Liver Disease Diagnoses Based on Serum Adenosine Deaminase Activity

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SUMMARY Serum adenosine deaminase activities were determined for 307 healthy controls, 105 patients with various liver diseases including acute hepatitis, chronic hepatitis, hepatoma and liver cirrhosis, and 34 patients with non-liver diseases. Determination of adenosine deaminase activity was effective for diagnosing all the liver diseases examined. The normal range of adenosine deaminase was 6.8 to 19.5 U/L, with the adenosine deaminase activity increasing in the order of acute hepatitis, chronic hepatitis, and liver cirrhosis. The validity (sensitivity + specificity) values of adenosine deaminase in acute hepatitis, hepatoma and chronic hepatitis were 1.00, 1.20 and 1.20, respectively. The highest validity (1.64) was found for liver cirrhosis when the cut-off level of adenosine deaminase activity was set at 40 U/L. Consecutive measurement of adenosine deaminase activity may be useful for diagnosing various liver diseases and the cut-off activity of 40 U/L seems to be especially important for predicting liver cirrhosis.

Introduction

Adenosine deaminase (AD, EC 3.5.4.4) catalyzes the conversion of adenosine to inosine and ammonia. It is widely distributed in human tissues with especially high activity being found in the spleen and intestines\(^1\). AD is important in the purine salvage pathway and the accumulation of adenosine caused by its absence is known to be toxic to cells such as fibroblasts and lymphoid cells in vitro\(^2\). Clinically, a lack of AD has been reported to be associated with severe combined immunodeficiency\(^3\). High activity of serum AD has been found in viral hepatitis\(^4\), infectious mononucleosis and liver cirrhosis\(^5,6\). We measured serum AD activity in various types of liver diseases and studied the usefulness of the AD test for diagnosing various types of hepatitis.
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Materials and Methods

Subjects

Three hundred and seven of healthy office workers were used as control (140 males, 167 females).

Their ages ranged from 20 to 65 years (mean 38). They completed a health questionnaire and underwent clinical laboratory tests to measure the pre-experiment levels of aspartate aminotransferase, alanine aminotransferase, calcium, creatinine, cholesterol, triglyceride and glucose in serum.

Patients with liver diseases were diagnosed by liver biopsy, laparotomy, angiography, increase of activity of liver enzymes such as aspartate aminotransferase and alanine aminotransferase and increase of bilirubin. The group consisted of 105 patients with various liver diseases; 30 with acute hepatitis; 20 with chronic hepatitis; 29 with liver cirrhosis; 26 with hepatoma. The 34 patients with non-liver diseases included eight with cardiovascular diseases such as congestive heart failure, constrictive endocarditis, valvular heart diseases, tetralogy of Fallot, angina of effort, aneurysm and lymphadenopathy, four with diabetes mellitus, four with hematopathy such as hypoblastic anemia, malignant cytosis and acute lymphocytic leukemia, four with cerebrospinal diseases such as meningitis, cerebral infarction, spondylitis and cephalhydrocele, four with respiratory diseases such as bronchial asthma, bronchogenic carcinoma, pneumococcosis and respiratory insufficiency, five with traumatopathy such as head injury, burns and transcervical fracture and five with other diseases such as stomach cancer, pancreatic head tumor, pyelitis, chronic nephritis and thyrophyma.

Enzyme assay

AD activity was determined according to the method of Giusti, in which ammonia produced by the action of AD from adenosine is measured by the Berthelot reaction. Activity of AD was expressed in International Unit at 37°C.

Statistical studies

From the results of AD activities in patients with various liver diseases and non-liver diseases, the predictive values (+) and (−) for each liver disease were calculated. The predictive values are the percentage true positives of the total number of patients with positive indicators and the percentage false negatives of the total number of patients with negative indicators. Patients with non-liver diseases were randomized with an equal number of cases of each liver disease. The risk ratio expresses the relative probability that subjects with the indicator have the specific disease compared with subjects without the indicator. It is calculated as follows:

\[
\text{Predictive value(+) \over \text{Predictive value(−)}}
\]

Sensitivity (Se) in each type of hepatitis was calculated with the cut-off levels of AD activity set at 10, 20, 30, 40, 50 and 60 U/L. In the same way, specificity (Sp) was calculated using the data of non-liver disease patients. Se and Sp curves were drawn for each hepatitis. AD activity at the intersecting point of Se and Sp curves show the same values of Se and Sp, i.e. the maximum validity (Se+Sp). We obtained the optimum cut-off levels of AD activity to show the maximum validity in each hepatitis type for effective diagnosis of liver diseases. From these data, we tried to establish a screening method for
various liver diseases based on measuring serum AD activity.

Results

Two lots of pooled sera were prepared for precision studies. The mean activities ±SD in the within-run assay were 16.0 ± 0.36 (CV = 2.25%) and 29.1 ± 0.78 (CV = 2.68 %) U/L (n = 20). In the between-run assay, the mean activities ±SD were 15.5 ± 0.75 (CV = 4.83%) and 30.2 ± 1.01 (CV = 3.30%) U/L (n = 20). Dilution studies with sera showed linearity up to 120 U/L.

Fig. 1 shows the distribution of serum AD activity of healthy control. The percentage of the relative cumulative frequency of AD activity in the control was plotted on log-normal probability paper. The distribution was of a log-normal type.
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and the normal range was from 6.8 to 19.5 U/L.

Fig. 2 shows the serum AD activities of various diseases. The mean activity in patients of liver diseases were significantly higher than that in the control. The highest mean activity was found for liver cirrhosis, with statistically significant differences from acute hepatitis (p<0.001), hepatoma and chronic hepatitis (p<0.05).

Table I shows the predictive value and risk ratio of various liver diseases. The predictive values (+) and (−) for liver cirrhosis were the highest (66%) and the lowest (0%), respectively, when compared with other liver diseases. Therefore, the risk ratio in liver cirrhosis was the highest.

Fig. 3 shows the Se and Sp curves at different cut-off levels of AD activity. When the cut-off level was set at 40 U/L in liver cirrhosis, Se and Sp were 82% and the validity (Se + Sp) was 0.82 + 0.82 = 1.64. In the same way, the points which showed the maximum validity and optimum cut-off levels were studied with other hepatitis varieties. The optimum cut-off levels were 21 in acute hepatitis, 28 in hepatoma and 27 U/L in chronic hepatitis and the validity values were 1.00, 1.20 and 1.20, respectively. Both the validity value and the cut-off activity decreased in the order of liver cirrhosis, chronic hepatitis, hepatoma, and acute hepatitis.

Discussion

We examined the changes in serum AD activity in various liver diseases and use of the probability values from the AD test to diagnose liver diseases. Zielhuis8) introduced the use of predictive value and risk ratio as indices of validity in biological tests. We calculated these values for groups of liver diseases. Although the predictive values (+) of AD were close to each other for the liver diseases examined, being from around 56% in acute hepatitis to 66% in liver cirrhosis, the predictive values (−) varied widely from 0% in liver cirrhosis to 42% in acute hepatitis. The risk ratio was the highest in liver cirrhosis, namely, the relative probability for subjects with indicator present com-

Table I Predictive values and risk ratios of the AD test in various liver diseases

<table>
<thead>
<tr>
<th></th>
<th>Predictive value (%)</th>
<th>Risk ratio</th>
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<tbody>
<tr>
<td></td>
<td>(+)</td>
<td>(-)</td>
</tr>
<tr>
<td>Acute hepatitis</td>
<td>56</td>
<td>42</td>
</tr>
<tr>
<td>Chronic hepatitis</td>
<td>58</td>
<td>38</td>
</tr>
<tr>
<td>Liver cirrhosis</td>
<td>66</td>
<td>0</td>
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<tr>
<td>Hepatoma</td>
<td>63</td>
<td>24</td>
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Fig. 3 Sensitivity and specificity of serum AD activity measurement in various liver diseases. ○ sensitivity, ○ specificity.
pared with subjects with indicator absent was higher in liver cirrhosis than in other liver diseases. Waaler\textsuperscript{10}) described the method of estimating Se and Sp for blood pressure measurements and gave the optimal cut-off level for defining hypertension in 1980.

We used the method of validity to decide whether the test of AD could be used for defining hepatitis. The optimum cut-off level is the value of AD activity used to judge the presence or absence of diseases with maximum validity. A validity value of 2.00 enables a clear decision as to the presence or absence of disease. According to our results, the mean activity increased in the order of acute hepatitis, chronic hepatitis and liver cirrhosis. In other words, the activity increased with the progress of the hepatitis. The highest validity (1.64) was observed with liver cirrhosis when the cut-off level was set at 40 U/L.

Therefore, the prognosis of a patient with hepatitis can be based on consecutive measurements of serum AD activity. The 40 U/L level may be an important value for diagnosing liver cirrhosis.

\textbf{References}

2) H. Green and T. S. Chan. : \textit{Science}, \textbf{182}, 836 (1973)