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Sensitivity and specificity of adenosine deaminase in diagnosis of juvenile idiopathic arthritis

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Abstract

Background: Juvenile Idiopathic Arthritis (JIA) is one of the most common chronic rheumatic diseases in children with unknown etiology and pathogenesis. It also has no diagnostic test and its clinical diagnosis is made through ruling out other types of arthritis. The aim of this study was to evaluate the level of ADA (Adenosine Deaminase) in the serum of JIA patients and to compare it with that of patients with Reactive Arthritis (RA). Evaluation of sensitivity and specificity of serum ADA level in JIA was another objective.

Methods: The study included 120 children with JIA (mean age= 7.6 ± 4.3 years) and 40 children with RA (mean age= 5.5 ± 3.1 years). The ADA was measured in the active phase of both diseases.

Results: The mean ADA serum level was obtained as 15.8 ± 11.8 U/l in JIA patients and 14.3 ± 7.5 U/l in RA patients. The difference was statistically insignificant (p= 0.4). Another finding of this study was the significant specificity (77.5%) of this laboratory parameter for JIA in comparison with its low sensitivity (36.7%). Positive predictive value was 83% and negative predictive value 29%.

Conclusion: Determination of ADA serum levels is a noninvasive reliable and easy biomarker for diagnosis of JIA and it can be used as alternative parameters representing disease activity.

Keywords: Juvenile idiopathic arthritis, Reactive arthritis, Adenosine deaminase, Chronic arthritis, Children.

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Introduction

Juvenile Idiopathic Arthritis (JIA) covers a heterogeneous group of diseases which are similar in onset which is before the age of 16 and that they last for at least 6 weeks (1). International League of Associations for Rheumatology (ILAR) replaced Juvenile Chroni c Arthritis (JCA), mostly used in Europe, and Juvenile Rheumatoid Arthritis, used in North America, by JIA (1). JIA is the most common rheumatic disease in children (2). The ILAR proposes the following definition for JIA: Arthritis with unknown etiology that starts before the age of 16 and lasts for at least 6 weeks while other causes of arthritis have been ruled out (1).

Hence, the first step in diagnosis is to rule out other known causes of arthritis (3). JIA diagnosis is fundamentally clinical and there is no lab test to confirm it. However, some lab parameters have been used to determine the amount of inflammation, the severity of the disease, treatment efficacy, and its prognosis. The parameters include: Count of Blood Cells (CBC), Erythrocyte Sedimentation Rate (ESR), C-Reactive Protein (CRP), serum immunoglobulins,

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Rheumatoid Factors (RF), Anti-Nuclear Antibody (ANA), and Anti-Cyclic Citrullinated Peptide (Anti-CCP) antibodies (2). Anti-CCP antibodies are not actually checked in JIA patients, but may sometimes show severe disease (4).

Besides the above-mentioned lab tests, some radiology techniques are commonly used in JIA patients: ultrasonography and standard Magnetic Resonance Imaging (MRI) which are easy to use and cost efficient are two ways of joint assessment (5). Since late diagnosis may lead to irreparable damages to joints and the skeleton, early diagnosis is of great importance (2).

One of the lab parameters that have been studied in JIA patients is the serum Adenosine Deaminase (ADA) level. A few studies showed that activity of this enzyme in the serum of JIA patients in the active phase of the disease increases significantly compared to the healthy people (6, 7). A study on Behcet's patients showed an increase in the plasma level of this enzyme in the active phase of the disease and introduced ADA activity as a good marker for evaluating Behcet's activity and the follow-up (8). Serum ADA also increases in infections, malignancy and liver diseases (9).

ADA is an important cytoplasmic enzyme for purine metabolism that irreversibly catalyzes the conversion of adenosine to inosine (9). It is necessary for duplication, maturity and distinction of the lymphocytes (10). The activity of this enzyme increases in diseases in which the number of T lymphocytes increase (11). Hence, it could be said that T lymphocytes activation may cause an increase in ADA activity (8). ADA exists in serum and most tissues, especially lymphoid tissues, and is necessary for monocyte-to-macrophage differentiation (12-14). Two iso-enzymes of ADA have been differentiated as ADA1 and ADA2 (15,16). ADA2 is the dominant enzyme in serum and ADA1 is the dominant intracellular enzyme in monocytes. The reason for this difference remains unknown; some have proposed ADA2 active secretion by monocytes or relatively higher serum lifespan of ADA2 compared to ADA1 as the reason (9,17). The presence of ADA1 and ADA2 enzymes in the serum has a diagnostic value. ADA2 activity increases in the serum of JIA and SLE patients (6).

This study aimed to compare the serum level of ADA in JIA patients with reactive arthritis (RA) patients in the active phase of arthritis. Evaluation of sensitivity and specificity of serum ADA level in JIA was another aim of this study.

Methods

The study recruited 160 children with rheumatic disease including 120 JIA patients and 40 RA patients. All patients were in the age group of 1 to 17 years. The patients in both groups were hospitalized, evaluated and treated between 2010 and 2011 in the rheumatology ward of Children's Medical Center of Tehran University of Medical Sciences. Other causes of chronic arthritis such as tuberculous arthritis were excluded in all patients of both groups. JIA was diagnosed in all patients according to the criteria of International League of Associations for Rheumatology (ILAR) (1) and RA was diagnosed according to the criteria of Berlin Diagnostic Criteria for Reactive Arthritis (18).

JIA patients were categorized into three groups based on the subtype of the disease: oligo-articular (arthritis of 1-4 joints in the first 6 months of the disease), poly-articular (arthritis in $\geq$ 5 joints in the first six months of the disease) and systemic onset (arthritis $\pm$ two weeks fever + a sign of rash, lymphadenopathy, serositis, hepatosplenomegaly). About 57.6%, 21.2% and 21.2% of the JIA patients were oligoarticular, polyarticular, and systemic onset type, respectively.

The patients were investigated in terms of the serum level of ADA enzyme in the active phase of the disease. The active phase of the disease means active arthritis in at least one joint on clinical examination, that is, inflammation, limited range of motion or tenderness. ADA enzyme serum level in patients of both groups was studied at the

time of hospitalization. Patients' venous blood ADA was measured in the laboratory of Children's Medical Center.

Serum ADA was measured by an ultraviolet kinetic method with NADH as substrate (19), adapted to a RA-1000 Analyzer (Technicon Ireland LTD., Dublin, Ireland). Each unit of ADA can convert 1µmol adenosine to inosine and ammonia per minute under standard assay conditions and is expressed as IU/l. Normal value for serum ADA level was less than 15 IU/L.

Some other parameters were also measured in JIA patients including white blood cell and platelet count, hemoglobin, ESR, CRP, Antinuclear Antibodies (ANA), anti-CCP antibodies, and Rheumatoid Factors (RFs).

This study was approved by the ethics committee of Tehran University of Medical Sciences. SPSS software was used for statistical analysis. Parametric values were expressed as mean ± SD. T-test, ANOVA (or their non-parametric equivalents-Mann-Whitney test and Kruskal-Wallis test), and Tukey's Post-hoc test were used for investigating the difference between means. Chi-square test was used for comparing groups and $p< 0.05$ was considered significant.

Results

This study recruited 160 children with rheumatic disease including 120 JIA patients (58 boys and 62 girls) and 40 RA patients (26 boys and 14 girls). The subtype of JIA was 57.6% pauciarticular, 21.2% polyarticular and 21.2% systemic JIA.

The mean age of the JIA and RA patients was 7.6 and 5.5 years, respectively. The mean of serum ADA level was 15.8 ± 11.8 and 14.3 ± 7.5 U/l in JIA and RA patients, respectively; their difference was not statistically significant ($p= 0.45$).

The mean of serum ADA level was higher in systemic onset JIA patients than pauciarticular and polyarticular JIA patients (25.6 ± 21.3 U/l vs. 14.2 ± 6.7 U/l in pauciarthicular and 13.7 ± 7.3 U/l in polyarticular). These differences was significant ($p= 0.009$). There was a significant difference between systemic JIA and polyarticular JIA ($p= 0.027$), however serum ADA level didn't have significant difference in poly and pauciarticular JIA ($p= 0.9$).

Serum ADA was positive in 33.8% pauciarticular JIA patients. This rate was 32% and 48% in polyarticular and systemic JIA, respectively. Out of the 40 RA patients, 31 (77.5%) were ADA negative. This rate in JIA group was 63.3%. Out of 53 patients with ADA positive 44 patients (83%) had JIA. The sensitivity and specificity of ADA for JIA diagnosis were 36.7 % and 77.5%, respectively. Positive predictive value of serum ADA level was 83% and its negative predictive value was 29%.

Of the JIA patients, 89%, 78%, 46%, and 2.6% had increased ESR, CRP, ANA, and RF, respectively. The relationship between ADA and ESR, CRP or anti-CCP antibody was not significant ($p> 0.05$).

Discussion

JIA, an oligogenic or polygenic autoimmune disease, is one of the most common rheumatoid diseases among children. There are no lab tests or definite diagnostic approaches for the disease; they can just support the clinical diagnosis by showing inflammation, and can be used for assessing the response to the applied treatment (1). The aim of this study was to find an answer to the question whether the serum ADA level in JIA patients increases significantly in comparison to RA patients.

ADA as an indicator of cellular immunity plays a significant role in the regulation of the immune system. Although, serum activity of this enzyme increases in infections and malignancy, it can be altered in rheumatologic disorders that cause a cell-mediated immune response such as JIA and SLE (6, 9, 20).

Although, overall there is no significant difference between serum ADA level in JIA and RA patients, its applications cannot be totally overlooked. The main finding of this study was high specificity of ADA in JIA patients. The true negative rate for

ADA was calculated to be 77.5%, which means that 77.5% of the JIA negative patients were ADA negative. The true positive rate of ADA was 36.7%, which means a low sensitivity of the test in JIA. The positive predictive value was 83%. This means that if the ADA test is positive, there is 83% probability that the patient has JIA. But, if the test is negative, JIA cannot be ruled out. Specificity and sensitivity of ADA in pleural and synovial fluids has been shown in other studies (21,22), but these indexes were not reported previously in JIA or chronic arthritis. In a study by Zakeri et al (23), they reported higher significant level of ADA in rheumatoid arthritis patients in comparison with osteoarthritis patients. They reported 93% sensitivity and 53.3% specificity for serum ADA level. An association and/or correlation between serum ADA level and disease activity have been reported in RA and JIA patients (7,24). Similar our study, there is no correlation between serum ADA level and CRP or ESR in the patient chronic arthritis in previous study (24).

Conclusion

According to the findings of the present study, determination of ADA serum levels is a noninvasive, reliable biomarker for diagnosis of JIA and it can be used as alternative parameters representing disease activity. In JIA patients, high level of serum ADA was seen in systemic onset of JIA.

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Conflict of interests

The authors declare that there is no conflict of interests.

References

1. Petty RE, Southwood TR, Manners P, Baum J, Glass DN, Goldenberg J, et al. International League of Associations for Rheumatology classification of juvenile idiopathic arthritis: second revision, Edmonton, 2001. J Rheumatol. 2004;31:390–2.
2. Ki Hwan Kim, Dong Soo Kim. Juvenile idiopathic arthritis: Diagnosis and differential diagnosis: Korean J Pediatr. 2010; 53:931–5.
3. Singh S, Mehra S. Approach to Polyarthritis. Indian J Pediatr. 2010;77:1005–1010.
4. Kuna AT, Lamot L, Miler M, Harjacek M, Simundic AM, Vrkic N. Antibodies to mutated citrullinated vimentin and antibodies to cyclic citrullinated peptides in juvenile idiopathic arthritis. Clin Chem Lab Med. 2009;47:1525–30.
5. Fedrizzi MS, Ronchezel MV, Hilario MO, Lederman HM, Sawaya S, Goldenberg J, et al. Ultrasonography in the early diagnosis of hip joint involvement in juvenile rheumatoid arthritis. J Rheumatol. 1997;24:1820–5.
6. Hitoglou S, Hatzistilianou M, Gougoustamou D, Athanassiadou F, Kotsis A, Catriu D. Adenosine deaminase activity and its isoenzyme pattern in patients with juvenile rheumatoid arthritis and systemic lupus erythematosus. Clin Rheumatol. 2001;20: 411-6.
7. Ziaee V, Amiran A, Moradinejad M, Haghi-Ashtiani M. Evaluation of Serum Adenosine Deaminase Changes Before and After Treatment in Patients with Systemic Lupus Erythematosus, Henoch-Schonlein Purpura and Juvenile Idiopathic Arthritis. Ann Paediatr Rheum. 2013;2:21-6.
8. Calis M, Ates F, Yazici C, Kose K, Kirnap M, Demir M, Borlu M, Evereklioglu C. Adenosine deaminase enzyme levels, their relation with disease activity, and the effect of colchicine on adenosine deaminase levels in patients with Behçet's disease. Rheumatol Int 2005;25:452-6.
9. Ungerer JPJ, Oosthuizen HM, Bissbort SH, Vermmak WJH. Serum adenosine deaminase: Isoenzymes and diagnostic application. Clin Chem 1992;38:1322-6.
10. Sullivan J, Osborne WR, Wedgewood RJ (1977) Adenosine deaminase activity in lymphocytes. Br J Haematol 37:157-8.
11. Kose K, Yazici C, Ascioglu O (2001) The evaluation of lipid peroxidation and adenosine deaminase activity in patients with Behcet □s disease. Clin Biochem 34:125-9.
12. Adams A, Harkness RA. Adenosine deaminase activity in thymus and other human tissues. Clin Exp. Immunol 1976;26:647-9.
13. Fischer D, van den Weyden MB, Snyderman R, Kelly WN. The role for adenosine deaminase in human monocyte maturation. J Clin Invest 1976;58:399-407.
14. Macdermott RP, Tritsch GL, Formeister JF. Adenosine deaminase and nucleoside phosphorylase activities in normal human blood mononuclear cell

populations. Clin Exp. Immunol 1980;42:303-7.

15. Hirschorn R, Ratech H. Isozymes of adenosine deaminase. In:Ratazzi MC, Scandalia JG, White GS, eds. Current topics in biological and medical research. Vol 1. New York: Alan R. Liss, 1980; Pp:132-57.

16. Gakis C. Adenosine deaminase (ADA) isoenzymes ADA1 and ADA2: diagnostic and biologic role. Eur Respir J 1996;9:632-3.

17. Oosthuizen HM, Ungerer JP, Bissbort SH. Kinetic determination of serum adenosine deaminase. Clin Chem 1993;39:2182-5.

18. Kingsley G, Sieper J. Third International Workshop on Reactive Arthritis: an overview, Ann Rheum Dis 1996;55:564-70.

19. Ellis G, Goldberg DM. A reduced nicotinamide adenine dinucleotide-linked kinetic assay for adenosine deaminase activity. J Lab Clin Med 1970;76:507–17.

20. Moon J, Han C, Kang S, Park M, Hwang S, Byun M, Chung W, Hwang H, Kim Y, Kim S, Chang J, Kim S. The relationship between age and pleural fluid adenosine deaminase activity in pleural tuberculosis. Tuberc Respir Dis 2005;58:459-64.

21. Foocharoen C, Sarntipipattana C, Foocharoen T, Mahakkanukrauh A, Paupairoj A, Teerajetgul Y, et al. Synovial fluid adenosine deaminase activity to diagnose tuberculous septic arthritis. Southeast Asian J Trop Med Public Health. 2011;42:331-7.

22. Mathur P.C, Tiwari K.k, Trikha S, Tiwari D. Diagnostic value of adenosine deaminase activity in tubercular serositis. Indian J Tuberc 2006;53:92-95.

23. Zakeri Z, Izadi S, Niazi A, Bari Z, Zendeboodi S, Shakiba M,et al. Comparison of adenosine deaminase levels in serum and synovial fluid between patients with rheumatoid arthritis and osteoarthritis. Int J Clin Exp Med. 2012;5:195–200.

24. Sari RA, Taysi S, Yilmaz O, Bakan N. Correlation of serum levels of adenosine deaminase activity and its isoenzymes with disease activity in rheumatoid arthritis. Clin Exp Rheumatol. 2003;21:87-90.