

Adenosine Deaminase Activity and Peripheral Immune Cells Ratios in a Sample of Inflammatory Bowel Disease Patients

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Abstract

Seventy inflammatory bowel disease (IBD) patients (35 ulcerative colitis; UC and 35 Crohn's disease; CD) and 20 controls were investigated for serum level of adenosine deaminase (ADA) and neutrophil-lymphocyte and monocyte-lymphocyte ratios. The results revealed that the mean of ADA was significantly increased in UC and CD patients (46.72 ± 8.99 and 41.17 ± 5.95 ng/ml, respectively) compared to controls (20.85 ± 2.79 ng/ml), but it was also significantly higher in UC patients than in CD patients. The neutrophil-lymphocyte ratio mean was also significantly increased UC patients (5.83 ± 3.10) as compared to CD patients (3.79 ± 2.02) or controls (1.66 ± 0.22). The ratio was also significantly increased in CD patients compared to controls. A similar pattern was also observed in monocyte-lymphocyte ratio. In conclusion ADA may have a role in the pathogenesis of IBD, especially UC, and consequently the peripheral immune cells might have been affected.

Keywords: Inflammatory bowel disease, ulcerative colitis, Crohn's disease, adenosine deaminase

INTRODUCTION

Inflammatory bowel disease (IBD) refers to two related but different diseases: ulcerative colitis (UC) and Crohn's disease (CD). These diseases cause chronic inflammation of the intestinal tract, which leads to a variety of symptoms (1). It is generally a disease of young people because it most commonly develops between the ages of 10 and 30. However, a second smaller peak of developing IBD is seen between ages of 50 and 60 (2).

Ulcerative colitis is primarily affecting the colonic mucosa; the extent and severity of colon involvement are variable (3). For CD, it is an ongoing disorder that causes inflammation of the digestive tract, and can affect any area of the gastrointestinal tract, from mouth to anus (4). The swelling extends deep into the lining of the affected organ and can cause pain and can make the intestines empty frequently, resulting in diarrhea (5).

The definitive etiology of IBD is not well-understood but immunological, genetic and environmental factors have been suspected. The immune system normally turns on and off to fight harmful substances like bacteria and viruses that pass through intestines, and in IBD, it

appears that there is an initial trigger such as an infection that activates the immune system (6). In addition, a genetic background is also involved in the development of IBD, and 10-20% of the patients have one or more other family members affected with IBD, and there is an increased risk of UC or CD of individuals in families having these diseases (7).

Although there is no definitive biological marker for diagnosis of IBD, some investigators suggested adenosine deaminase (ADA) activity is altered in IBD patients, especially if we consider that a deficiency in ADA is associated with a severe combined immune deficiency syndrome (8). Adenosine deaminase is a cytoplasmic enzyme involved in the catabolism of purine bases, capable of catalyzing the deamination of adenosine (9). Adenosine is a purine molecule necessary for normal cell metabolism and growth, and adenosine has been recognized as a potential anti-inflammatory molecule. In general, cellular adenosine is produced by both *de novo* synthesis and by absorption from the diet into the body through transporters in the gastrointestinal tract. It is thought that activation of adenosine receptors deactivates the synthesis of critical components necessary for activation of chronic inflammatory

diseases, including IBD (10). In addition, as ADA is an enzyme that is associated with immunological functions, it has also been suggested that an alternation in its activity may affect the peripheral immune cell ratios (neutrophil-lymphocyte and monocyte-lymphocyte ratios), which are important in maintaining an adequate immune response (11).

Aim of the study

This study was planned with aims to evaluate the total activity of ADA in sera of IBD (UC and CD) patients, and to determine the neutrophil-lymphocyte and monocyte-lymphocyte ratios in the peripheral blood of Iraqi patients and normal people as control group.

This research was part of the research plan in Al-Rafidain University collage, and it has been approved conducted in the Medical city in Baghdad.

PATIENTS AND METHODS

Seventy IBD patients (35 UC and 35 CD) were enrolled in the study. The patients were referred to the Gastrointestinal and Liver Diseases hospital in Baghdad during the period January-April 2014 for diagnosis and treatment, and their age range was 20-45 years. The diagnosis was made by the consultant medical staff at the hospital, which was based on a clinical examination and endoscopy. In addition, 20 apparently healthy volunteers age matched to patient's (19-40 years), were involved in the study and they were never had signs or symptoms of IBD, after take the agreement from patients and controls to participation in this study.

From each participating subject, 4 ml of venous blood were collected and distributed into two aliquots, each of 2 ml. The first aliquot was dispensed in EDTA tube, and used within an hour for differential count of leukocytes. The second aliquot was left to clot in the refrigerator (4°C) for 30 minutes, and then it was centrifuged (3000 rpm for 15 minutes) in a cooled centrifuge (4°C), and after centrifugation, the serum was frozen at -20°C until assessment of ADA total activity.

The differential count of leukocytes was determined by smearing a drop of the blood on a clean and washed slide, and after air-drying; it was stained with Leishman's stain for 10 minutes and then buffered with Leishman's buffer for a similar time. The slide was examined using oil-immersion lens and at least 200 leukocytes were inspected and their percentages were recorded. The ratio was obtained by dividing the percentage of the first type of cells by the second type (10).Serum level of ADA was determined using an ELISA kit (MyBioSource, USA). The test principle applied in this kit was a sandwich enzyme immunoassay. The microtiter plate provided in this kit

was pre-coated with an antibody specific to ADA. Standards or samples were then added to the appropriate microtiter plate wells with a biotin-conjugated antibody specific to ADA. Next, avidin conjugated to horseradish peroxidase (HRP) was added to each microplate well and incubated. After TMB substrate solution was added, only those wells that contain ADA, biotin-conjugated antibody and enzyme-conjugated avidin will exhibit a change in color. The enzyme-substrate reaction was terminated by the addition of sulphuric acid solution and the color change was measured spectrophotometrically at a wavelength of 450 nm. The sample results were calculated by interpolation from a standard curve that was performed in the same assay as that for the samples by using a standard curve fitting equation.

Data were presented as means ± standard deviations (SD), and significant differences between means were assessed by ANOVA (analysis of variance), followed by Duncan test, in which the difference was considered significant when $P \leq 0.05$. Such analyses were carried out using the SPSS (statistical package for social sciences) version 13.

RESULT

Serum level mean of ADA was significantly increased UC and CD patients (46.72 ± 8.99 and 41.17 ± 5.95 ng/ml, respectively) compared to controls (20.85 ± 2.79 ng/ml), but it was also significantly higher in UC patients than in CD patients. Therefore, UC patients recorded 43.0 of the total sum of ADA, followed by CD patients (37.9%) (Table1).

Table 1. Serum level mean of adenosine deaminase in ulcerative colitis, Crohn's disease patients and controls.

Groups	Number	ADA Mean ± SD (ng/ml)	Percentage of Total Sum
Ulcerative colitis	35	46.72 ± 8.99^A	43.0
Crohn's disease	35	41.17 ± 5.95^B	37.9
Controls	20	20.85 ± 2.79^C	19.2

Different superscript letters: Significant difference ($P \leq 0.05$) between means (Duncan test).

The neutrophil-lymphocyte ratio mean was significantly increased UC patients (5.83 ± 3.10) as compared to CD patients (3.79 ± 2.02) or controls (1.66 ± 0.22), and this contributed to an increase in the percentage of total sum in UC patients, in whom the percentage reached 51.7%). The ratio was also significantly increased in CD patients compared to controls (Table 2). A similar pattern was also observed in monocyte-lymphocyte ratio, and again UC patients scored the highest percentage of the total sum, which was 43.6% (Table 3).

Table 2. Mean of neutrophil-lymphocyte ratio in ulcerative colitis, Crohn's disease patients and controls.

Groups	Number	Neutrophil-Lymphocyte Ratio Mean ± SD	Percentage of Total Sum
Ulcerative colitis	35	5.83 ± 3.10 ^A	51.7
Crohn's disease	35	3.79 ± 2.02 ^B	33.6
Controls	20	1.66 ± 0.22 ^C	14.7

Different superscript letters: Significant difference ($P \leq 0.05$) between means (Duncan test).

Table 3. Mean of monocyte-lymphocyte ratio in ulcerative colitis, Crohn's disease patients and controls.

Groups	Number	Monocyte-Lymphocyte Ratio Mean ± SD	Percentage of Total Sum
Ulcerative colitis	35	0.34 ± 0.11 ^A	43.6
Crohn's disease	35	0.26 ± 0.07 ^B	33.3
Controls	20	0.18 ± 0.02 ^C	23.1

Different superscript letters: Significant difference ($P \leq 0.05$) between means (Duncan test).

DISCUSSION

The present study shows a significant increase in the mean of ADA values for the different categories are shown in (table1). The mean serum ADA levels was high in UC and CD compared with control groups at probability level ($P \leq 0.05$), also found in this study evaluated the activity of total ADA in serum of UC patients compared with CD patients at probability level ($P \leq 0.05$). The results in this study are in agreement with (Beyazit et al) (10) and in a recent study by (Maor et al) (13) It has been demonstrated that serum total ADA levels were also elevated in active CD. Therefore those support the hypothesis that ADA may have a role in the cytokine network of the inflammatory cascade of UC and CD with activated T-cell response in the disease pathophysiology. 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. (14) ADA catalyzes deamination of both adenosine and 2'-deoxyadenosine to inosine and 2'-deoxyinosine, respectively (15). Elevated ADA activity reflects a cell-mediated immune response in disease pathogenesis. With positive association with lymphocyte differentiation and proliferation, ADA is crucial for the differentiation and maturation of the immune cells including lymphocytes and monocyte. (16). The present study also shows a significant increase in Neutrophil-Lymphocyte Ratio among groups of the patients as compared to the control group in probability level ($P \leq 0.05$). NLR were found to be elevated

compared to UC patients and controls (5.83 ± 3.10 , 1.66 ± 0.22) respectively, and these values were also found to be elevated compared to CD patients and controls (3.79 ± 2.02 , 1.66 ± 0.22) respectively (Table 2). The present results are consistent with the finding of (Torun et al).(11), and also shows a significant increase in Monocyte-Lymphocyte Ratio among groups of the patients as compared to the control group in probability level ($P \leq 0.05$). MLR were found to be elevated compared to UC patients and controls (0.34 ± 0.11 , 0.18 ± 0.02) respectively, and these values were also found to be elevated compared to CD patients and controls (0.26 ± 0.07 , 0.18 ± 0.02) respectively (Table 3). More generally used laboratory markers include white blood cells count, red blood cells count, and platelets. White blood cell count will increase as part of the acute phase response. Increased leucocytosis is therefore not a specific feature of IBD and may be seen in other inflammatory conditions and stressful events.(17-18)

Reference through review of studies and research in Iraq and the Arab world turned out to be a study in Iraq, This research deals with the possibility of assaying the activity of adenosin deaminase (ADA) for Tuberculosis diagnosis. Results indicated that its activity increases by Tuberculosis infection (19). However there are no study on the subject of research in Iraq.

Through a search be clarified that the effectiveness of disease for patients with UC and CD increases with the studied indicators, which increased both the ADA and the proportions of lymphocyte, neutrophil and monocyte. This is due because of the important activities carried out by the enzyme ADA where it plays an important role in the events metabolic (construction and demolition) of nucleic acids in the nucleus which find in all cells of the body, but the deficiency in the body, the most cells affected are white blood cells especially lymphocyte cells, because the lack of an enzyme ADA leads to the accumulation of large amounts of construction materials moderation such as adenosine and 2-chlorodeoxy adenosine, and this material is considered toxic to lymphocyte cells especially T lymphocyte. This in turn could lead to an imbalance in the immune system is likely directs immune cells to attack the lining of the intestine.

Conclusions

The present study revealed that NLR and MLR are increased in both UC and CD. Peripheral blood NLR, and MLR, can reflect disease activity and can be used as an additional marker for Inflammatory bowel disease (IBD). In addition for that, serum ADA levels were found to be elevated in UC patients and CD patients,

suggesting a partial role of activated T-cell response in the disease pathophysiology.

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