# Adenosine Deaminase and Guanase Activities in Sera of Patients with Liver Cirrhosis

## *KARACİĞER SİROZLU HASTALARDA SERUM ADENOZİN DEAMİNAZ VE GUANAZ AKTİVİTELERİ*

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**Summary**

Adenosine deaminase (ADA) and guanase activities in sera of patients with liver cirrhosis were investigated and compared with the control group of healthy individuals. Correlations amongst activity levels of the enzymes, ethiology of cirrhosis, clinical manifestations and liver functional tests were determined. Forty-two patients with cirrhosis and 40 healthy individuals were administrated to this study. Activity levels of ADA was found to be 33.9±13.3 U/L in sera of patients with cirrhosis, 18.2±3.4U/L in healthy individuals ($p<0.0001$), and guanase activities were 3.19±1.58 and 1.61±0.21 IU/L, respectively ($p<0.0001$). There was a significant positive correlation between ADA and guanase in sera. There were also significant positive correlations amongst guanase activity, alanin aminotransferase (ALT), aspartate aminotransferase (AST) and bilirubin concentrations. Among the groups with different stages of cirrhosis, significant difference was statistically present in serum ADA activities, but not in guanase activities. It was determined that there was no correlation between ADA, guanase activities in sera and the ethiology of cirrhosis. In consequence, ADA and guanase activities in sera of patients with cirrhosis increases, and it may be originated from compensatory elevated rate of the pathway of purine nucleotide salvage in other tissues resulted from inadequacy in the synthesis of nucleotides in the cirrhotic liver. Therefore, clinical stage of the disease with ADA activity and hepatocyte degeneration with guanase activities might be related to each other.

**Key Words:** Adenosine deaminase, Guanase, Cirrhosis

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**Özet**

Karaciğer sirozlu hastalarda serum adenozin deaminaz (ADA) ve guanaz aktiviteleri araştırılarak, sağlıklı bireylerin değerleriyle karşılaştırıldı. Sirozlu hastalarda bu enzimlerin aktivite düzeyleriyle, sirozun etyolojisi, klinik evreleri, ve karaciğer testleri arasındaki ilişki belirlendi. Çalışmaya 42 siroz hastası ve 40 sağlıklı birey alındı. Serum ADA aktivitesi sirozlu hastalarda 33,9±13,3 U/L, sağlıklı bireylerde 18,2±3,4 U/l ($P<0.0001$) guanaz aktivitesi sırasıyla 3,19±1.58 ve 1,61±0.21 U/L ($p<0.0001$) saptandı. Serum ADA ve guanaz aktiviteleri arasında önemli pozitif ilişki vardı. Serum guanaz aktivitesi ile serum alanin aminotransferaz ve bilirubin konsantrasyonları arasında önemli pozitif ilişkiler bulundu. Sirozun evreleri arasında, serum ADA aktivitesinde önemli fark varken, guanaz aktivitesinde yoktu. Serum ADA ve guanaz aktivitelerinin sirozun etyolojisi ile ilişkili olmadığı belirlendi.

Sonuç olarak sirozda ADA ve guanaz aktiviteleri artmaktadır, bu artışın nedeni sirotik karaciğerin nükleotid sentezinde yetersiz kalması sonucu, diğer dokularda pürin nükleotid kurtarma yolunun kompanzasyonel olarak artmış olmasından kayanaklanabilir. Serum ADA aktivitesiyle hastalığın klinik evresi, guanaz aktivitesi ile hepatosit degenerasyonu ilişkilendirilebilinir.

**Anahtar Kelimeler:** Guanaz, Adenozin Deaminaz, Siroz

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Adenosine deaminase (ADA, Adenosine aminohydrolase, E.C. 3.5.4.4.) and guanase (guanin aminohydrolase, EC 3.5.4.3) are important enzymes involved in the catabolism of purine nucleotides. While adenosine deaminase irreversibly

converts adenosine and dexyadenosine into inosine and deoxyinosine, respectively, guanase converts guanine into xanthin by deamination, (1,2). ADA activity is required for proliferation and differentiation of lymphocytes, and it was suggested that it might be a marker of cellular immunity (3). High serum ADA activities were observed in patients with acute hepatitis (4), haematological malignensiler (5) and certain infectious diseases in which the cellular immunity is required (6-8). It has a very invaluable diagnostic specificity in tuberculosis pleurisy (9). Although high serum activity of ADA was reported, The mechanism that cause its increase has not been cleared yet (10).

Guanase enzyme has a low measurable activity in serum, and there are very limited number of reports about the enzyme. A few reports indicated that serum activity of this this enzyme is increased in the hepatotoxicity (11). However, no sufficient information is present about its value in the cirrhosis. In human, red blood cells, polimorphonuclear leukocytes, brain and some other tissues can not synthesise purines de novo. De novo synthesis of purine nucleotides is mainly made in the liver in mammals, and these nucleotides are supplied for the tissues unable to synthesise de novo. Since the synthesis decreases in a cirrhotic liver, peripheral tissues obtain purines via their salvation.

We determined the correlation of parameters measured in laboratories and clinical findings with ADA and guanase activities in sera of cirrhotic patients in the study presented here.

## Materials and Methods

Subjects: The groups of patients (n=42) with liver cirrhosis, including 30 males and 12 females aged from 20 to 72, were occurred from Firat Medical Centre in Elazig. As a control group, 40 healthy individuals were 25 males and 15 females aged of 24-47 years from the same area. Ethical consents were obtained from all participants administrated to this study.

Clinical diagnosis of liver cirrhosis was obtained by histopathological techniques. In the diagnosis of the patients with a contraindication for biopsy, clinical and laboratory findings were used. Clinical stage of liver cirrhosis was determined in regard with Child's classification. Out of patients, fifteen (35.7%) were in Child A, 14 (33.4%) were in Child B and 13 (31%) were in Child C. For the diagnosis of cirrhosis, HBsAg, anti-HBs, HBeAg, anti-HBe, antiHBc, anti HCV and anti-Delta tests were run by using ELISA. Gamma glutamiltranspeptidase (GGT), protrombin time (PTT), alanine aminitransferase (ALT), aspartate aminotransferase (AST), bilirubines, total proteins and albumin were measured by Technicon autoanalyser.

Measurement of serum ADA and guanase activities: Ten ml of venous blood was withdrawn and transferred into dry tubes. Having centrifuged at 400 x g for 5 min, serum was separated for serum ADA activity assessment.

Serum ADA activity was determined at 37oC by a method as described by Giusti and Galanti (12) that was based on the Bertholet reaction. In brief, the formation of coloured indophenol complex from ammonia liberated from adenosine and quantified spectrophotometrically. One unit of ADA is defined as the amount of enzyme required to release 1 µmol of ammonia/min from adenosine at standard assay conditions. Results were expressed as international unit (IU) of enzyme activity of serum.

Guanase activity was measured as described. Ammonia formed during 30 min incubation at 37°C in the presence of guanine in a phosphate buffer (pH:7.4) is estimated colorimetrically using a modified phenol-alkaline hypochlorite procedure (13).

Statistical analysis: Student's t test, analysis of variance (ANOVA) test and correlation analyses were performed, using a computer program named SPSS for Windows Release 6.0. The value $p<0.05$ was considered as significant.

## Results

Means of ages of the cirrhotic cases and the control groups were 45.6±18.3 (20-72) and 42.2±12.6 (24-65) years, respectively.

Serum ADA activities in the cases with cirrhosis and in the controls were found to be 33.9±13.3 U/L and 18.2±3.4 U/L, respectively, shown in the Table 1 and the Figure 1. Difference between the groups was statistically significant ($p<0.001$). Serum guanase activities were determined in the

**Table 1.** Adenosine deaminase and guanase activities in the cases with the liver cirrhosis and in the controls; means in regard with etiology and Child's classification.

| | ADA (u/l) | Guanase activities (u/l) |
|---|---|---|
| Control (n=40) | 18.2±3.4 (12.2-31) | 1.61±0.21(1.23-2.15) |
| Cirrhosis (n=42) | 33.9±13.3 (12.4-63)* | 3.19±1.58 (1.04-6.82)* |
| Etiology | | |
| Hepatitis B (n=32) | 31.67±19.50 | 3.08±1.39 |
| Hepatitis C (n=6) | 44.32±14.22 | 4.86±1.60 |
| Hepatitis B+D (n=4) | 34.67±19.50 | 2.55±0.08 |
| Child class | | |
| A (n= 15) | 26.74±12.67 | 3.53±1.74 |
| B (n= 14) | 33.31±10.48 | 3.17±1.96 |
| C (n= 13) | 43.10±12.58** | 2.76±0.87 |

* Significant difference between the liver cirrhosis group and the controls ($p<0.001$; Student t test).
** Significance in the Child C group compared with Child B and Child A groups ($p<0.05$; ANOVA test ).

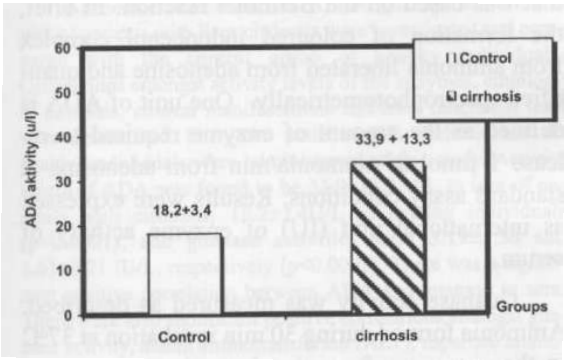


**Figure 1.** ADA activity in the liver cirrhosis and the control group.

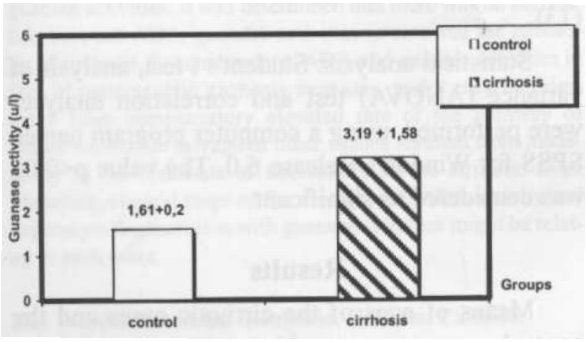


**Figure 2.** Guanase activity in the liver cirrhosis and the control group.

groups as 3.19±1.58 U/L and 1.61±0.21, respectively (Table 1, Figure 2). Difference between the groups was statistically significant ($p<0.001$). In cirrhotic cases, there was a positive correlation ($r=0.27$; $p<0.05$).

When evaluated in regard with the Child's Staging, applying variant analysis, ADA activity was significantly higher in the Class C and B than in the Class A ($p<0.05$). However, there was no difference among the activities of guanase of the Class A, B and C groups (Table 1). No correlation was found between etiological factors of ADA and guanase activities in sera ($p>0.05$). There was no significant correlation amongst albumin levels, ADA and guanase activities in sera, either. Moreover, no correlation was present among serum ADA activity, ALT, AST, bilirubin, alkaline phosphatase, g-GT and protrombin time. While there are correlations between serum guanase activity, ALT, AST and bilirubin, no correlation was found between serum guanase activity, alkaline phosphatase, g-GT, and protrombin time (Table 2).

## Discussion

Adenosine deaminase is one of important enzymes of catabolism of purine nucleotides. It irreversibly converts adenosine and deoxyadenosine into inosine and deoxyinosine. In physiological conditions, inosine is degraded to uric acid through xanthine oxidase pathway. It is alternatively turned into adenosine monophosphate and guanosine monophosphate via oxidation and amination reactions in all the cells.

Guanase converts guanine, which is formed from guanosine by the cleavage of ribose-1-phosphate, into xanthine by deamination. Enzyme is abundantly found in bowels, kidney and especially in the liver. It is a molibden possessing flavoprotein. Xanthine originated from guanine is shed in urine by being converted into uric acid. Xanthine is not used in the synthesis of guanosine monophosphate. De novo synthesis of guanosine monophosphate occurs via a salvage pathway from inosine.

Serum ADA activities were demonstrated to be elevated in many infectious diseases (6-9) and hepatitis (4,10) in which the cellular immunity is active (6-8). While it is observed that the elevation of ADA-2 isoenzyme produced from macrophages is responsible for high ADA levels, increase in ADA-1 isoenzyme produced from the cytosols of liver cells is considered to be responsible for ADA elevation in hepatitis (4,14). Elevated levels of ADA activity in sera of cirrhosis cases were also report-

**Table 2.** Some parameters in the cases with cirrhosis and their correlation with ADA and guanase activities in sera.

| Parameters | Means of values (ranges) | ADA | | Guanase | |
|---|---|---|---|---|---|
| | | r | p | r | p |
| Albumin (g/dl) | 3.23±0.82 (1-4.50) | -0.27 | 0.081 | 0.12 | 0.42 |
| ALT (u/dl) | 104.48±91.64 (12-342) | 0.22 | 0.15 | 0.84 | 0.000 |
| AST (u/dl) | 108.29±64.17 (23-271) | 0.21 | 0.17 | 0.44 | 0.004 |
| ALP (u/dl) | 164.12±112.67 (56-590) | 0.01 | 0.93 | -0.11 | 0.45 |
| Bilirubin (u/dl) | 1.55±1.72 (1-7.7) | 0.04 | 0.78 | 0.44 | 0.003 |
| l-GT (u/dl) | 67.88±60.44 (12-230) | 0.13 | 0.37 | 0.02 | 0.86 |
| Protrombin time (sn) | 15.29±4.57±4.57(11-30) | 0.21 | 0.17 | -0.11 | 0.48 |

ed (10,15). Nevertheless, its mechanism has not been established yet in the literature. Therefore, we intended to explore the mechanism of elevated ADA activity in the patients with cirrhosis in the study presented here.

In human, red blood cells, leukocytes and brain can not synthesise purines De novo and mainly use exogen purines, since they can not produce 5-phosphoribosilamin, which is the first step of purine nucleotide synthesis. De novo synthesis of purine nucleotides is mainly made in the liver in mammals. When the synthesis of purines can not be supplied adequately, these cells capture back nucleotide products in the inosine step, which originated from intracellular purine catabolism, then converts it into inosine monophosphate, adenosine monophosphate and finally guanosine monophosphate, inturn. As mentioned previously, these cells also use exogen purines. This pathway is called as purine salvage pathway. This pathway resembles in a limited manner salvage of iron removed from hem, not excreting in urine, and its use in De novo hem synthesis in the bone marrow.

In the report of Kobayashi and his colleagues, it was found that ADA-1 isoenzyme originated from the liver increased in acute hepatitis and ADA-2 isoenzyme mainly originated from lymphocytes increased in chronic active hepatitis and liver cirrhosis (10). We suggest that the mechanism that cause the elevation of ADA activity in sera of cirrhosis cases is increase the result of purine salvage pathways which are active in peripheral tissues. Therefore, purine synthesis in the liver will elevate in correlation with the stage of cirrhosis, and peripheral compensatory mechanism will also develop. We found a positive correlation between ADA activity levels and the stages of cirrhosis (Table 1). However, we could not observe the same correlation between albumin concentration, protrombin time and other biochemical parameters. These finding are in agreement with the study of Nardiello And his colleagues (16). According to these findings, serum ADA activities might be a useful parameter in laboratories in evaluation of clinical cirrhosis stages.

Guanase has low level of activity in sera. Therefore, its measurement requires time consuming and hard working techniques in laboratories compared to ADA measurement. It is probably the reason of limited reports about guanase activity. Guanase is a cytosolic enzyme and its activities are found to be high in hepatitis, and it was reported that it presents a correlation with ADA activity levels (11). There is no report indicating its importance in cirrhosis. This study is the first study serum guanase activity in the patients with cirrhosis. Nevertheless, it does not seem to be that high guanase activity detected in sera of cirrhotic patients by us is unlikely to resulted with as we established for the elevated ADA activities. Synthesis of guanosine monophosphate via the salvage mechanism also occurs through inosine, i.e.., ADA activity is required for this mechanism. However, very significant positive correlation between serum guanase activity and ALT activities ($r=0.84$, $p<0.0001$) indicates that elevated guanase activity reflects the liver damage.

Consequently, ADA and guanase activities in sera of cirrhotic cases increased. Elevation in ADA activity might be useful tool to determine the stage

of cirrhosis in clinics, and increased guanase activity might reflect the level of damage in hepatocytes.

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