Adenosine deaminase in patients with primary immunodeficiency syndromes: The analysis of serum ADA1 and ADA2 activities

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Abstract

Objectives: We aimed to investigate the activity of ADA and its isoenzymes in serum of patients with various primary immunodeficiency (PID) syndromes.

Design and methods: Total ADA (tADA) and its isoenzymes were measured in 76 children with PID syndromes and 30 healthy controls using the Ellis method.

Results: Our results indicated that tADA and ADA2 levels were higher in patients with Chronic Granulomatous Disease (CGD), Leukocyte Adhesion Deficiency (LAD), hyper IgM (HIM) and Wiskott–Aldrich Syndrome (WAS) than those of corresponding controls (P<0.01). There was a significant elevation of tADA and ADA1 activities in IgA deficiency patients as compared to healthy individuals (P<0.01).

Conclusions: Our results hypothesized that altered ADA activity may be associated with altered immunity. Therefore, serum ADA level could be used as an indicator along with other parameters in follow up of patients with CGD, LAD, IgA deficiency, HIM and WAS.

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Keywords: Adenosine deaminase; Isoenzyme; Primary immunodeficiency

Introduction

Primary immunodeficiencies (PID) are a heterogeneous group of disorders, with defects in cellular and humoral immunity or non-specific host defense mechanisms mediated by complement proteins, and cells such as phagocytes and natural killer (NK) cells. A primary immunodeficiency (PID) disease results whenever one or more essential parts of the immune system is missing or not working properly at birth because of a genetic defect. They are relatively rare and occur in approximately one in 2000–10,000 live births [1]. All immunodeficiency syndromes predispose individuals to infections that are recurrent or due to unusual organisms [2–4]. PIDs are also often associated with autoimmune, haematological, and malignant disorders [5–7].

Adenosine deaminase (ADA, EC 3.5.4.4) catalyzes the deamination of adenosine and deoxyadenosine to inosine and deoxyinosine, respectively. ADA, an enzyme essential for the differentiation and proliferation of lymphocytes and the monocyte–macrophage system [8], has been used for monitoring several diseases in which immunity has been altered [9]. Two isoenzymes of ADA coded by different gene loci exist, namely ADA1 and ADA2, each with unique biochemical properties [10]. The ADA1 isoenzyme is found in all cells, with the highest activity in lymphocytes and monocytes, whereas ADA2 is the predominant isoenzyme in the serum of normal subjects [11]. Most human cells contain very small amounts of ADA2 and its major source is likely to be a monocyte–macrophage cell system [12]. Current interest in this enzyme has been stimulated by the finding that patients with inherited deficiency of ADA have a combined immunodeficiency [13], which is one of the most severe of the PIDs in humans. The metabolic basis for this immunodeficiency is likely related to the sensitivity of lymphocytes to the
accumulation of the ADA substrates adenosine and deoxyadenosine. Both humoral and cell-mediated immunity in these patients is severely impaired and have decreased numbers of peripheral blood lymphocytes (PBL). ADA is also associated with extracellular domain of CD26, one of many T-cell activation antigens [14–16]. Therefore, ADA activity is required for differentiation, normal growth, and proliferation of lymphocytes [8].

As ADA plays a significant and vital role in the mechanisms of the immune system, the genetic deficiency of the immune system components in various PID syndromes could affect its level in serum of these patients. Even though there are some controversial reports available on ADA levels in immunodeficient subjects [17,18], the patterns of ADA1, ADA2 and total ADA (tADA) and their correlation with various defects in immune system has not been elucidated.

To determine whether abnormal ADA levels are present in serum from PID patients and whether such abnormalities correlate with their impaired immune status, we decided to investigate the activity of tADA and its isoenzymes, ADA1 and ADA2, and compared the results with healthy individuals. The results obtained from this study, might imply new possibilities for the biochemical basis of these rare inherited disorders.

Methods

Patients

Patients, who were referred to the Children Hospital Medical Center, affiliated to Tehran University of Medical Sciences (TUMS) from September 2007 to April 2008, were included in this study. This hospital is the main pediatric referral center for children and infants with immunological disorders in Iran. A total of 76 pediatric patients with various PIDs were diagnosed and classified according to the general recommendations of the World Health Organization (WHO) committee [19,20]. Patients were diagnosed by the specialists based on the specific and appropriate laboratory assays and clinical studies. They were enrolled into the study by considering the local ethical approved and informed consent. Included were 30 patients with phagocyte defects, 25 with various antibody deficiencies and 21 with combined immunodeficiencies who had severe to moderate impairment of B-cell and T-cell function. For comparison, 30 normal healthy children in the same age group were included in the study. The age distribution of all subjects was 6 months to 18 years.

Blood sampling protocol

For collection of serum, blood was drawn into pyrogen-free tubes without additives. The tubes were immediately immersed in ice water, the blood was allowed to clot for 2 h, and centrifuged at 1000 × g for 10 min, and serum was stored at −80 °C until analyzed.

Measurement of adenosine deaminase

The ADA activity was assayed by the modified Ellis method [21] with a model 7070 type Automatic Analyzer (Hitachi Co. Ltd., Tokyo, Japan). This method is based on the indirect quantitation of the decreased amount of NADH by the release of ammonia, a product of the transformation of adenosine to inosine. One enzyme unit is defined as the amount of enzyme that converts 1 mM adenosine to inosine and ammonia per minute at standard assay conditions.

To distinguish between the ADA1 and ADA2 forms, the ADA activity was measured using the same technique with and without erythro-9-(2-hydroxy-3-nonyl) adenine (EHNA), obtained from Sigma-Aldrich (St. Louis, MO, USA.). EHNA, a potent selective inhibitor of ADA1, was used at a final concentration of 0.1 mmol/L. In its presence, only the ADA2 isoenzyme is active. The ADA1 activity is then calculated by subtracting the ADA2 activity from the total ADA activity [22].

Statistical analysis

All results were expressed in terms of mean ± standard deviation (S.D.). Comparison between the controls and all the

| Table 1: Adenosine deaminase activity in PIDs |
|-----------------|---|---|---|---|---|
|                | Total ADA activity, IU/L | ADA1, IU/L | ADA2, IU/L | %ADA1 | %ADA2 |
| CGD             | Median 16                 | 2          | 14.5       | 10    | 90    |
|                | Range 13–21               | 1–4        | 12–19      | 6–15  | 80–95 |
| LAD             | Median 19                 | 7.5        | 12         | 38    | 62    |
|                | Range 17–21               | 2–9        | 10–16      | 25–47 | 52–75 |
| Hyper IgE       | Median 14                 | 3          | 11.5       | 20    | 80    |
|                | Range 13–16               | 2–4        | 10–13      | 15–28 | 71–84 |
| CVID            | Median 14                 | 4          | 10         | 29    | 71    |
|                | Range 10–17               | 3–5        | 7–13       | 22–33 | 65–78 |
| IgA deficiency  | Median 19                 | 8          | 11         | 39    | 61    |
|                | Range 18–20               | 7–9        | 11–12      | 38–45 | 55–62 |
| IgG deficiency  | Median 13                 | 3          | 11         | 25    | 75    |
|                | Range 12–19               | 2–4        | 8–16       | 20–30 | 69–80 |
| HIM             | Median 23                 | 4          | 19         | 15    | 85    |
| WAS             | Median 17.5               | 3.5        | 14         | 20    | 80    |
|                | Range 12–25               | 3–10       | 9–16       | 16–25 | 70–84 |

* %ADA1 and %ADA2 represent the percentage contribution of ADA1 and ADA2 to total ADA activity, respectively.
patient groups were performed by one-way ANOVA test. A \( P \) value < 0.05 was considered statistically significant. Correlations between the variables of the study (tADA, ADA1 and ADA2), were measured by means of the Pearson’s Correlation Coefficient (\( r \)). All analyses were done with the Statistical Package for the Social Sciences (SPSS, Version 10.0).

**Results**

The results of the ADA activity studies, including the total ADA activity, medians, ranges and the percentage contribution of its isoenzymes to total ADA activity are shown in Table 1.

The mean tADA, ADA1 and ADA2 activities in healthy control subjects were found to be 13.83 ± 2.61 IU/L, 3.8 ± 1.09 IU/L and 10.03 ± 2.45 IU/L, respectively.

All enzyme assays for both the test and the control group, were carried out in triplicate, results represent the mean value.

The median ADA value was highest in the patients with HIM (23 IU/L). However, the lowest value was found for those with IgG deficiency (Table 1).

**Phagocyte defects**

As illustrated in Fig. 1, serum tADA and ADA2 in patients with CGD showed significant increase in levels (\( P < 0.01 \),
while ADA1 remained significantly lower when compared with controls ($P<0.01$). Serum tADA and both of its isoenzymes were significantly higher in LAD patients than the normal group (Fig. 2). No significant increase was found in serum tADA, ADA1, and ADA2 activities in hyper IgE patients in comparison to healthy control group.

**Antibody deficiencies**

There was a significant elevation of tADA and ADA2 activities in patients with IgA deficiency as compared to healthy individuals ($P<0.01$), however, ADA2 activity was not significantly increased ($P>0.05$) (Fig. 3). ADA and its isoenzymes in CVID and IgG deficiency patients were not significantly different from normal controls ($P>0.05$).

**Combined deficiencies**

Compared to healthy controls, serum tADA and ADA2 levels were significantly higher ($P<0.01$) in HIM and WAS patients, but the increase of ADA1 level was not statistically significant ($P>0.05$) (Figs. 4, 5).

**Discussion**

PIDs, a diverse group of disorders, affect cellular and humoral immunity. They mostly present in childhood with long-term infections and multiple recurrences that are resistant to antibiotics. This makes the early and prompt diagnosis of the disease a prerequisite to decrease the seriousness of their infections.

An enzyme in purine salvage pathway, ADA, is found to be present in higher concentrations in lymphoid tissues and is considered to play an important role in the proliferation and differentiation of lymphoid cells [23]. Previous studies claimed that changes in ADA activity reflect alteration in immunity [11,24,25], though the genetic deficiency of immune system components in PID syndromes might affect ADA level in serum of these patients.

In serum, ADA is known to be divided into two isoenzymes, ADA1 and ADA2, which have different molecular weights and kinetic properties. There are several reports that serum ADA1 and ADA2 activities has been increased or decreased in several diseases where cellular immunity is stimulated [26–28]. The congenital and genetically determined deficiency of ADA1 is usually associated with a severe form of combined immunodeficiency and is responsible for an increase in toxic nucleotides, which prevent the differentiation and/or proliferation of lymphocytes, causing a diminished immune response [13]. The high activity of this enzyme was considered a reflection of immunological disturbances observed in tuberculosis, infectious mononucleosis, leukemia, and other conditions [11,29–31]. Even though there are some controversial reports available on ADA levels in immunodeficient subjects [17,18], the patterns of ADA1 and ADA2 activity in serum of various PID patients have not been studied.

The present study was undertaken to clarify the pattern of two ADA isozymes, ADA1 and ADA2, in serum of patients with ADA and its isoenzymes activities in SCID patients showed variability: serum tADA, ADA1 and ADA2 in three SCID cases were within the normal range. We observed abnormalities in two SCID patients. One of these two had no detectable ADA1 activity in her serum, while the other patient had elevated serum tADA and ADA1 activities (22 IU/L and 7 IU/L, respectively) (unpublished data).

The correlation coefficient ($r$) between tADA, ADA1 and ADA2, are presented in Table 2.

**Table 2**

<table>
<thead>
<tr>
<th></th>
<th>CGD</th>
<th>LAD</th>
<th>Hyper IgE</th>
<th>CVID</th>
<th>IgA deficiency</th>
<th>IgG deficiency</th>
<th>HIM</th>
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<tr>
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</table>

$^a$ Significant.

$^b$ Extremely significant.

Fig. 5. Serum tADA, ADA1 and ADA2 activities (mean±S.D.) in WAS patients and healthy controls (**$P<0.01$).
with PID syndromes and comparing the results with those of healthy individuals. In order to be included in this study, patients had to present with clinical manifestations suggestive of various PID disorders, including CGD, LAD, hyper IgE, CVID, IgA deficiency, IgG deficiency, hyper IgM, WAS and SCID.

In this study, all of the patients, who met the criteria for the diagnosis of CGD, were found to have significantly higher activity of tADA, which is largely caused by an increased level of ADA2. It is corroborated by our findings that ADA2 contributed 90% of total ADA activity in patients with CGD and a significant correlation ($r=0.92; p<0.0001$) was found between tADA and ADA2. However, no positive correlation was determined between tADA and ADA1 activity ($r=-0.32; p=0.30$), and ADA1 and ADA2 activity ($r=-0.05; p=0.86$). In such patients, there is a defect in phagocytes to produce reactive oxygen to destroy the bacteria in the phagocytosis process [32]. Although ADA might play an important role in the induction of macrophage phagocytosis [33], CGD results from a defect in another enzyme, NADPH oxidase (PHOX). The significant increase in serum ADA and ADA2 in CGD patients can be attributed to the stimulation of T lymphocytes. The Granulomatosis in such patients shows the response of the body by enhancing T-cells response, which might be met by augmenting gamma interferon production. It in turn increases the activity of macrophages to partially compensate for the defect in hydrogen peroxide production to kill the pathogens by some other approaches. It has already been shown that the normal serum ADA2 may originate from monocyte/macrophage cells [12], so such findings could be explained by the higher macrophage activity. Our results regarding CGD were supported by previous studies on Systemic Lupus Erythematosus (SLE) [27,34]. Taysi et al. and Stancikova et al. concluded that the increased amount of serum ADA in SLE patients was due to the elevated ADA2 value, which was in turn the outcome of increased macrophage and T-cell activity [27,34].

The inherited molecular defect in patients with LAD is a deficiency of β-2 integrin subunit. This molecule helps neutrophils to make their way from the blood stream into the infected areas [35]. WBC differential in LAD patients reveals extremely elevated levels of neutrophils because of their defect in leaving the blood vessels. Ungerer et al. [11] reported that neutrophils are one of the major sources of ADA1 in blood, so the increased level of ADA1 serum in LAD patients, could be explained by the increased number of neutrophils in blood. There was a very strong correlation between ADA1 and tADA ($r=0.87; p=0.01$), comparing to this value between tADA and ADA2 ($r=0.63; p=0.71$). The finding indicates that the increase in tADA in patients with LAD could be the result of ADA1 elevated activity. Although the elevated ADA1 value in such subjects was reasonable, the increased ADA2 activity remains to be more investigated. The differences in isoenzyme patterns between the two groups of PID syndromes, CGD and LAD, could be indicative of different origins of ADA release.

Our investigations on CVID, IgG deficient and Hyper IgE patients failed to make any correlation between serum ADA activity and these syndromes. No individual with such disorders was found to have abnormal tADA, ADA1 and ADA2 activities comparing to that of healthy controls. Fleischman et al. concluded the same results regarding CVID patients (18).

Our study revealed that tADA level in the serum of IgA deficient cases is due to the elevated ADA1 activity, as the percentage of its contribution was investigated to be 61%. A strong correlation was found between tADA and ADA1 ($r=0.89; p=0.03$). Further studies are needed to elucidate the correlation between ADA levels and pathophysiology of IgA deficiency syndrome.

ADA activity in serum of HIM cases was found to be significantly higher than normal values in which ADA2 is the predominant isoenzyme (with 85% percentage of contribution). In these patients ADA2 activity is significantly correlated with the serum tADA levels ($r=0.87; p=0.01$). The highest median value of tADA among studied PID syndromes (23 IU/L), was found in the patients with Hyper IgM.

HIM results from the defects in the genes that are involved in the CD40 signaling pathway. However, the defects in two other genes (AID and UNG) have also been identified in HIM syndrome. All of these genes are necessary for antibody switching process in B-cells. Patients with HIM have severely reduced IgG, IgA, and IgE serum levels with normal or even raised IgM levels [36]. In these patients, T-cells might be more active to make the immune system balanced. It can be speculated that an elevated tADA value in HIM cases, which is due to the increased level of serum ADA2, might be the outcome of increased T-cell activities, leading macrophages to be more activated.

WAS is associated with a mutated protein which affects immune cell motility and trafficking. The abnormality affects all immune cells, including dendritic cells, macrophages, B- and T-cells, leading to abnormal immune responses. The data presented here, revealed that tADA activity in patients with WAS is significantly elevated, which is mainly due to increased ADA2 isoenzymes. ADA2 percentage of contribution to tADA in these patients was proved to be 80%. The correlation between two values (ADA2 and tADA) was found to be extremely significant ($r=0.85; p=0.007$). Therefore, this may indicate that the increased level in tADA was probably due to macrophage activation in addition to T-cell activation.

Our results on SCID patients agreed with the previous reports [37] and was submitted elsewhere.

Of note, no correlation was found between ADA1 and ADA2 isoenzymes in any of the studied syndromes. Hence, it can be considered that these two isoenzymes are acting independently in PIDs and could be viewed as separate diagnostic parameters.

In CGD, HIM and WAS cases, ADA2 was responsible for increased serum tADA. On the other hand, ADA1 caused elevated tADA activity in serum of patients with LAD and Hyper IgE. It might be concluded that the knowledge of the contribution of ADA isoenzymes to tADA activity is essential for the optimal interpretation of the results.

This research showed the usefulness of ADA assay in studying diverse PIDs. Even though several approaches are available for the diagnosis of PIDs, a more rapid precise
approach leading to early diagnosis might be useful with special respect to its low price and simplicity.

In conclusion, serum TADA and ADA2 may serve as useful indicators for diagnosis of CGD, LADs, IgA deficiency, SCID, HIM and WAS patients as a supplementary laboratory test in combination with clinical, immunological, and other laboratory findings.

Noteworthy, the overall diagnostic value of serum ADA determination might be enhanced by the scanning of its isoenzymes patterns. It might also be of a great usefulness in detection of the disease severity and treatment response, which needs to be elucidated by further studies.

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References


