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Elevated Adenosine Deaminase Levels in Celiac Disease

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Celiac disease (CD) is a genetically based chronic inflammatory disorder of the small bowel induced by the dietary gluten and possibly other environmental cofactors. The objective of this study was to investigate the relation of adenosine deaminase (ADA), a cytoplasmic enzyme involved in the catabolism of purine bases, as an index of altered immune response, with adult CD patients. ADA has been shown to increase in several inflammatory conditions, but there is no literature data indicating an alteration in CD. Serum levels of ADA were investigated in newly diagnosed 20 CD patients. ADA levels were compared in patients with CD and in healthy controls. Correlation analysis was also performed between ADA and other serum markers of CD (anti-gliadin and anti-endomysial antibodies) Mean serum ADA levels were significantly elevated in CD patients compared with control group. ROC curve analysis suggested that the optimum ADA level cut-off point for CD was 12.27 U/l. At a cut-off value of 12.27 U/l, the sensitivity was 80% and specificity was 100%. There was no statistically significant correlation between ADA and anti-gliadin and anti-endomisium antibodies. Serum ADA levels elevated significantly in CD patients, suggesting a partial role in activated T-cell response in the disease pathophysiology. ADA can be used as a supportive diagnostic marker in patients with CD. J. Clin. Lab. Anal. 24:323–326, 2010.

Key words: celiac disease; gluten; adenosine deaminase

INTRODUCTION

Celiac disease (CD) is a common but often under diagnosed chronic inflammatory disorder of the small bowel triggered in susceptible individuals by the ingestion of proline-rich and glutamine-rich proteins of wheat, gluten, or related rye and barley proteins (1). It results from complex interactions between the immune system, an adaptive B and T cell-mediated immune response and the mucosal barrier where inflammation is eventually manifested. In recent years major advances have occured in the clinical understanding of CD containing the role of the major histocompatibility gene complexes and the significance of autoantibodies found in the disease (2,3).

Adenosine deaminase (ADA) is a cytoplasmic enzyme involved in the catabolism of purine bases, capable of catalyzing the deamination of adenosine, forming inosine in the result process (4). ADA is widely distributed in human tissues and body fluid and its activity is higher in the lymphoid tissues, with the principal biological activity of ADA being found in T lymphocytes (5). It is essential for proliferation and differentiation of T lymphocytes as well as for the maturation and function of blood monocytes and macrophages (6,7). Serum activity of ADA has been suggested to be altered in diseases such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), pancreatic disorders, acute appendicitis, and tuberculosis (8–11). Although ADA has been considered as an indicator of a nonspecific marker of T-cell activation, the precise mechanisms by which serum ADA activity is altered has not been clearly identified yet (12).

In this study, we aimed to explore whether the levels of ADA alter in CD patients and its correlation with serum markers of CD. To the best of our knowledge, this study is the first to investigate the level of ADA in

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CD patients; therefore, we consider that, this study is important because it facilitates additional research into the immunopathogenesis of CD.

MATERIAL AND METHODS

The study was carried out in September 2008 and April 2009 on newly diagnosed 20 CD patients admitted to the gastroenterology department of Ankara Training and Research Hospital. The diagnosis was established by both histological findings of duodenum biopsy (total villous atrophy and lymphocytic infiltration) and positive anti-endomysial (EMA) or antigliadin antibodies (AGA). Ten milliliter venous blood samples were taken to measure ADA. None of the patients had tuberculosis or any other underlying inflammatory diseases that could influence the ADA levels.

Twenty-one healthy subjects were included as a control group. These were cases who were diagnosed with functional dyspepsia but were otherwise normal. All had a normal hemogram, biochemical tests, sedimentation rate and CRP and negative antibodies for antigliadin and endomisium.

ADA activity was measured with an enzymatic spectrophotometric method using a CL-770 clinical spectrophotometer (Shimadzu Corporation, Kyoto, Japan). Briefly, duplicate 25 ml samples were incubated for 60 min at 37 uC with 500 ml 21 mM adenosine (one sample) in 50 mM phosphate buffer and the ammonium ion released was determined by reaction for 30 min with 1.5 ml phenol nitroprusside in the presence of 1.5 ml sodium hypochlorite absorption at 628 nm, which was read in a spectrophotometer. To control for ammonium present before addition of exogenous adenosine, untreated samples were run in parallel.

SPSS for Windows Version 10.0 was used to analyze the data. All data are presented as mean (SD). For continuous variables the Mann–Whitney U test was used to compare differences between groups. The χ^2 test was used for comparison of catogorical variables. Receiver operating characteristic (ROC) curve analysis was used to identify optimal cut-off values of the ADA level to detect CD. Spearman's correlation analysis was used to analyze the data; P values of 0.05 were considered statistically significant.

RESULTS

There were 16 females and 4 males in the CD group and 13 females and 8 males in the control group ($P = 0.200$). The mean (SD) age of the patients with CD and the controls was 40.4 (15.7) years and 36.4 (10.4) years, respectively ($P = 0.354$). Serum ADA levels were 15.0 (4.9) U/l for patients with CD and 9.3 (1.2) U/l in the healthy control group; this difference was statistically

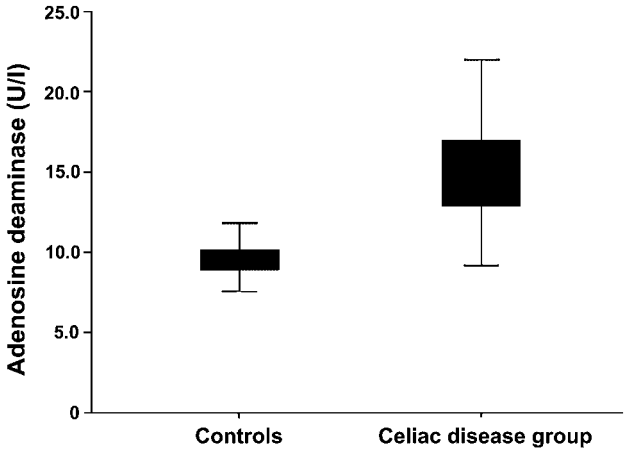

Fig. 1. Comparison of serum adenosine deaminase levels among CD patients and control subjects.

significant ($P < 0.001$, Fig. 1). When covariate analysis including age was used, the difference in the ADA level between the two groups was still significant ($P = 0.001$). ROC curve analysis suggested that the optimum ADA level cut-off point for CD was 12.27 U/l (area under the curve 0.874 (95% confidence interval (CI) 0.746–1.001). At a cut-off value of 12.27 U/l, the sensitivity was 80% (95% CI 55–93%), specificity 100% (95% CI 81–100%), positive predictive value 100% (95% CI 75–100%), and negative predictive value 84% (95% CI 64–94%). Calculated power of the study was 90% for $P < 0.05$.

At a cut-off value of 10.30 U/l, the sensitivity was 85% (95% CI 61–96%), specificity 81% (95% CI 57–93%), positive predictive value 85% (95% CI 57–93%), and negative predictive value 84% (95% CI 61–96%).

The mean level of ADA in patients with CD was 14.8 (5.5) U/l in women and 15.7 (1.5) U/l in men; the difference between the sexes was not significant ($P = 0.765$).

There was no statistically significant correlation between ADA and the other serum markers investigated (red blood cell distribution width: $r = 0.05$, $P = 0.834$; white blood cell count: $r = 0.232$, $P = 0.324$; Antigliadin IgA: $r = 0.332$, $P = 0.153$; Antigliadin IgG: $r = 0.068$, $P = 0.777$; EMA: $r = 0.041$, $P = 0.848$).

DISCUSSION

In this study, we examined ADA levels in CD patients. Serum ADA level is found to have high sensitivity, specificity, and predictive values in CD. Elevated ADA levels considered to having a role in the cytokine network of the inflammatory cascade of CD and a partial part of the activated T-cell response in the disease pathophysiology. Hence, evaluation of ADA activity in the serum of CD patients can be considered as a supportive diagnostic tool in CD.

Elevated ADA activity reflects a cell-mediated immune response in disease pathogenesis. During positive association with lymphocyte differentiation and proliferation, ADA level increases during mitogenic and antigenic responses of these cells. It is a polymorphic enzyme ubiquitous in mammalian tissue with the highest concentration in lymphoid tissues. It catalyzes deamination of both adenosine and 2′-deoxyadenosine to inosine and 2′-deoxyinosine, respectively (13).

ADA is essential for the differentiation of lymphoid cells, and has been used for monitoring various diseases in which immunity has been altered (14). As a sign of cell-mediated immune response, the serum activity of this enzyme has been proposed to be elevated in several inflammatory conditions, including infectious diseases, Behçet's disease, SLE, acute appendicitis, Graves disease, RA, and tuberculosis (10,15–17). Although lymphocytes 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 has not been clarified yet (18). To our knowledge, there is no study investigating the changes in serum ADA activity in CD patients, which could have led a new understanding of disease pathogenesis.

CD is an autoimmune enteropathy that is triggered in susceptible individuals by the ingestion of gliadin-containing grains. Although the disorder is characterized by a diferrent clinical heterogeneity that ranges from asymptomatic to severely symptomatic, it can be difcult to make a diagnosis based on clinical symptoms alone. Serological testing with EMA, transglutaminase antibody (tTG), and AGA has a well-established place in the diagnosis of CD, although definitive diagnosis always requires small bowel biopsy (19). After positive serologic test results for EMA and/or AGA, all of our patients underwent small bowel biopsy to confirm the diagnosis.

Aberrant T-cell populations play a key role in the pathogenesis of CD. Activated T-cells secrete cytokines such as INF-Gamma, as well as activated B-cells produce antibodies against gliadin and transglutaminase 2 resulting villous atrophy (20). In this study, high levels of ADA in CD patients suggest an action by cytokine release via T-cell activation, playing a major role in the inflammation process.

Among several causes which may lead to elevated ADA levels, the possibility of tuberculosis (TB) must be considered in the differential diagnosis because of the association with CD. The increased prevalence of TB among CD patients is considered to be due to a common genetic predisposition which leads to depressed cell-mediated immunity and/or malnutrition (21). ADA levels were also studied in malignant disorders and found to be elevated in malignancies such as laryngeal, head and neck, breast and lung cancers, as well as colorectal carcinomas (22–26). It is speculated that elevated ADA activity might be a physiological attempt by cancer cells to provide more substrate to accelerate salvage pathway activity (27,28). Although CD is an inflammatory disorder, it carries an increased risk of gastrointestinal malignancy. The most common neoplasm in celiac patients is jejunal T-cell lymphoma but also an increased frequency of small intestinal adenocarcinoma and squamous carcinoma of the esophagus has been reported. Most small intestinal lymphomas in the general population are of B-cell origin but intestinal lymphoma complicating CD is usually T-cell origin. Carcinoma, particularly of the oropharynx, esophagus, and small bowel accounts for more than one half of the remaining malignancies that may complicate CD (29). Unraveling the mechanisms that contribute to the development of lymphoma and other tumors in CD may well contribute to a wider understanding of oncogenesis. In this point of view this research may contribute a novel understanding of disease pathophysiology.

In conclusion, this study revealed that ADA levels were significantly elevated in CD patients. If our data can be confirmed with further studies, we believe that a standardized cut-off value would facilitate the diagnosis of CD. Moreover, extensive studies dealing with T-cells and ADA levels in CD patients are required to document the role of ADA in the immunopathogenesis of CD.

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