Original Article

Adenosine deaminase (ADA) activity and isozymes in the serum of patients with hepatitis B compared with healthy people: a useful method in diagnosis clinical status

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Abstract

Background: Adenosine deaminase (ADA) specifications (EC3.5.4.4) is an enzyme is involved in purine metabolism that breaks adenosine and deoxy-adenosine and produce inosine and deoxy-inosine and ammonia. The highest levels are of adenosine deaminase activity in monocytes and lymphocytes. High serum ADA activity with increased serum levels of AST, ALT and immunoglobulins were reported in variety of diseases including hepatitis. We aimed to investigate the activity of total ADA in serum of patients with hepatitis B and were determined molecular weight ADA1 and ADA2 isozymes in serum and RBC of hepatitis patients. **Materials and Methods:** We were defining experiments by electrophoresis on SDS-PAGE, for isozymes of ADA; ADA1 and ADA2 in serum and red blood cells. ADA1 molecular weight was estimated at about 35 KDa and ADA2 about 110 KDa. The total ADA activity was measured by the modified Ellis method in 37 patients with hepatitis B and 40 healthy controls in the age range (20-60 years). **Results:** The normal values for serum ADA in humans have been studied by various workers and found in serum samples to be in the range of 0-15 U/L, whiles in our analysis total ADA (tADA) enzyme activity is in controls and patients with hepatitis B, respectively, 13.35 ± 1.62 and 27.05 ± 8.49. Our results indicated that tADA level was higher in patients with hepatitis B than those of corresponding controls ($P < 0.05$).Total ADA enzyme activity shows a significant increase compared to the control group in all age groups tested. **Conclusion:** Therefore, the serum ADA level could be used as an index along with other parameters in follow up of patients with hepatitis B.

Keywords: Adenosine deaminase (ADA), hepatitis B, SDS-PAGE electrophoresis

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Introduction

Hepatitis B is characterized as an inflammation of the liver and a potentially life-threatening liver infection which caused by the hepatitis B virus (HBV). It is a major global health problem and it can cause chronic infection and puts people at high risk of death from cirrhosis and liver cancer [1]. The virus is transmitted by exposure to blood, semen, or another body fluid of an infected person. This can happen through sexual intercourse; working in healthcare; tattooing and acupuncture; sharing needles, syringes, or other drug-injection equipment; or from mother to baby at birth [2]. Viral

hepatitis is usually monitored by repeated measurements of aminotransferase and viral load levels. Quantitation of viral load by polymerase chain reaction (PCR) may offer a more reliable marker of disease status. About 350 million people are chronically infected with hepatitis B. More than 780,000 people die every year due to complications of hepatitis B, including cirrhosis and liver cancer [3, 4]. Hepatitis B is an important occupational hazard for health workers. However, it can be prevented by currently available safe and effective vaccine. Most people, particularly children, don't experience any symptoms during the acute infection phase [5]. However, some people have acute illness with symptoms that last a few weeks, including yellowish skin and eyes (jaundice), dark urine, tiredness, vomiting and abdominal pain [6].

Adenosine deaminase (EC 3.5.4.4) is an amino hydrolytic enzyme that is involved in the deamination of adenosine and deoxy-adenosine nucleosides, forming inosine and deoxy-inosine, respectively. ADA is widely distributed in human tissues included in spleen, kidney, serum, lymphocytes, leucocytes and erythrocytes. In particularly, ADA has an important role in proliferation and differentiation of lymphocyte cells [7-9]. The enzyme is a glycoprotein (molecular weight=32.5-33kDa) which contains galactose and glucosamine. Optimum PH of 3.6, and the isoelectric point of is 4.85. The receptor activator show not been reported and no metal ion as a cofactor. The deamination specificity is same of the adenosine and deoxy-adenosine. In humans, the highest ADA activity exists in the thymus and other lymphoid tissues (800 IU/ mg) and the lowest activity in erythrocytes (1 Iu/ mg). Also, ADA activity in T cells are much higher than B cells[10, 11].The normal values for serum ADA in humans have been studied by various workers and found in serum samples to be in the range of 0-15 U/L. pleural fluid, values were found to be in the range of 6.8-30 U/L, and for CSF , values were found to be in the range of <5 U/L [12-14]. altered serum ADA activity were reported in portal cirrhosis, acute hepatitis, tuberculosis, leishmaniosis, arthritis rheumatoid, HIV, hepatitis, jaundice, hematoma and primary immunodeficiency [9, 15, 16]. Two ADA isozymes are identify as ADA1 and ADA2.The ADA1 isozyme is found in all cells, with the highest activity in lymphocytes and monocytes, while ADA2 is the principal isozyme in the serum of normal subjects. Most human cells contain very small amounts of ADA2 and its major source is likely to be a monocyte macrophage cell system [7]. ADA deficiency in humans is an autosomal recessive disorder that results in severe combined immunodeficiency disease, ADA deficiency caused a widely of diseases, increase or decrease the enzyme activity exists in many diseases and in some disease-specific isozymes are changed [17]. Therefore, the methods and techniques designed to measure the ADA as an important complementary test to help diagnose many diseases. Since molecular weight and activity of these isozymes different with each other, it is possible to investigate and isolate them in the laboratory. the purpose of this study were to determine the serum ADA activity in patients with hepatitis and compare with the control group and this work shows a method of ADA isozymes detection in SDS-PAGE gel electrophoresis.

Methods

Patients. After confirming a specialist in medical center (Tehran Heart Center), we first of 37 patients with hepatitis B (16 men and 21 women) were studied between August 2012 and March 2013. For comparison, 40 similar sex and age-matched healthy controls were also included in this study. Serum samples were then centrifuged at rpm 2500 rpm for 10 minutes apart in the same way we obtain the serum of 10 healthy people as well.

Blood sampling protocol. For accumulation of serum, blood was drawn into pyrogen-free tubes without additives. The blood was allowed to clot for 1 hour and centrifuged at 1000× g for 10 min at 4 °C, and serum was stored at −80 °C until analyzed.

Measurement of adenosine deaminase. Total serum ADA activity was measured by the modified Ellis method[18] with a model 912 type Automatic Analyzer (Hitachi Co. Ltd., Tokyo, Japan). ADA assay kit is for the determination of ADA activity in human serum, plasma samples, CSF and pleural fluid. This kit has been prepared based on an enzyme adenosine to inosine in the substrate conversion and ammonia is

released. In the second stage the ammonia, alpha Keto-glutarate and NADPH is converted to glutamate and NADP+ by glutamate dehydrogenase, as absorption at 340 nm was measured in the sample is proportional to the amount of ADA. The rate of absorption at 340 nm will be a direct relationship with the ADA enzyme activity. One unit of ADA activity is defined as the amount of enzyme that converts 1 mM adenosine to inosine and ammonia per minute under standard assay conditions and is expressed as IU/l. The enzyme is stable for at least 24 h at 25°C, 7 days at 4°C, and 3 months at -20°C[7, 18].To minimize the measurement errors all the assays were performed two times and the mean value is presented.

Preparation of Serum and Lysate of Erythrocytes from electrophoresis. A pool of venous blood samples was used. The RBC were obtained after blood centrifugation 2000 rpm during 10 min at 4°C. Red blood cells were washed three times in saline solution (NaCl 150 mM) and then lysated with distilled water (1:3) using a vortex mixer for 5 minutes. After lysate centrifugation at 2000 rpm (10 min / 4°C), the supernatant was separated and were loaded in SDS-PAGE Gel for electrophoresis procedure. An appropriate dilution of the sample to a ratio of 1 to 10 and 1 to 50 are produced at a rate of 1 to 4 to make and loading buffer are added. The SDS-PAGE gel (8% and 10%) for the electrophoresis was prepared in 50 mM phosphate buffer (pH=6.7). A 100 mM phosphate buffer (pH= 6.7) was used as running buffer. The run was performed using 100VFor 4 hours. The environmental temperature was maintained at 4°C. 20 µL of sample were used in each well.

Statistical Analysis. All results were described in terms of mean ± standard deviation (SD). Statistical significance of differences between mean values was tested by one way analysis of variance (ANOVA) test. All analyses were performed using the Statistical Package for the Social Sciences (SPSS, version 23). A P Value less than 0.05 were considered to be significant.

Results

ADA activity assay. The comparison of enzymatic levels in the sera of viral hepatitis cases with the control group is shown in the Table 1. As the table shows results, ADA enzyme activity in comparing the two groups (patients and controls), were found to be significantly higher in the cases with hepatitis than the controls ($p<0.05$), but a significant amount were obtained in the hepatitis and control groups is larger than 0.05, so the tADA no significant difference in the men and women in the patient and healthy groups.

Table1: Adenosine deaminase activity in hepatitis B Total ADA activity was assayed in serum of healthy control and hepatitis B patients. In hepatitis B, the total activity of ADA in serum increased as compared to healthy controls. The difference was significant between control and hepatitis B patients ($p< 0.001$).

Subject information parameters	Healthy controls	Hepatitis B
Female	13.33±3.37	26.77±4.59
Male	13.42±3.21	27.43±9.11
Total ADA activity	13.35±1.62	27.05±8.49

Electrophoresis analysis

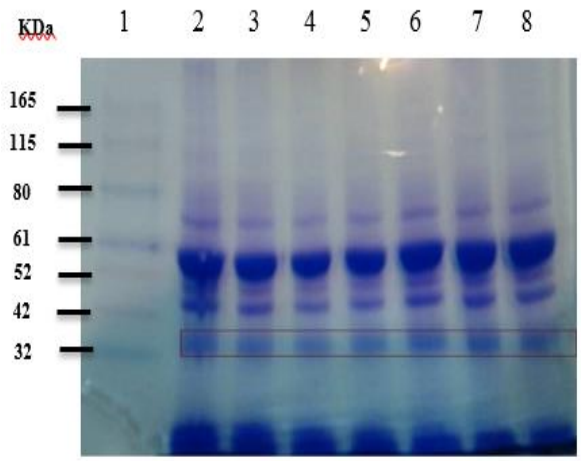

Figure 1. SDS-PAGE analysis of determined molecular weight ADA1 (10% gel).Line 1 Prestained ladder, line 2 to 8 contains RBC lysates with HBV patients in the presence of 2-mercaptoethanol (+2ME), which binds to free SH groups but does not alter the electrophoretic mobility of the RBC-ADA isozyme.

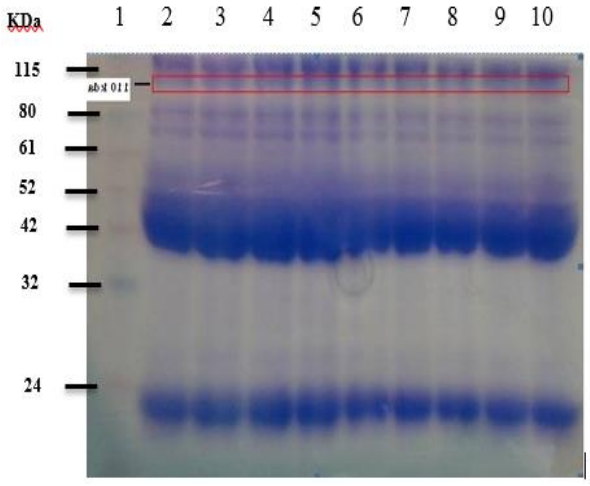

Figure 2. SDS-PAGE analysis of determined molecular weight ADA2 (8% gel). Line 1: Prestained ladder, line 2 to 10 contains pool serum with HBV patients in the presence of 2-mercaptoethanol (+2ME), which binds to free SH groups but does not alter the electrophoretic mobility of the serum-ADA isozyme.

Discussion

ADA is the major enzyme of adenosine metabolism. It plays a critical role in the differentiation and maturation of the immune cells including lymphocytes and monocyte-macrophage cell lines [7, 8]. An increases in serum ADA activities hepatitis B virus infected subjects [9, 19, 20], acquired immune deficiency syndrome (AIDS) [21], and other Infectious diseases such as tuberculosis, leishmaniosis, brucellosis, typhoid fever and human immunodeficiency virus infection [22-24], reflect the amplified in phagocytic activity of the macrophages and may be valuable in monitoring on the pathogenesis of hepatitis [9, 25, 26]. There are few reports available on ADA levels in hepatitis subjects, however, only one of them focused on ADA activity in different phases of the disease based on a body of experimental proof that revealed importance of ADA [15]. In another study, kaya et al, suggested that the evaluation of ADA activity in serum of patients with hepatitis B and C could be considered as a useful tool for monitoring their clinical status, and they reported that serum ADA levels were significant higher in HBV and HCV patients with high viral load than in controls, although there was no statistical difference in the ADA levels of HBV and HCV in patients with low viral load [27]. In agreement with previous studies, in this survey we observed the level of total ADA activity in serum with HBV patients clearly higher than in controls, and there was no significant difference between men and women with hepatitis patient.

Adenosine and 2'deoxyadenosine are molecules with many effects on human cells . Isozymes of ADA; ADA1 and ADA2 deaminated inosine and 2'deoxy-inosine. Thus, the homeostasis of these substances and the activity of the isozymes ADA1 and ADA2 in human cells are of extreme importance [28-30].

The isozyme ADA1 is ubiquitous and guarantees the adjustment of the substrates adenosine and 2'deoxyadenosine. PH of 7–7.5 and a similar affinity for both adenosine and 2'deoxyadenosine. These features make ADA1 highly efficient in deamination the substrates (adenosine and

2'deoxyadenosine), whiles the isozyme ADA2 is not ubiquitous, but coexists with ADA1 only in monocytes-macrophages. ADA2 has an optimum pH of 6.5 and a weak affinity for this homeostasis is particularly important because a low level of 2'deoxyadenosine is essential for a proper function in immune cells. ADA1 has an optimal 2'deoxyadenosine, these features make ADA2 inefficient in deamination 2'deoxyadenosine in biological sites [30-32].

However, prognosis and outcome of acute hepatitis B infection are variable. As a result of subclinical infection,

Chronic infection occurs frequently in 10% of infected individuals. In general, it is believed that immune system mediated processes play roles in the pathogenesis of hepatitis B infection. The mechanisms involved in liver cell injury may be an HLA class I restricted cytotoxic T-cell response directed at HBcAg/HBeAg on HBV- infected hepatocytes, direct cytopathic effect of HBcAg expression in infected hepatocytes in hepatitis, a direct toxic effect of HBV on liver cells, liver cell injury results from high-level expression and inefficient secretion of HBsAg, and finally co-infection with a second cytopathic virus, the hepatitis delta virus (HDV) [9].

In summary, we believe ADA activity may be considered as a more efficient marker of hepatitis in comparison with healthy. Subsequently, we suggest that serum ADA activity and molecular weight can be used in the diagnosis of HBV patient as a supportive laboratory test in combination with the other clinical and laboratory findings.

Conflicts of Interest

There is no conflict of interest among authors.

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