J Microbiol Immunol Infect.
2007;40:288-292

Original Article

Adenosine deaminase activity in serum of patients with hepatitis — a useful tool in monitoring clinical status

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Received: November 14, 2006 Revised: January 20, 2007 Accepted: February 15, 2007

Background and Purpose: The evaluation of adenosine deaminase (ADA) activity in sera of patients with hepatitis should be considered a useful tool in the monitoring of their clinical status. In this study, we aimed to determine the relationship between viral load, transaminase levels, and serum ADA levels in hepatitis B virus (HBV)- and hepatitis C virus (HCV)-infected patients.

Methods: Seventy three patients with hepatitis B, 71 patients with hepatitis C and 40 healthy individuals were included. Patients with HBV and HCV infections were classified into 3 groups according to viral load. Serum ADA levels were investigated by colorimetric assays.

Results: Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and ADA levels of HBV- and HCV-infected patients were higher than those of the control group. These differences were statistically significant for the levels of all enzymes in HCV-infected patients ($p<0.05$), and all except AST ($p>0.05$) in HBV-infected patients. ADA levels of HBV-infected patients with high viral loads were higher than those in HBV-infected patients with intermediate and low viral loads, and the difference was detectably significant between patients with high and intermediate viral loads. Evaluation of HCV-infected patients according to viral load showed no statistically significant relationship between viral load and serum ADA, ALT, and AST levels ($p>0.05$). HBV- and HCV-infected patients with high ALT and AST levels showed statistically significantly higher levels of ADA than patients with normal ALT and AST levels ($p<0.001$).

Conclusions: We suggest that serum ADA levels are associated more with the level of serum transaminases than viral load in HBV- and HCV-infected patients. In the treatment of patients with hepatitis, serum ADA levels should be considered a useful tool for the monitoring of liver condition.

Key words: Adenosine deaminase; Hepacivirus; Hepatitis B virus; Viral load

Introduction

Hepatitis B and C are diseases characterized by a high global prevalence, complex clinical course, and limited effectiveness of currently available antiviral therapy. Approximately 350 million people are infected with hepatitis B virus (HBV) worldwide, and the World Health Organization estimates that approximately 170 million people are infected with hepatitis C virus (HCV) [1,2]. HBV and HCV infections account for a substantial proportion of liver diseases worldwide. The most important biological characteristic of HBVs and HCVs is their ability to cause chronic hepatitis [3,4]. The natural course of HBV infection is variable, ranging from an inactive hepatitis B surface antigen (HBsAg) carrier state to the progressive chronic hepatitis that can evolve into liver cirrhosis and hepatocellular carcinoma [5]. HCV infection becomes chronic in 80% of infected individuals resulting in different degrees of chronic hepatitis, with 20-30% of these cases progressing to cirrhosis within a period of 20 years [6]. The mechanisms involved in liver damage induced by viral hepatitis are not fully understood, though both viral and host factors are believed to be implicated. Liver lesions could be the result of immune responses or the cytopathic actions of the virus. Cytotoxic T cells and

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cytokines, produced by both CD4+ (T helper) and cytotoxic T cells, may be responsible for much of the damage that occurs in the livers of infected patients [7]. Viral hepatitis is usually monitored by repeated measurements of aminotransferase and viral load levels [8]. Quantitation of viral load by polymerase chain reaction (PCR) may offer a more reliable marker of disease status [9].

Adenosine deaminase (ADA) is an enzyme involved in the catabolism of purine bases, capable of catalyzing the deamination of adenosine, forming inosine in the process [10,11]. ADA is widely distributed in human tissues and is higher in the lymphoid tissues, with the principal biological activity of ADA being detected in T lymphocytes [12]. Its main physiological activity is related to lymphocytic proliferation and differentiation. As a marker for cellular immunity, its plasma activity is found to be elevated in diseases eliciting a cell-mediated immune response [13,14]. The evaluation of ADA activity in the sera of patients with hepatitis should be considered a useful tool in the monitoring of their clinical status. In this study, we aimed to determine the relationship between viral load, and the levels of serum transaminases and ADA in HBV- and HCV-infected patients.

Methods

Patients

Seventy three patients with HBV (43 males, 30 females; mean age, 42.8 ± 12.5 years), 71 patients with HCV (34 males, 37 females; mean age [± SD], 41.5 ± 12.7 years), and 40 healthy individuals (20 males, 20 females; mean age, 38.4 ± 7.8 years; normal medical histories, physical examinations, blood biochemistry, and negative anti-HCV and HBsAg) were included in the present cross-sectional study. Informed consent was obtained from all subjects prior to their inclusion in the study. Patients were excluded from the study if they had a history of alcohol abuse, other known causes of liver disease (such as metabolic diseases, non-alcoholic steatohepatitis, or any other infectious cause of liver disease), or if the subjects had chronic diseases, such as diabetes mellitus, and cardiac or renal failure.

Anti-HCV, HBsAg, anti-HBs, anti-hepatitis B core antigen (HBc; total and anti-HBc immunoglobulin M) were assayed by the microparticle enzyme immunoassay (MEIA; Abbott AxSYM System, Chicago, IL, USA). HCV RNA and HBV DNA were investigated using the real-time PCR method (Roboscreen, Leipzig, Germany; GeneAmp 7700 Sequence Detector System, Perkin Elmer, Norwalk, USA). Lower and upper limits of HBV DNA levels with real-time PCR were 1×10^2 and 1×10^8 copies/mL, respectively. By the same method, these limits for HCV RNA were 6×10^2 and 6×10^8 copies/mL, respectively.

Patients with HBV and HCV infections were classified into 3 groups according to viral load (low viral load, $<10^3$ copies/mL; intermediate viral load, 1×10^3 to 9.9×10^4 copies/mL; high viral load, $>10^5$ copies/mL). HBV- and HCV-infected patients were also classified into 2 groups according to their alanine aminotransferase (ALT; normal range, 10-35 U/L) and aspartate aminotransferase (AST; normal range, 10-40 U/L) levels.

Serum ADA level investigations were carried out by colorimetric assays (Diazyme Laboratories, San Diego, USA) at 550 nm. The assay is linear in the range of 0-200 U/L, and ADA activity in healthy subjects is usually within the 4-20 U/L range.

Statistical analysis

All results are reported as mean ± SD. Parametric analysis of variance (ANOVA) and Student's *t* test were used to compare results in both patients and controls, and Spearman's rank correlation was used to test for association between variables. Statistical comparison of discontinuous variables was made using the chi-squared test. A p value <0.05 was considered significant.

Results

According to age and gender distribution, no statistically significant difference between HBV- and HCV-infected patients and the control group was detected ($p>0.05$). Serum ALT, AST, and ADA levels in the control group and patients are shown in Table 1. Serum ALT, AST and ADA levels in HBV- and HCV-infected patients were higher than those in the control group. While there were statistically significant differences in the levels of all of these enzymes for HCV-infected patients ($p<0.05$), HBV-infected patients showed an exception in only their AST levels ($p>0.05$).

ADA levels in HBV-infected patients with high viral load were higher than in those with intermediate and low viral loads. This difference was statistically significant between patients with high and intermediate viral loads according to Scheffe's one-way Post Hoc test. Only HBV-infected patients with high viral load showed statistically significantly higher levels of ADA ($p<0.001$), AST ($p<0.05$), and ALT ($p<0.001$) in comparison with the control group.

Table 1. Adenosine deaminase (ADA), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels of hepatitis B virus (HBV)- and hepatitis C virus (HCV)-infected patients

Variable (mean ± standard deviation)	HBV (n = 73)	HCV (n = 71)	Control (n = 40)	*p*
Age	42.84 ± 12.52	41.49 ± 12.70	38.35 ± 7.79	>0.05
Male:female (n)	43:30	34:37	20:20	>0.05
ALT	64.15 ± 88.74	70.41 ± 109.00	21.33 ± 6.79	<0.05
AST	46.55 ± 47.00[a]	62.59 ± 108.40	21.3 ± 7.36	<0.05
ADA	26.68 ± 11.66	30.01 ± 13.86	18.83 ± 3.12	<0.001

[a]$p>0.05$.

Serum ADA, ALT, and AST levels in patients with viral hepatitis B and C according to viral load are shown in Table 2. Evaluation of HCV-infected patients according to viral load showed no significant correlation between viral load and the levels of serum ADA, ALT, and AST ($p>0.05$). Serum ADA levels of patients with high and intermediate viral loads were higher than those of the control group and this difference was statistically significant ($p<0.001$). Furthermore, only HCV-infected patients with high viral load showed statistically significantly higher AST levels than the control group ($p<0.05$).

HCV- and HBV-infected patients with high ALT and AST levels had statistically significantly higher levels of ADA than in patients with normal ALT and AST levels ($p<0.001$) [Table 3]. Positive correlation was detected between the levels of ADA and those of ALT and AST in HBV- and HCV-infected patients ($p<0.001$).

Discussion

This positive correlation between serum ADA activity and liver disease is an original finding; highest levels of ADA are seen in cirrhosis and acute viral hepatitis. Hence, evaluation of ADA activity in the serum of patients with hepatitis can be considered a useful tool for monitoring their clinical status.

Increased serum ADA activities have been observed in infectious diseases caused by microorganisms infecting mainly the macrophages, like tuberculosis, leprosy, visceral and cutaneous leishmaniasis, brucellosis, typhoid fever and human immunodeficiency virus infection [15-19]. Earlier studies found that there was increased ADA activity in liver diseases, such as chronic active hepatitis and liver cirrhosis [20-22]. In this study, while serum ADA levels in HBV- and HCV-infected patients were higher than those in the control group, there was no significant difference between the serum ADA levels of HBV- and HCV-infected patients. Similarly, elevation of serum ADA levels has been reported in cases of alcohol liver disease with chronic hepatitis C complications rather than alcohol liver disease alone [23]. However, this is the first report of serum ADA levels being higher in chronic hepatitis C than chronic hepatitis B [21].

Barnes et al [24] reported that adenosine can lessen the potentially damaging activity of neutrophils at sites of infections. On the other hand, ADA activity neutralizes the effect of adenosine by utilizing it. Elevated serum ADA activity in patients with hepatitis is proposed to reflect the amplified phagocytic activity of macrophages, and may provide useful diagnostic information on the pathogenesis of hepatitis [23,25]. Early studies on ADA activity in serum of patients

Table 2. Serum adenosine deaminase (ADA), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels in hepatitis B virus (HBV)- and hepatitis C virus (HCV)-infected patients according to viral load

Variable (mean ± standard deviation)	ADA	ALT	AST
HBV (n = 73)			
Low (n = 15)	26.60 ± 9.64	70.87 ± 119.60	35.87 ± 25.25
Intermediate (n = 30)	23.33 ± 9.52	49.33 ± 65.73	39.07 ± 48.33
High (n = 28)	30.32 ± 13.82	76.43 ± 92.26	60.29 ± 52.25
HCV (n = 71)			
Low (n = 15)	25.20 ± 10.89	89.47 ± 150.20	42.40 ± 25.88
Intermediate (n = 29)	31.60 ± 16.70	53.66 ± 36.15	48.76 ± 28.15
High (n = 27)	30.93 ± 11.65	77.81 ± 133.60	88.67 ± 171.20
Control (n = 40)	18.83 ± 3.12	21.33 ± 6.79	21.30 ± 7.36

Table 3. Serum adenosine deaminase (ADA) levels in hepatitis B virus (HBV)- and hepatitis C virus (HCV)-infected patients according to alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels

Variable (mean ± standard deviation)	ADA (HBV-infected patients)	ADA (HCV-infected patients)
ALT		
High level (n = 39-32)	30.59 ± 12.46	36.13 ± 14.32
Normal level (n = 34-39)	22.21 ± 8.91[a]	22.56 ± 8.83[a]
AST		
High level (n = 39-31)	30.59 ± 12.46	38.26 ± 14.70
Normal level (n = 34-40)	22.21 ± 8.91[a]	23.63 ± 9.10[a]

[a]Student's *t* test: $p<0.001$ normal level vs high level group.

with active and chronic liver diseases suggested that monitoring ADA levels in chronic liver diseases might provide a useful guide for the interpretation of liver status [26]. Kalkan et al [25] reported that the evaluation of ADA activity in serum of patients with hepatitis could be considered a useful tool for monitoring their clinical status, although they found no difference in the ADA levels of acute and chronic hepatitis B cases. For this reason, we evaluated the ADA levels of HBV- and HCV-infected patients together with viral load and transaminase levels, without taking the chronicity of the disease into consideration. We observed that serum ADA levels in patients with hepatitis B and C infections are associated more with higher rather than lower viral load. The ADA levels were statistically 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.

The utility of evaluating serum aminotransferases in hepatitis has been highlighted in many earlier studies [8,9]. The degree of elevation in ALT values is interpreted as an indicator of liver damage. Zechini et al [8] suggested that aminotransferase values, particularly AST, may correlate with histological parameters of disease severity. The cause of this direct correlation between high AST values and an increasing degree of liver damage is not clear. Fanning et al [9] indicated a relationship between the degree of inflammation and serum viral load and transaminase levels in patients with chronic HCV infections.

Changes in serum ALT levels have been found to correspond with changes in viral load in HBV infections, and it will be interesting to see if this correlation is also seen in HCV infections. In chronic HBV infection, the increase of viral load and the subsequent immune response are thought to be responsible for initiating the acute exacerbation in liver injury [27]. HCV- and HBV-infected patients with high ALT and AST levels showed statistically higher ADA levels than patients with normal ALT and AST levels. Positive correlation was observed between the levels of ADA and those of ALT and AST in HBV- and HCV-infected patients in the current study.

In conclusion, we suggest that serum ADA levels are associated more with the levels of serum transaminases than with viral load in hepatitis B- and C-infected patients. However, further wide-ranging studies, including the histological parameters of patients, are necessary for serum ADA levels to be a useful tool for monitoring the liver condition of hepatitis patients.

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