EVALUATION OF SERUM ADENOSINE DEAMINASE LEVEL IN CORRELATION TO THE ACTIVITY OF ULCERATIVE COLITIS

By

Abdo Mabrouk El-Shafae, Sherif Ali Abd El-Aziz, Nagah Mohamed Abu Mohamed*, and Mostafa Mohamed Abd El-Raaof

Departments of Hepatology, gastroenterology & infectious diseases, and Clinical Pathology*, Faculty of Medicine, Al-Azhar University-Cairo, Egypt

Corresponding author email: Mostafa Mohamed Abd El-Raaof, E-mail: smart4doc@gmail.com

ABSTRACT

**Background:** Ulcerative colitis (UC) is a chronic inflammatory disease characterized by recurrent inflammation and ulcerations of colonic mucosa causing frequent episodes of bleeding per rectum.

The diagnosis of UC is best made with colonoscopy and mucosal biopsy for histopathology. Noninvasive tests, including C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), white blood cells (WBC), fecal calprotectin are being widely recognized, both for initial diagnosis and for accurately monitoring disease activity in UC.

**Objective:** Evaluation of the level of serum adenosine deaminase in correlation to the activity of Ulcerative colitis.

**Patient and Methods:** In this Prospective study evaluation the level of serum adenosine deaminase in correlation to the activity of UC was done in 80 patients. They were divided into 3 groups, Group I: 30 patients confirmed and well known cases of ulcerative colitis (by colonoscopy and histopathological examination), during remission state. Group II: 30 patients confirmed and well known cases of ulcerative colitis (by colonoscopy and histopathological examination), during exacerbation state (activity state). Group III: 20 patients with colonic manifestations other than ulcerative colitis.

**Results:** The current study showed that ADA, the cut-off was 9.32 U/L to differentiate between exacerbation and remission states with sensitivity of 82.3%, specificity of 84.5%.

**Conclusion:** Serum ADA is more accurate than CRP, WBCs and ESR to differentiate between exacerbation and remission states of UC.

**Keywords:** ADA, CRP, WBCs, ESR, Ulcerative colitis.

INTRODUCTION

Ulcerative colitis is a chronic inflammatory disease characterized by recurrent inflammation and ulcerations of colonic mucosa causing frequent episodes of bleeding per rectum (Annese et al., 2013).
The diagnosis of UC is best made with colonoscopy and mucosal biopsy for histopathology. Laboratory studies and imaging tests are also helpful to establish the precise diagnosis. Although medical therapy has advanced during the past decades and colectomy rates are decreasing (Annese et al., 2013).

It is therefore not surprising that the early detection of disease activity will significantly reduce the surgery rate, and therefore will reduce mortality and morbidity in patients with serious Ulcerative colitis (Poggioli et al., 2019).

Noninvasive tests, including C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), white blood cells (WBC), fecal calprotectin are being widely recognized, both for initial diagnosis and for accurately monitoring disease activity in UC (Langhorst et al., 2016).

Nevertheless, an ideal test has not yet been developed. For this reason the adjunctive use of additional serum markers add significant benefit for predicting disease severity and achieving diagnostic accuracy (Mosli et al., 2015).

Adenosine deaminase (ADA) is a purine catabolic enzyme, capable of catalyzing the deamination of adenosine, forming inosine in the result process. It is widely distributed in tissues and body fluids. The most important biological activity of ADA is related to lymphoid tissue and is necessary for proliferation and differentiation of T lymphocytes as well as for the maturation and function of blood monocytes and macrophages. The assay of ADA activity in the serum and other biologic fluids is very important for a precise diagnosis of many pathological situations. In this respect ADA has been shown to increase in several inflammatory conditions such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), pancreatic disorders, acute appendicitis, celiac disease and tuberculosis. Total serum ADA was measured with an automatic spectrophotometric analyzer (cobas and ELISA) (Camici et al., 2018).

**PATIENTS AND METHODS**

The current study included 80 patients attended at Al-Azhar university hospitals from December 2018 to April 2019. Informed consents were written by patients who agreed to participate in this study. They were divided into three groups:

- **Group I**: 30 patients confirmed and well known cases of ulcerative colitis (by colonoscopy and histopathological examination), during remission state.
- **Group II**: 30 patients confirmed and well known cases of ulcerative colitis (by colonoscopy and histopathological examination), during exacerbation state (activity state).
- **Group III**: 20 patients with colonic manifestations other than ulcerative colitis.

We included patients who previously diagnosed with ulcerative colitis during activity and remission states and patients with colonic manifestations other than ulcerative colitis.

We excluded patients who diagnosed with crohn’s disease, Systemic lupus erythematosis, Rheumatoid arthritis and colonic malignancies.
All patients were subjected to:

1. Full history taking.
2. General examination.
3. Local abdominal examination
4. Laboratory investigations including:
   • Complete blood count (CBC).
   • Liver function tests including: alanine transferase (ALT), aspartate transferase (AST), Serum albumin, total bilirubin, direct bilirubin, Prothrombin time (PT), international normalization ratio (INR).
   • Renal function tests.
   • Serum adenosine deaminase (ADA) by cobas and ELISA. Samples were incubated with adenosine and the released ammonium ions were determined, to control for ammonium present before the addition of exogenous adenosine, untreated samples were run in parallel, ADA activity was defined as the concentration of ammonium ions formed in 1 min and expressed as units per liter.
   • C-reactive protein (CRP).
   • Erythrocyte sedimentation rate (ESR).
   • Pregnancy test.
   • Stool analysis to exclude parasitic infestation.
5. Colonoscopy and histopathology.


Statistical analysis:

Data were analyzed using Statistical Program for Social Science (SPSS) version 15.0. Quantitative data were expressed as mean± standard deviation (SD). Qualitative data were expressed as frequency and percentage.

Chi-square test was used on comparing between non-parametric data.

Mann Whitney U test was used to compare differences between two independent groups when dependant variable is either ordinal or continuous, but not normally distributed.

Post Hoc test was used for multiple comparisons between different variables.

• P1: statistical difference between all groups.
• P2: statistical difference between group I and group II.
• P3: statistical difference between group I and group III.
• P4: statistical difference between group II and group III.
• P value < 0.05 was considered significant.
RESULTS

In this study, the age range of participants was 29 to 55 years. Males were 40, and females were 40. The duration of symptoms ranged between 28 months and 32 months. There was higher percentage of disease in non-smokers than smokers. However, there was no statistical significant difference between studied groups as regard demographic data (Table 1).

Table (1): Comparison between studied groups as regard demographic data

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>Group I (n = 30)</th>
<th>Group II (n = 30)</th>
<th>Group III (n = 20)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>Mean</td>
<td>44.9</td>
<td>41.8</td>
<td>46.1</td>
<td>0.621</td>
</tr>
<tr>
<td></td>
<td>±SD</td>
<td>11.5</td>
<td>12.4</td>
<td>10.7</td>
<td>NS</td>
</tr>
<tr>
<td>Duration (months)</td>
<td>Mean</td>
<td>28.7</td>
<td>32.8</td>
<td>----</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>±SD</td>
<td>10.6</td>
<td>11.2</td>
<td>----</td>
<td>NS</td>
</tr>
<tr>
<td>Sex (n, %)</td>
<td>Male</td>
<td>18 60%</td>
<td>13 43.3%</td>
<td>9 45%</td>
<td>0.38</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>12 40%</td>
<td>17 56.7%</td>
<td>11 55%</td>
<td>NS</td>
</tr>
<tr>
<td>Smoking (n, %)</td>
<td>Non</td>
<td>22 73.3%</td>
<td>21 70%</td>
<td>13 65%</td>
<td>0.82</td>
</tr>
<tr>
<td></td>
<td>Smoker</td>
<td>8 26.7%</td>
<td>9 30%</td>
<td>7 35%</td>
<td>NS</td>
</tr>
<tr>
<td>ADA (U/L)</td>
<td>Mean</td>
<td>7.86</td>
<td>12.3</td>
<td>8.2</td>
<td>P1 &lt; 0.00</td>
</tr>
<tr>
<td></td>
<td>±SD</td>
<td>2.3</td>
<td>2.6</td>
<td>2.5</td>
<td>P2 &lt; 0.001 P3=0.623 P4 &lt; 0.00</td>
</tr>
</tbody>
</table>

Diagnostic performance of ADA and other inflammation markers to differentiate active from inactive UC showed that (Table 2):

For ADA, the cut-off was 9.32 U/L with sensitivity of 82.3%, specificity of 84.5%, PPV of 84.15%, NPV of 82.68% and accuracy of 76.9%.

For CRP, the cut-off was 4.2 mg/dl with sensitivity of 69.5%, specificity of 90.1%, PPV of 87.53%, NPV of 74.71% and accuracy of 79.6%.

For ESR, the cut-off was 14 mm/h with sensitivity of 56.7%, specificity of 86.4%, PPV of 80.65%, NPV of 66.62% and accuracy of 65.4%.
Table (2): Diagnostic performance of ADA and other inflammation markers to differentiate active from inactive UC

<table>
<thead>
<tr>
<th>Performance Parameters</th>
<th>Cut off</th>
<th>AUC</th>
<th>Sensitivity %</th>
<th>Specificity %</th>
<th>PPV %</th>
<th>NPV %</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADA</td>
<td>9.32</td>
<td>0.83</td>
<td>82.3</td>
<td>84.5</td>
<td>84.15</td>
<td>82.68</td>
<td>84.5</td>
</tr>
<tr>
<td>WBCs</td>
<td>8.8</td>
<td>0.79</td>
<td>72.1</td>
<td>76.9</td>
<td>75.74</td>
<td>73.38</td>
<td>76.7</td>
</tr>
<tr>
<td>CRP</td>
<td>4.2</td>
<td>0.77</td>
<td>69.5</td>
<td>90.1</td>
<td>87.53</td>
<td>74.71</td>
<td>79.6</td>
</tr>
<tr>
<td>ESR</td>
<td>14</td>
<td>0.63</td>
<td>56.7</td>
<td>86.4</td>
<td>80.65</td>
<td>66.62</td>
<td>65.4</td>
</tr>
</tbody>
</table>

**DISCUSSION**

In the present study, we demonstrated that patients with active UC have elevated ADA concentrations in comparison with inactive UC and healthy controls. Serum ADA activity is found to have high sensitivity, specificity and predictive values in active UC. The overall accuracy of ADA for disease activity in UC was comparable with CRP, ESR and white blood cells.

High levels of ADA activity in the sera of active UC patients, compared to inactive UC and controls, support the hypothesis that ADA may have a role in the cytokine network of the inflammatory cascade of UC with activated T-cell response in the disease pathophysiology.

UC is a chronic inflammatory disease primarily affecting the colonic mucosa with variable extent and severity. The clinical course is marked by exacerbations and remissions, which may develop spontaneously or in response to medical treatment (Clarke and Chintanaboina, 2018).

A great majority of patients are generally mildly active and have a self-limiting disease; some will develop severe 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 (Yates and Finnel, 2019).

Although colonoscopy, histologic 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 (Laube et al., 2018).

Unfortunately, the role of ADA in the UC pathophysiology has not been clearly elucidated. In this setting, the primary aim of this study was to evaluate the role of ADA in UC pathogenesis in correlation with clinical and biochemical severity indexes. Elucidation of the associations between serum ADA activity and UC may help a better understanding toward the
enigmatic pathogenesis of inflammatory bowel disease (IBD).

White blood cell count, CRP and ESR are the most commonly used inflammatory indices in clinical practice for determining UC activity. These parameters can alter according to degree of inflammatory states, but they don't adequately reflect disease activity because of low sensitivity and specificity for intestinal inflammation (Matsuoka et al., 2018).

Yuksel et al. (2009) reported that overall accuracy of white blood cell count and ESR in determining disease activity was 57% (sensitivity 58%) and 65% (sensitivity 70%) respectively. In the present study overall accuracy of white blood cell count and ESR was 76.7% (sensitivity 72.1%) and 65.4% (sensitivity 56.7%) respectively.

Although CRP seems to be more promising for determining disease activity, it has some limitations because it correlates well with disease activity in Crohn's disease, but less well in UC (Mumolo et al., 2018).

Although expensive or practically difficult to measure, different markers of severity shown to be useful for predicting severity on admission are fecal lactoferrin, lysozyme, elastase, myeloperoxidase, and calprotectin. Recently, lactoferrin, calprotectin, and PMN-elastase have been found to be superior to CRP in several studies (Ayling and Kok, 2018).

ADA is a polymorphic enzyme that is involved in purine metabolism which is widely distributed in tissues and body fluids. It is ubiquitous in mammalian tissue with the highest concentration in lymphoid tissues. ADA activity of the lymphocytes is ten times higher than that of the erythrocytes and B-lymphocytes. ADA catalyzes deamination of both adenosine and 2′-deoxyadenosine to inosine and 2′ deoxyinosine, respectively. Elevated ADA activity reflects a cell-mediated immune response in disease pathogenesis. With positive association with lymphocyte differentiation and proliferation, ADA level increases during mitogenic and antigenic responses of these cells (Ahmed, 2018).

ADA is crucial for the differentiation and maturation of the immune cells including lymphocytes and monocyte-macrophage cell lines. ADA seems to maintain the proper function of the human immune system and has been used for monitoring various diseases in which immunity has been altered. 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, SLE, celiac disease, acute appendicitis, Graves’ disease, RA and tuberculosis (Antonioli et al., 2018).

In the study by Maor et al. (2011), it has been demonstrated that serum total ADA levels were also elevated in active CD. They mentioned that after remission ADA levels decrease and approaches to normal values. On the basis of their findings, they proposed that ADA activity in serum may serve as a marker of inflammation, providing additional information to markers such as CRP and erythrocyte sedimentation rate.
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 (Borea et al., 2018).

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 (Lee et al., 2018).

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. Moreover this local immune response is characterized by CD1-reactive natural killer T cell production of IL-13. 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 (Tatiya-Aphiradee et al., 2018).

CONCLUSION

Serum ADA levels are significantly elevated in active UC patients. Its activity may be considered as an efficient marker of UC and it could probably be a potential indicator of disease activation.

REFERENCES


EVALUATION OF SERUM ADENOSINE DEAMINASE LEVEL IN... 309

تقييم مستوي الأدينوزين ذي أمينيز في نشاط إلتهاب القولون التقرحي

عبده مبروك الشافعي، شريف علي عبد العزيز، ناجح محمد أبو محمد، مصطفى محمد عبد الروف

قسم أمراض الكبد والجهاز الهضمي والأمراض المناعية والباثولوجيا الإكلينيكية - كلية الطب - جامعة الأزهر

خليفة البحث: إلتهاب القولون التقرحي هو مرض إلتهابي مزمن يتميز بالتهاب متكرر وترحبات في الغشاء المخاطي للقولون مما يسبب نوبات متكررة من النزيف من القولون. ويتم تشخيص مرض إلتهاب القولون التقرحي عن طريق الاختبارات المعملية واستخدام تقييمات تظاهر الجهاز الهضمي والفحص بالأشعة واختبارات الأنسجة. وهناك العديد من الدوافع الداعية إلى دراسة وتقييم الدبلاء البيوكيميائية لنشاط مرض إلتهاب القولون التقرحي وعلى رأسها الحصول على مقياس رقمي لدرجة نشاط المرض دون الحاجة إلى الاعتماد كلياً على الأعراض والبعض قدر المستطاع عن تكرار التنظير للقولون والأمراض بما يسبب من عديد مادى ومعنوياً، وإرهاق مستمر للمريض، وإنهاك للقواد الطبية.

الهدف من البحث: تقييم مدى ارتباط الأدينوزين ذي أمينيز في نشاط إلتهابات القولون التقرحية.

المريض وطرق البحث: في هذا التقييم دراسة مستقبالية تم القيام بها على مستوي مصل الأدينوزين ذي أمينيز في 80 مريضا، تم تقسيمهم إلى ثلاث مجموعات:

المجموعة الأولى: 30 مريضا، سباق تشخيصهم بالإلتهاب القولون التقرحي عن طريق المنظار القولوني وتحليل الأنسجة وهم أثناء الدراسة في حالة عدم نشاط للمرض.

المجموعة الثانية: 30 مريضا، سباق تشخيصهم بالإلتهاب القولون التقرحي عن طريق المنظار القولوني وتحليل الأنسجة وهم أثناء الدراسة في حالة نشاط للمرض.
المجموعة الثالثة: 20 مريضاً يعانون من أعراض التهاب القولون غير
إلتئام القولون التقرحي.

نتائج البحث: أظهرت نتائج هذه الدراسة أن متوسط مستوى الأدينوزين في أمينيز فی مرضى القولون التقرحي النشاط 12.3±2.6، ومرضى القولون التقرحي غير النشط 7.86±2.3، بينما كان المتوسط للمجموعة الحاكمة 8.2 ± 2.5. لهذا هناك دلائل إحصائية واضحة لارتفاع مستوى الأدينوزين في أمينيز مرضى التهاب القولون التقرحي مقارنة بمرضي التهاب القولون التقرحي الغير نشط.

الإرث характеристик: يمكن استخدام مستوى الأدينوزين في الدم كدالة تشخيصية للتمييز بين مرضى التهاب القولون التقرحي النشط والتهاب القولون التقرحي الغير نشط دون الحاجة للتنظير المتكرر لتحديد درجة نشاط المرض.