine deaminase inactivates adenosine and hence activates lipolysis and markedly potentiates the increase in cAMP accumulation due to norepinephrine.\(^{[6,28]}\) As discussed in the pathophysiology of diabetes, deregulated fat metabolism and consequent elevation of free fatty acids leads to subsequent development of type 2 Diabetes Mellitus.\(^{[26,27]}\)

CONCLUSION

A case-control study using age and sex matched controls is used to evaluate serum Adenosine Deaminase (ADA) levels in patients with type 2 DM. Serum levels of ADA were found to be significantly higher in type 2DM when compared to controls. ADA activity was positively correlated with HBA1c and blood glucose values. Age, gender and duration of diabetes were not found to significantly influence the ADA level.

Though there is a clear cut elevation of serum ADA in type 2 diabetes mellitus, due to short half life and diurnal variations of ADA, the role of serum ADA as a marker of glycemic status in patients with type 2 diabetes mellitus need further studies.

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Source of Support: Nil Conflict of Interest: Nil
Performance Evaluations

AS A BREAST CANCER MARKER

ADA-MTB®
For the determination of ADA activity in serum, plasma and biological fluids
ACTIVITIES OF SERUM ADA, GGT AND ALP IN CARCINOMA BREAST-A CASE CONTROL STUDY FOR DIAGNOSTIC AND PROGNOSTIC SIGNIFICANCE

ARCHANA CHOUDHARI, PRAKASH DESAI¹, INDUMATI V², SUMANGALA KADI³

ABSTRACT

AIM: To assess the clinical utility of Serum adenosine deaminase, gamma glutamyl transferase and alkaline phosphatase in carcinoma breast patients for diagnostic and prognostic purpose. MATERIALS AND METHODS: Thirty clinically and histopathologically confirmed female patients of the age group of 30-65 years served as cases and 30 normal healthy females in the same age group served as controls. The parameters were estimated by standard biochemical methods. RESULTS: The activities of serum ADA, GGT and ALP were significantly increased in carcinoma breast patients when compared to controls. When all the 4 stages of carcinoma breast were compared with controls ADA and GGT were increased significantly. Whereas ALP showed a significant increase only in stage II, III and IV. Interstage comparison yielded a steady and progressive increase in the activities of these enzymes from stage I-IV. CONCLUSION: The study concludes that enzyme markers like serum ADA and GGT could be sensitive, specific and cost effective biomarkers for diagnosing carcinoma breast and for monitoring its progression. Serum ALP level can be used as important biomarker for detecting metastasis and for differentiation of carcinoma breast with and without metastasis.

Key words: Adenosine deaminase, alkaline phosphatase, carcinoma breast, gamma glutamyl transferase.

INTRODUCTION

Despite centuries of theoretical meanderings and scientific inquiry carcinoma breast with its uncertain etiology remains one of the most dreaded of human diseases and is one of the common causes of death in many developed countries in middle aged women. About 9,00,000 women are diagnosed every year with the disease and it causes 5,19,000 deaths per year worldwide.[1] The incidence of carcinoma...
breast is increasing even in developing countries like India due to change in lifestyle like late marriage, birth of the first child at a later age and shorter period of breastfeeding and industrialization. The incidence among Indian women is 23.2 per 1,00,000 population and is the second most common cancer in females in India next only to carcinoma cervix.\(^2\)
The story of efforts to cope with carcinoma breast is complex, and there is no happy ending as in diseases for which cause and cure have not been found. However progress has been made in lessening the horrors that formerly devastated the body and psyche.

Breast cancer that is detected early can potentially be cured when the tumor is small enough which can be surgically removed completely. So for the early detection of carcinoma breast a number of biochemical markers have been studied, however the analytical methods of many of these are unapproachable for general population as the facilities for these are available only at sophisticated and well equipped centres with latest technology and they are expensive.\(^3\) In view of this the present study is undertaken at providing some of the promising enzyme markers namely adenosine deaminase(ADA), gamma glutamyl transferase(GGT) and alkaline phosphatase(ALP) which are inexpensive, accurate, identified by easy method of detection and validated that may be of diagnostic and prognostic significance.

**MATERIALS AND METHODS**

The present study comprised 30 clinically and histopathologically confirmed female patients of carcinoma breast in the age group of 30-65 years admitted to the hospital inpatient ward as cases and 30 normal healthy females in the same age group served as controls. The study was conducted over a period of one year. Ethical clearance was obtained from the institutional ethical committee. An informed consent was obtained from all the participants before collecting the blood samples.

**Criteria for selecting patients with carcinoma breast**

**Inclusion criteria:** Patients with clinically and histopathologically confirmed carcinoma breast were included in the study. The patients of carcinoma breast selected for the interventional group were of 4 stages, stage I \((n=6)\), stage II \((n=8)\), stage III \((n=7)\) and stage IV \((n=9)\). The staging was done according to TNM staging of carcinoma breast based on clinical observation related to tumor size (T), involvement of regional lymph nodes (N) and metastasis (M).\(^4\)

**Exclusion criteria:** Patients suffering from tuberculosis, rheumatic fever, hemolytic anemia, jaundice and hepatobiliary disease, bone diseases, pancreatic disease, congestive cardiac failure, myocardial infarction, ulcerative colitis, other malignancies and patients who had already received or were under treatment for malignancy were excluded from study.

About 3 ml of venous blood was collected from antecubital vein under aseptic precautions and was allowed to clot. Serum was separated by centrifugation to estimate ADA, GGT and ALP. The estimation of parameters was carried out immediately. Serum ADA was estimated by colorimetric method of Galanti.
and Giusti,[5] using ADA-MTB Microxpress Tulip Diagnostics(P) Ltd Kit, serum GGT by Szacz and Rosalki method,[6] using kits supplied by Tecodiagnostics, Anaheim, CA 92807, USA and serum ALP by Kind and King’s method,[7] using kits of Span Diagnostics Ltd. The above mentioned parameters were recorded as mean and standard deviation. Statistical analysis of all the obtained parameters in patients of carcinoma breast and control groups were done by student’s ‘t’ test and ANOVA. The comparison between the controls and various stages of Carcinoma breast was done using Dunnet’s test. Interstage comparison of above mentioned parameters was done by multiple comparison test of Bonferroni. The observations were tabulated and conclusions were obtained by biochemical data.

RESULTS

In our study a highly significant increase in mean serum ADA and GGT was observed in carcinoma breast patients when compared to controls (P < 0.000) as shown in [Table 1]. When the serum ADA and GGT levels of various stages of Carcinoma breast were compared with controls, stage I showed a significant increase (P < 0.001), further in stage II, III and IV the raise was highly significant (P < 0.000) as shown in [Table 2].

Comparison of stage I with stage II, III and IV showed a non significant increase in stage II (P > 0.05) but highly significant increase in stage III and IV (P < 0.000). Comparison of stage II with stage III and IV showed a highly significant increase in both stage III and IV (P < 0.000). Comparison of stage III with IV yielded a significant increase in stage IV (P < 0.05).

A highly significant increase in serum ALP of carcinoma breast was observed when compared to controls (P < 0.000) [Table 1]. When compared to controls stage I showed a non-significant increase (P > 0.05) where as raise in stage II, III and IV was significant (P < 0.000) [Table 2]. Comparison of stage I with stage II, III and IV yielded a non-significant increase (P > 0.05) in stage II and III and a significant increase in stage IV (P < 0.000). Comparison of stage II with stage III and IV showed a significant increase in stage IV (P < 0.000).

Table 1: Comparison of serum levels of ADA, GGT and ALP between controls and carcinoma breast cases and their ‘P’ values

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Controls (n = 30)</th>
<th>Cases (n = 30)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADA (U/L)</td>
<td>21.60±4.62</td>
<td>51.26±9.05</td>
<td>&lt;0.000</td>
</tr>
<tr>
<td>GGT (IU/L)</td>
<td>21.10±8.31</td>
<td>51.48±16.13</td>
<td>&lt;0.000</td>
</tr>
<tr>
<td>ALP (KA UNITS)</td>
<td>6.52±2.12</td>
<td>15.19±4.48</td>
<td>&lt;0.000</td>
</tr>
</tbody>
</table>

The values are expressed as their Mean ± SD

Table 2: Comparison of serum levels of ADA, GGT and ALP between controls and different stages of carcinoma breast patients and their ‘P’ values

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Controls (n = 30)</th>
<th>Stage I (n = 6)</th>
<th>Stage II (n = 8)</th>
<th>Stage III (n = 7)</th>
<th>Stage IV (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADA (U/L)</td>
<td>21.60±4.62</td>
<td>40.93±1.06*</td>
<td>45.29±2.53**</td>
<td>56.19±7.45**</td>
<td>58.99±6.32**</td>
</tr>
<tr>
<td>GGT (IU/L)</td>
<td>21.10±8.31</td>
<td>38.71±5.30*</td>
<td>39.45±7.74**</td>
<td>40.40±10.9**</td>
<td>67.68±8.13**</td>
</tr>
<tr>
<td>ALP (KA UNITS)</td>
<td>6.52±2.12</td>
<td>10.08±0.16***</td>
<td>12.67±3.84**</td>
<td>15.24±2.16**</td>
<td>19.28±2.81**</td>
</tr>
</tbody>
</table>

The values are expressed as their Mean ± SD

*P < 0.001 compared with controls significant

**P < 0.000 compared with controls highly significant

***P > 0.05 compared with controls nonsignificant

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stage IV showed a significant increase in stage IV ($P < 0.05$).

**DISCUSSION**

The cytoplasm contains most of the enzymes that catalyze all biosynthetic and degradative metabolisms. Under normal conditions enzymes are retained within their cells of origin by the plasma membrane surrounding the cell. Each tissue maintains a steady and consistent enzymatic pattern which is significantly altered in malignancy. The plasma membrane is a metabolically active part of the cell, and its integrity depends on the cell’s production of ATP. Any process that impairs ATP production either by depriving the cell of oxidizable substrates or by reducing the efficiency of energy production by restricting the access of oxygen (Anoxia) promotes deterioration of membrane. The membrane becomes leaky and membrane constituents are shed into the surrounding milieu at increased rate when cells replicate more rapidly. Small molecules are first to leak followed by larger molecules such as enzymes. Cytosolic enzymes appear early on serum followed much later by mitochondrial and membrane bound enzymes. Ultimately the content of the cells are discharged leading to raised enzyme activity in malignancy and the enzymatic changes in malignant tissues may result from genetic reprogramming to malignant behaviour, a likely strategy for survival of tumors. ADA is the major enzyme of purine salvage pathway and catalyses the irreversible deamination and hydrolytic cleavage of adenosine and deoxyadenosine to inosine and deoxyinosine respectively with liberation of ammonia, there by plays a role in maintaining cellular pools of these important purine bases. The purine nucleoside adenosine is produced at increased levels in the tissues of solid cancers as a result of local hypoxia and in cancer there is an increased turnover of malignant cells and an associated increase in nucleotide metabolism leading to an increase in purine metabolizing enzyme ADA. Acceleration of the salvage pathway provides a selective advantage to cancerous cells to grow and develop more rapidly.

Adenosine inhibits the cell mediated antitumor immune response, promotes tumor cell migration and angiogenesis and stimulates the proliferation of tumor cells. As a result serum ADA activity is also increased to detoxify the high amounts of toxic adenosine and deoxyadenosine substrates produced from accelerated purine metabolism in the cancerous tissues. It has also been suggested that increased ADA activity might be physiologic attempt of the cancer cells to provide more substrates needed by cancer cells to accelerate the salvage pathway activity.

In our study a highly significant increase in serum ADA was observed in carcinoma breast patients as compared controls. It was also observed that serum ADA increased significantly in all the four stages when compared to controls. An inter stage comparison yielded a progressive increase from stage I-IV. Similar results were documented by Majoomdar M, Borzenko BG, Walia M, and Aghaei M.

GGT is a microsomal glycoprotein enzyme that catalyzes the transfer of gamma-glutamyl group from a peptide to an acceptor peptide or...
an L-amino acid. In most biological systems glutathione (GSH) serves as glutamyl donor. GSH is a most abundant non-protein thiol in most cells which plays key role in protection against oxidative stress and in detoxification of endogenous and exogenous compounds including carcinogens. GGT plays an important role in glutathione homeostasis by catalyzing hydrolysis and trans-peptidation of extracellular GSH. The expression of GGT is increased as an adaptive response upon the exposure of oxidative stress which causes induction of GGT mRNAs by multiple signalling pathways. GSH/GGT dependent pro-oxidant reaction has been found to modulate the transduction of proliferative and apoptotic signals.

Oxidative mechanism in carcinogenesis: All most all biological macromolecules are damaged by the free radicals including DNA damage which may directly cause inhibition of protein and enzyme biosynthesis and indirectly cause cell death or mutation. This ubiquitous stress can stimulate changes in gene expression which may function in the stimulation of the initiated cell during tumor promotion. Further, oxidant induced toxicity in the normal population may facilitate the clonal expansion of the more resistant initiated cell during promotion. Thus the higher levels of serum GGT from rapidly multiplying tumor cells may be due to response of increased reactive oxygen species (ROS) production in the blood. The increased activity of the serum GGT in metastatic carcinoma breast cases is due to hepatocellular damage and biliary obstruction.

According to our study serum GGT level was significantly increased in carcinoma breast patients when compared to controls. It was also observed that serum GGT increased significantly in all the four stages when compared to controls. An inter stage comparison yielded a progressive increase from stage I-IV. Similar results were documented by Mishra S, Seth LR, Buamah PK. Where as in a cohort study conducted by Fentiman IS, and Allen DS it was shown that there is an increased risk of breast cancer in premenopausal women with elevated levels of serum GGT and might benefit from close surveillance.

Alkaline phosphatases are a group of enzymes which catalyse hydrolysis of a variety of ester orthophosphates under alkaline conditions by splitting a terminal phosphate group from an organic phosphate with the concomitant production of an alcohol. Elevation of serum ALP in carcinoma breast occurs due to neoplastic metastasis of the liver resulting from localized intra-hepatic cholestasis with increased enzyme synthesis in liver tissue adjacent to the neoplasm, through pressure necrosis of liver cells and enzyme formation in proliferating endothelial cells may also be contributory. The bones are third only to liver and lung as the most probable sites of secondary infiltration by carcinoma breast which produces osteolytic lesions and a moderate increase in ALP activity.

In the present study a significant increase in the serum ALP was observed in carcinoma breast patients compared to controls. It was also observed that there was no significant increase in the activity of serum ALP in localized tumor where as there was a significant increase in patients with distant metastasis. Our findings correlate well with the studies conducted
CONCLUSION

In conclusion, our present study suggests that estimation of serum ADA and GGT level could be used as an important biochemical aid for diagnosing and monitoring the progression of carcinoma breast and serum ALP level might be used for detecting metastasis and differentiation of carcinoma breast with and without metastasis. The advantages of these parameters are that, they are simple, accurate, cost effective, and can be easily assayed in smaller laboratories which have not yet been exposed to any sophisticated technology.

Further studies on a larger sample with longer follow up are needed to substantiate our findings which can establish strong guidelines for the utility of these enzymes for the diagnosis and assessment of progression of carcinoma breast.

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Performance Evaluations

AS A GASTRIC CANCER MARKER

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For the determination of ADA activity in serum, plasma and biological fluids
Evaluation of Serum Adenosine Deaminase as a Tumor Marker in Gastric Cancer

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Abstract: Currently recommended tumor markers in gastric cancer are being used to monitor treatment or detect recurrences and serum carcinoembryonic antigen (CEA) is a widely accepted tumor marker in the management of gastric cancer. Hence there is a need for identifying a tumor marker which will be useful for detecting gastric cancer. Alterations in enzyme levels in cancer are well known, and adenosine deaminase (ADA), an enzyme of the purine salvage pathway, has been found to be elevated in various malignancies. The present study was carried out on 30 healthy controls and 22 gastric cancer patients. ADA in serum was estimated by colorimetric method of Galanti and Giusti. The diagnostic accuracy of ADA was assessed by receiver operating characteristics (ROC) curves. The diagnostic relevance of independent and two marker combination was analyzed by logistic regression model. Increase in serum ADA (p<0.001) and CEA (p<0.005) activity was found in the patients when compared to controls. Areas under the ROC curve were 0.908 for ADA, 0.865 for CEA. The area under the ROC curve when both ADA and CEA were combined was 0.952. ADA was found to have an independent strong predictor outcome and hence may be considered as a potential tumor marker in gastric cancer.

Key words: Gastric cancer, tumor markers, adenosine deaminase, carcinoembryonic antigen.

INTRODUCTION

Gastric cancer is the second most frequent cancer in the world after lung cancer, with 60% of cases occurring in developing countries and with about 800,000 new cases diagnosed every year [1]. It traditionally carries a poor prognosis with 79% of the tumors diagnosed at an advanced stage, when the five year survival rate is less than 5%. If diagnosed earlier, gastric cancer has 95% cure rate. In contrast advanced stages are generally refractory to chemotherapy leading to poor prognosis. Lack of a simple, inexpensive, non-invasive and reliable screening test has been quoted as the main reason. Traditional methods of diagnosis include biopsy, barium x-ray, gastroscopy, computer tomography and cytology, all of which are cumbersome and invasive except for barium x-ray. In this scenario minimally invasive cancer specific tests are urgently sought and recently serological tumor markers have been included and actively pursued to obtain an easy, simple, reliable diagnostic tool for the detection of gastric cancer.

Tumor markers are substances that are detected in blood, urine, or body tissues of some patients with certain types of cancer. Most tumor markers can be produced by cancer cells as well as normal cells and may not be elevated in every person with cancer especially in the early stages. Many are not specific to a particular type of cancer as elevated levels are found in more than one type of cancer. For several reasons, tumor markers by themselves are usually not enough to diagnose or rule out cancer. Gastrointestinal tumor markers were developed for screening of colorectal cancer and were also studied in other gastrointestinal malignancies, but no marker has yet been found to be useful for gastric cancer. Carcinoembryonic antigen (CEA) was first used as a specific marker for colonic cancer, but it was also found to be elevated in various other malignancies like stomach, breast, lung, pancreatic, bladder, hepatic cancers, lymphoma and melanoma and also in other benign conditions like cigarette smoking, peptic ulcer disease, inflammatory bowel disease, pancreatitis, cirrhosis and in biliary obstruction [2]. Other tumor markers used in the diagnosis and prognosis of gastric cancer include CA125, CA19-9, CA72-4 and AFP. Among these CEA, CA19-9 and CA72-4 are the three most widely studied tumor markers for their individual or combined predictability, with the main focus being on monitoring treatment to detect recurrence. Serum CEA and CA19-9 are reported to be good prognostic factors in gastric cancer [3]. However none of these markers meet the
original goal of discovering cancer at an early stage and hence the pursuit for newer markers has continued. The National Academy of Clinical Biochemistry (NACB) Guidelines for the Use of Tumor Markers in Gastric Cancer says that the use of markers for the diagnosis of gastric cancer cannot be recommended as none are specific and sensitive enough to be included in the diagnostic procedure [4].

It has been known for many years that the enzyme complement of a tumor cell differs in many ways from that of its normal counterpart reflecting its altered metabolism. Elevated enzyme levels in cancer patients frequently decrease to normal levels following successful treatment, with unchanged or increasing levels indicating a lack of response. Attempts to exploit this information clinically have led to the assay of a wide variety of enzymes in the search for both serum and tissue tumor markers. Serum enzymes are useful for monitoring the effects of therapy, to detect recurrences and also have prognostic value as their level frequently reflects tumor burden. Adenosine deaminase (ADA) an enzyme of the purine salvage pathway is widely distributed in tissues and relatively high levels are found in the villi of epithelial cells lining the duodenum. Many studies have demonstrated alterations of ADA activity in the tumor tissue and serum in patients with lung, head and neck, breast and ovarian cancer [5 - 8]. In colon carcinoma [9] and in colorectal cancer [10], ADA was found to be elevated in the cancerous large bowel tissue. Studies done in gastric tissues in patients with gastric cancer have shown increased ADA activity in the cancerous tissues [11, 12]. But to the best of our knowledge, we could not get any report on serum ADA activity in gastric cancer.

The aim of the present study was to define the role of ADA as a tumor marker in gastric cancer, to assess its individual diagnostic accuracy in comparison with the conventional CEA and also to analyze the diagnostic relevance with the combination of both markers.

MATERIALS AND METHODS

The case-control study comprised of 22 patients with gastric cancer and 30 healthy individuals as control group. Newly diagnosed patients with gastric cancer who were not treated with any type of therapy were recruited from the Medical Oncology department of Sri Venkateswara Institute Of Medical Sciences (Tirupati, India) from 2003-2004. The control group was recruited from the people attending the master health checkup program of the hospital and staff belonging to Biochemistry clinical laboratory of the Institute during the same period and none of them had any type of cancer at that time or previously. Smokers and alcoholics and those with diabetes, hypertension, liver diseases, renal failure and active infection were excluded from the study. All the members were recruited with informed consent.

Sample Collection: A 5 ml venous blood sample from patients and controls was collected in plain tubes and centrifuged at 2,500rpm for 15 minutes at room temperature. Serum obtained was stored at -80°C until analysis.

Marker Determination: ADA was estimated by the colorimetric method of Galanti and Giusti using ADA–MTB Microxpress, Tulip Diagnostics (P) Ltd kit. CEA was estimated by quantitative solid phase enzyme linked immunosorbent assay, using UBI Magiwel, United biotech inc kit.

Statistical Analysis: The results were expressed as mean ± SD. Difference between parameters for control and patient group was assessed using unpaired student’s ‘t’ test and p < 0.05 was considered statistically significant. Receiver operating characteristic (ROC) curves were constructed to study the diagnostic accuracy of the parameters as tumor markers. Their independent diagnostic relevance was assessed by performing Logistic regression analysis with the various cut off values. Correlation between the markers was assessed with the Spearman Rank test. Statistical analysis was performed using Microsoft excel spread sheets and SPSS for windows version 11.5.

RESULTS AND DISCUSSIONS

Result: Serum ADA and CEA levels were found to be significantly higher in the patient group as shown in table 1. ROC curve analysis revealed statistically significant areas under the curve (AUC) for both, with ADA having more AUC when compared to CEA. When both markers were combined, AUC was found to be higher, when compared to that obtained individually as shown in table 2.

Applying the various cut off values for ADA and CEA in logistic regression model, ADA was found to have a higher odds ratio, with a higher outcome predictor variable with the ROC curve identified cut off value when compared to CEA. Combination of both ADA and CEA also gave a higher odds ratio when compared to that of CEA alone, as shown in table 3.

Spearman’s rank correlation analysis between ADA and CEA revealed a highly significant correlation with r 0.440 (p<0.01).
Activity
In the present study serum ADA was differentiated tumor

does not express pronounced ADA as a tumor marker, while slow growing well-
differentiated tumors do not express pronounced ADA activity. In the present study serum ADA was found to be significantly elevated in gastric cancer patients. The individual diagnostic accuracy of ADA as tumor marker was assessed by ROC curves which showed a more significant AUC for ADA, indicating ADA to have a better diagnostic utility than CEA. Cut off values with the best combination of sensitivity and specificity obtained from the ROC curves were 19.5 IU/L for ADA with 82% sensitivity and 90% specificity and 6.4 ng/ml for CEA with 85% sensitivity and 81% specificity. When both were combined and ROC curve was constructed the AUC was found to be more than that obtained individually for ADA and CEA, which signifies the increase in diagnostic accuracy with the combination of the markers.

When both ADA and CEA were introduced into the logistic regression model as covariates, it was found that ADA had a higher Wald statistic (12.682 for ADA as compared to 6.976 for CEA) which shows the importance of ADA as a predictor variable. Similarly when the ROC curve identified cut offs were applied in the logistic regression model as categorical variables, ADA had a higher Wald statistic (20.268 for ADA as compared to 14.583 for CEA). The predictor outcome obtained by ADA ROC curve cut off was given by the combination of both ADA and CEA applied as continuous variables (86.5%). Among them the contribution of ADA was more as evidenced by a
higher wald statistic (8.643 for ADA as compared to 3.377 for CEA). With a well identified ROC cut off value ADA was found to perform better than CEA as a tumor marker. When combination of both of these markers was used, the performance was similarly found to be better than that of CEA alone. This signifies that ADA has a pronounced role in gastric cancer, either used alone or in combination with CEA. Studies have shown significant ADA activity in various cancerous tissues like breast, bladder, kidney, colon including gastric tissue, which may point to the source of ADA, as being secreted from the tissues and its consequent elevation in the blood. These results probably reflect the changes in purine metabolism due to increase in DNA turnover in the cancerous tissues. Acceleration of the salvage pathway provides a selective advantage to cancerous cells to grow and develop more rapidly.

The simplicity of measuring ADA activity combined with its cost effectiveness gives an added advantage to consider ADA as a tumor marker in gastric cancer. ADA independently has a stronger predictor outcome than that of CEA as found in this study, which gives strength to support the inclusion of ADA as a tumor marker in gastric cancer. Further studies on these lines in a larger number of patients are needed to evaluate the role of ADA as a tumor marker, in the early detection of gastric cancer which remains the goal of identifying a tumor marker which can be included in the diagnostic panel.

REFERENCES
