A NEW BIOCHEMICAL MARKER: SERUM ADENOSINE DEAMINASE ACTIVITY FOR ASSESSMENT OF SEVERITY IN ULCERATIVE COLITIS PATIENTS

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ABSTRACT

Objective: Adenosine deaminase (ADA) is known as a key enzyme in purine metabolism and DNA turnover. Although ADA has been shown to increase in several inflammatory conditions, there are less studies signifying a change in UC. Ulcerative colitis (UC) is a chronic inflammatory disease characterized by recurrent inflammation and ulcerations of colonic mucosa and an inappropriate and delayed healing.

Material and Methods: This study evaluated the activity of total ADA in serum of 50 patients with UC and 25 healthy controls. Patients’ age, disease duration, drug intake, and other medical history were all noted for each subject. Complete blood count, and ADA were determined for both patients and controls.

Result: Serum mean ADA levels were 16.22 ± 5.91 and 9.24 ± 2.19 U/l for patients with UC in active state and in remission and 9.64 ± 3.37 U/l in the healthy control group. Mean serum ADA levels were significantly elevated in active UC patients compared with patients with UC in remission and control groups.

Conclusion: Serum ADA levels were found to be elevated in UC patients in active state suggesting a partial role of activated T-cell response in the disease pathophysiology. Further randomized controlled studies are warranted to demonstrate the role of the ADA in UC patients, with a special interest in specifically targeted therapies against ADA for achieving disease remission.

Keywords: Adenosine deaminase, Ulcerative colitis, Disease activity.

Introduction

Ulcerative colitis (Colitis ulcerosa, UC) is a form of colitis which is classified as inflammatory bowel disease (IBD). Ulcer or open sores on colon is characteristic feature of UC. The Periods of remission interpose by clinical exacerbations during the clinical course is a challenge for treating clinicians. The differential diagnosis of UC can be done with endoscopy and mucosal biopsy for histopathology. Laboratory investigations and imaging tests are also useful for the correct diagnosis. (1) Severity of UC is defined as mild moderate severe and fulminant. (2) Severity decides the management of UC that UC is managed by either medical therapy or surgery.
Though availability of advance medical therapy from last decades, the rate of colectomy has decreased but the surgery maintains its importance in the therapeutic management in severe UC. (3) It is expected that the early recognition of disease severity will considerably bring down the surgery rate and hence will decrease mortality in serious UC patients. (4) Laboratory tests, Like white blood cells (WBC), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) are being broadly accepted as routine investigation, for preliminary diagnosis and to monitor activity in UC. However, a specific test is not available till yet. (5) This is the reason which motivates us to find out an additional serum biomarker for assessment of severity and approaching a precise diagnosis.

Adenosine deaminase (ADA) is a key enzyme in purine catabolism. (6) ADA is biologically active in lymphoid tissue and plays an important role for proliferation and differentiation of T lymphocytes, blood monocytes and macrophages. (7) The estimation of ADA activity in the serum and other biologic fluids is very helpful in diagnosis of many inflammatory conditions and disease such as rheumatoid arthritis (RA), acute appendicitis, systemic lupus erythematosus (SLE), celiac disease (CD) and tuberculosis. (8, 9, 10, 11, 12)

MATERIALS AND METHODS

Patients
Fifty patients with UC and 25 healthy controls coming at the Gastroenterology outdoor of Geetanjali Medical College and Hospital, Udaipur between February 2013 and August 2013 were included in the study. The diagnosis of UC was made on basis of clinical history, typical radiological, endoscopic and histological criteria. Patient’s age, disease duration, drug intake, other medical and personal history was recorded for every subject. Complete blood count and ADA were measured for both patients and controls. Colonoscopy was done for all patients.

All patients of UC were classified in two groups according to Disease activities and it was calculated according to endoscopic activity index (EAI) scores. (13)
Active UC - Patients with EAI scores ≥4
Inactive UC - Patients with EAI scores < 4

Serum samples
Blood samples were collected from a peripheral vein after an overnight fast without using any anticoagulant and were subjected to centrifugation with the speed of 3000 rounds per minute for 10 min (Remi). Serum was analyzed immediately.

ADA assay
Total serum ADA was estimated with an auto analyzer (Cobas c311, Roche, Basel, Switzerland). Serum ADA activities were estimated by the method of Giusti and Galanti. (14) Briefly, samples were incubated with adenosine and the released ammonium ions were determined. ADA activity was defined as the concentration of ammonium ions (mol/l) formed in 1 min and expressed as units per litre.

Statistical analyses
Values were demonstrated as mean ± standard deviations. Comparisons of percentages between different groups of patients were carried out using the chi-squared test. All normally-distributed data were analyzed using unpaired Student t test or 1-way analysis of variance (ANOVA; for 3 groups). A p-value of <0.05 was deemed statistically significant.
RESULTS
Fifty patients with UC and 25 control subjects were included in this study. There were 20 females and 32 males in the UC group and 11 females and 14 males in the control group (p= 0.485). Table 1 represents the demographic and clinical characteristics of patients and control subjects. The age, gender and smoking of the subjects were not found statistically significant differences

**Table 1. Demographic features of the patients and controls.**

<table>
<thead>
<tr>
<th></th>
<th>Active UC (n=25)</th>
<th>Inactive UC (n=25)</th>
<th>Controls (n=25)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (Year)</strong></td>
<td>54.24±14.86</td>
<td>57.6±15.56</td>
<td>63.32±11.24</td>
<td>0.075</td>
</tr>
<tr>
<td><strong>Gender (F/M)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>12(48%)</td>
<td>8(32%)</td>
<td>11(44%)</td>
<td>0.508</td>
</tr>
<tr>
<td>Male</td>
<td>15(60%)</td>
<td>17(68%)</td>
<td>14(56%)</td>
<td></td>
</tr>
<tr>
<td><strong>Disease duration (months)</strong></td>
<td>29.36±11.50</td>
<td>27.96±9.58</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td><strong>Smoking</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>9</td>
<td>4</td>
<td>8</td>
<td>0.431</td>
</tr>
<tr>
<td>No</td>
<td>16</td>
<td>21</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td><strong>Disease localization</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Distal colitis, n (%)</td>
<td>9</td>
<td>11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left, n (%)</td>
<td>10</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pancolitis, n (%)</td>
<td>6</td>
<td>5</td>
<td></td>
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</tr>
</tbody>
</table>

The mean serum ADA levels of UC patients and controls were 12.73 ± 5.65 and 9.64 ±3.37 respectively (p=0. 0.0141). Serum ADA levels of active UC (16.22 ± 5.91 U/l) patients were significantly higher than those of inactive UC (9.24 ± 2.19U/l (p<0.001). Table 2 shows serum ADA and the other laboratory values of study participants at onset of the study. No correlation was found between ADA and WBC and also ADA and HB. (WBC: r=0.026, p<0.902; HB: r= 0.004, p=0.98).

**Table 2. Comparison of serum ADA levels with other laboratory markers between patients and controls.**

<table>
<thead>
<tr>
<th></th>
<th>Active UC (n:25)</th>
<th>Inactive UC (n:25)</th>
<th>Controls (n:25)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ADA (U/l)</strong></td>
<td>16.22 ± 5.91</td>
<td>9.24±2.19</td>
<td>9.64±3.37</td>
<td>0.0001*</td>
</tr>
<tr>
<td><strong>Hemoglobin (g/dl)</strong></td>
<td>13.18±2.06</td>
<td>13.99±2.53</td>
<td>14.33±2.6</td>
<td>0.201</td>
</tr>
<tr>
<td><strong>WBC (/mm3x103)</strong></td>
<td>9.73±1.99</td>
<td>6.85±1.99</td>
<td>6.52±2.02</td>
<td>0.0001*</td>
</tr>
</tbody>
</table>

ADA: Adenosine deaminase, WBC: White blood cell,
DISCUSSION

Our study showed that concentration of ADA was significantly elevated in active UC in relation to inactive UC and healthy controls. The serum ADA was more accurate to define activity of UC than Hb and white blood cells. A high value in the serum ADA activity in UC patients are likely to reflect, at least in part, changes in the immunological status that occurs throughout the course of UC. However, to our current knowledge, there have only been few studies providing the reference values of serum ADA activity during UC. (15)

The clinical course is marked by exacerbations and remissions, which may develop spontaneously or in response to medical treatment. (16) A great majority of patients are generally mildly active and have a self-limiting disease; some will develop disease associated with serious complications. Approximately 30% of UC patients will need to undergo surgery at some point during their lifetimes for these complications. The determination of inflammatory activity has a significant role for the assessment of disease severity and for the therapeutic management. Since effective therapy significantly diminishes mortality in patients with severe UC, determination of inflammatory activity is therefore crucial for the assessment of disease activity and also for the tailoring of therapy. (17,18) Although lower gastrointestinal endoscopy, histological findings and radiological imaging modalities are commonly used to monitor intestinal inflammation, a great number of invasive/non-invasive methods have also been studied for UC diagnosis and determining the disease activity. (19,20) Elucidation of the associations between serum ADA activity and UC may help a better understanding toward the enigmatic pathogenesis of inflammatory bowel disease (IBD).

In our study, serum ADA levels were found to be significantly high in patients with active UC and patients with inactive UC to control group. Beyazit Y et.al also found increased serum ADA level in patients with active UC and patients with inactive UC to control group. (15) However the rise was much higher in patients of active UC. The significant finding in our study was, clinically inactive UC have also shown high serum ADA level but serum ADA level at par with active UC.

ADA is a polymorphic enzyme that is involved in purine metabolism and DNA turnover which is widely distributed in tissues and body fluids. (21) It is ubiquitous in mammalian tissue with the highest concentration in lymphoid tissues. Adenosine deaminase plays a crucial role in lymphocyte proliferation and differentiation and shows its highest activity in T-lymphocytes. (7,22) The high plasma adenosine deaminase activity might be due to abnormal T-lymphocyte responses or proliferation; may point towards a mechanism that involves its release into circulation. (22) Giblett et al. was the first study that demonstrated the vital and putative role of ADA on the immune system's function in patients with severe combined immunodeficiency. (23) Moreover as a sign of cell-mediated immune response, serum concentrations of ADA have been proposed to be elevated in several inflammatory and autoimmune conditions including infectious
diseases, rheumatoid arthritis (RA), acute appendicitis, systemic lupus erythematosus (SLE), celiac disease (CD) and tuberculosis and Graves’ disease.\(^{(8,9,10,11,12,24)}\)

In a recent study by Maor et al, it has been demonstrated that serum total ADA levels were also elevated in active CD. \(^{(25)}\) They mentioned that after remission ADA levels decrease and approaches to normal values. Although lymphocytic differentiation and proliferation or the monocyte-macrophage cell system have been considered to be responsible for the alterations in serum ADA activity, the precise mechanisms by which serum ADA activity is changed have not been clarified yet.\(^{(26)}\)

Ulcerative colitis and CD are both characterized by enhanced recruitment and retention of effector macrophages, neutrophils and T cells into the inflamed areas of intestine, where they are activated and release proinflammatory cytokines. Accumulation of effector cells in the inflamed intestine is a result of enhanced recruitment as well as prolonged survival triggered by decreased cellular apoptosis. \(^{(27)}\) Although these immunological reactions set off in the course of immune disturbances in UC are imperfectly understood; activated macrophages and enhanced stimulation of T cells seem to be implicated in inflammation in UC. In the present study high levels of ADA in active UC patients suggest an action by cytokine release via T-cell activation, playing a key role in the inflammation process.

**CONCLUSION:**

The present study demonstrated that ADA levels were significantly elevated in active UC patients. We believe that ADA activity may be considered as an efficient marker of UC and it could probably be a potential indicator of disease activation. If our data can be confirmed with further studies, we believe that a standardized cut-off value would facilitate the diagnosis of UC activation. Extensive studies dealing with T-cells and ADA activity are required to document the role of ADA in the immunopathogenesis of UC.

**Funding:** No funding sources

**Conflict of interest:** None declared

**Ethical approval:** The study was approved by the institutional ethics committee

**REFERENCES:**


12. Lakshmi V1, Rao RR, Joshi N, Rao PN. Serum adenosine deaminase activity in bacillary or paucibacillary pulmonary tuberculosis. Indian J Pathol Microbiol. 1992 Jan;35(1):48-52.). The aim of present study is to investigate the level of ADA in UC patients; and to evaluate its usefulness as possible marker of UC Severity and find the relationship between ADA and UC.


