ORIGINAL

Clinical value of the determination of serum guanase activity in patients with chronic hepatitis type C

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Abstract : The study examines the clinical significance of guanase (GU) measurement in patients with hepatitis C. 688 patients in whom either ALT was abnormal, or in whom HBsAg or HCVAb was detected in the serum, were enrolled into this study. The percentage of cases in which normal ALT while elevated GU was compared among the different disease groups. Then, the percentage of cases with normal ALT but elevated GU was compared between HBV and HCV groups. For the entire population, a significant correlation was observed between ALT and GU (r=0.872). The overall percentage of cases with normal ALT but elevated GU activity was 11.4%. In HCV group, 449 cases had normal ALT. Of these cases, 20.3% had elevated GU, while ALT was normal. Before 1989, no test to check donated blood for HCV antibody was available. However, screening of donated blood for high GU was associated with a reduced incidence of post-transfusion hepatitis. This is probably because following the screening, blood donated by patients with hepatitis C who had normal ALT but elevated GU was rejected. After the introduction of HCV antibody measurement, GU measurement is still useful to reveal the pathophysiological condition in-patients with chronic hepatitis type C. J. Med. Invest. 50 : 64-71, 2003

Keywords **:** *chronic hepatitis, HCV, guanase, ALT*

INTRODUCTION

No reliable screening method for donated blood had been established before 1972. The incidence of post-transfusion hepatitis was very high (20-40%) before that year (1). HBs antigen measurement was introduced in 1972 as a means of screening blood donated for transfusion. Since 1981, alanine aminotransferase (ALT) activity measurement has been additionally performed to check donated blood, resulting in a reduction in the incidence of post-transfusion hepatitis (2). However, non-A, non-B post-transfusion hepatitis was still seen in about 15% of blood recipients. Under these circumstances, serum guanase (guanine deaminase ; GU) measurement began to attract attention as a more effective means of screening donated blood (3-5).

GU is abundantly expressed in the liver, brain and kidney. It is quite scarce in the skeletal muscles, myocardium, pancreas and other organs in which aspartate aminotransferase (AST) and ALT are relatively abundant (6-7). Therefore, it has been reported that a rise in blood GU activity may be a sign specific to liver disease, and that measurement of his enzyme may serve as an excellent means of screening for liver disease (8-10). It has also been

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shown that the GU activity in transfused blood is significantly correlated with the incidence of post-transfusion hepatitis (3-5), and that discarding donated blood showing high GU activity can reduce the incidence of post-transfusion hepatitis (11). Based on these findings, the introduction of GU measurement as a screening test for blood donated for transfusion was discussed. However, in 1989, a method for measuring hepatitis C virus (HCV) antibody was published (12), which hampered the introduction of GU measurement as a screening test for donated blood.

In November 1989, checking donated blood for HCV antibody (C100-3 antibody) and HBc antibody was started. Since then, a sharp decrease in the incidence of post-transfusion hepatitis has been reported. Since February 1992, when second-generation HCV antibody measurement was introduced, the incidence of post-transfusion hepatitis has decreased to almost zero (13-15). Data collected from HCV antibody measurements revealed that most cases of post-transfusion non-A, non-B hepatitis were cases of hepatitis C. Therefore, it was thought that there could be some relationship between the serum GU activity and hepatitis C. To date, however, this relationship has not been studied. After the introduction of HCV antibody measurement, the significance of GU measurement has to be revealed.

The present study was undertaken to follow the time-course of changes in the serum GU activity in patients with hepatitis, and to examine the clinical significance of serum GU activity measurement in patients with hepatitis C.

SUBJECTS AND METHODS

Subjects

Of the patients who visited our department between January 1991 and December 1996, 688 patients in whom either serum ALT activity was abnormal, or in whom HBsAg or HCVAb was detected in the serum, were enrolled into this study. Fig. 1 shows the clinical details of these subjects. Elevated serum ALT activity was seen in 599 cases, HBsAg was positive in 91 cases, and HCVAb was positive in 436 cases. Thus, many patients had two or more of the three abnormalities. The subjects underwent biochemical tests and diagnostic imaging. Biopsy was additionally performed in some of the subjects. Based on the results, the subjects were diagnosed as being asymptomatic carriers (ASC), or as having chronic hepatitis (CH), liver cirrhosis (LC) or hepatocellular carcinoma (HCC) ; of all the subjects, 36 were diagnosed as being ASC, 372 as having CH, 130 as having LC, 53 as having HCC, and 116 as having "other disease". In those patients in whom liver biopsy was not performed, the diagnosis of ASC, CH, LC or HCC was made based on the following criteria. The diagnosis of ASC was made in cases where the serum ALT activity had never been abnormal. The diagnosis of CH was made in cases where the serum ALT activity had remained abnormal for 6 months or longer, but the criteria for diagnosis of LC and HCC were not satisfied. The diagnosis of LC was made in cases where three or more of the following criteria were satisfied, and diagnostic imaging also indicated signs of LC : 1) serum AST/ALT ratio>1.0, 2) serum cholinesterase activity (ChE) below 0.50△pH, 3) serum albumin level (Alb) below 3.5 g/dl, 4) blood platelet count (Plt) below 100,000/ml, 5) serum hepaplastin activity (HPT) below 70%, and 6) ICG R15 over 15%. The diagnosis of HCC was made in cases where diagnostic imaging (abdominal ultrasonography, dynamic CT, or angiography) revealed signs suggestive of HCC. The diagnosis of "other disease" was made in cases where none of the aforementioned liver conditions were diagnosed. The subjects were divided into the HBsAg-positive group (HBV group, excluding patients who were both HBsAg-and HCVAb-positive), the HCVAb-positive group (HCV

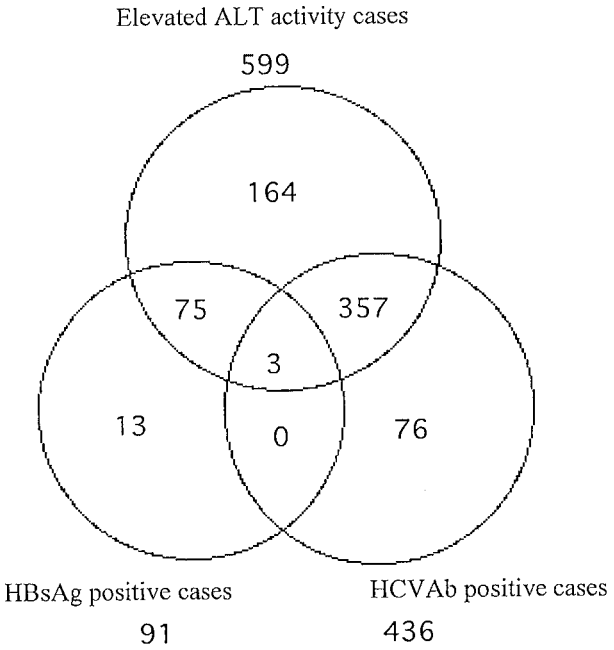

Fig. 1. The clinical details of the subjects (total : 688 cases)

group, excluding patients who were both HBsAg- and HCVAb-positive), the HBsAg + HCVAb-positive group (comprising patients who were both HBsAg- and HCVAb-positive), and the HBsAg, HCVAb-negative group (the NBNC group), as shown in Table 1. Of all the patients, 90 were assigned to the HBV group, 449 patients to the HCV group, 3 patients to the HBsAg + HCVAb-positive group, and 165 patients to the NBNC group. During long-term follow-up, CH progressed to LC in 7 cases, CH advanced to HCC in 1 case, and LC progressed to HCC in 11 cases. These 19 cases were counted in duplicate. For each of the followed up cases, hematological testing was conducted once every 1-6 months. When reporting the results, the number of cases was expressed as N, and the number of samples as n.

Measurement

HBs antigen was measured by the reversed passive hemagglutination (R-PHA) method ; HCV antibody was measured by enzyme immunoassay (EIA) ; ChE was measured by the enzyme method ; Alb was measured by bromcresol green (BCG) ; HPT was measured automatically with an MDA ; AST and ALT were measured by ultraviolet (UV) ; GU was measured by the enzyme method, using a Hitachi 736 automated analyzer.

Methods

1) Correlation between serum ALT and serum GU activity

For all samples (n=3744) obtained from 688 patients (707 cases, including duplicate counts), the correlation between the serum ALT and serum GU activity was evaluated. For each disease group, the correlation between the two was analyzed by further subdividing the group into the HBV group and HCV group.

2) Analysis of cases not showing a relationship between the serum ALT and serum GU activity

The percentage of cases in which the serum ALT activity was high while the serum GU activity was normal was compared between the HBV group and the HCV group, and also among the different disease groups. Then, the percentage of cases with normal serum ALT activity but elevated serum GU activity was compared between the HBV and the HCV groups. Patients with chronic hepatitis C in whom a normal ALT activity was recorded at least once during the follow-up period were deemed as cases showing normal ALT activity. The incidence of elevated GU activity during periods of normal ALT activity was investigated in these patients.

3) Testing the significance of differences

The F-test was used to test the coefficient of cor-

Table 1. Incidence of HBV and HCV in patients with liver disease

	ASC	CH	LC	HCC	Another	Total
B(+)C(-)	11	58	9 (10) from CH : 1 case	7 (8) from LC : 1 case	3	88 (90)
B(-)C(+)	25	310	70 (76) from CH : 6 cases	25 (35) from CH : 1 case from LC : 9 cases	3	433 (449)
B(+)C(+)	0	1	1	1	0	3
B(-)C(-)	0	3	43	8 (9) from LC : 1 case	110	164 (165)
Total	36	372	123 (130)	41 (53)	116	688 (707)

(counted in duplicate)

B : HBV C : HCV
ASC : asymptomatic carrier CH : chronic hepatitis LC : liver cirrhosis HCC : hepatocellular carcinoma
B(+)C(-) : HBV group
B(-)C(+) : HCV group
B(-)C(-) : NBNC group

relation. P<0.05 was regarded as denoting statistical significance. Fisher's exact test was employed to test the percentage of cases not showing any correlation between the serum ALT and serum GU activity, and Student's t-test was used for other tests. P<0.01 was regarded as denoting significance in these tests.

RESULTS

1) Correlation between the serum ALT and serum GU activity

Table 2 shows the coefficient of correlation between the serum ALT and serum GU activity and the p value for all samples (n=3744) obtained from 688 patients (707 cases if some patients were counted in duplicate). For the entire population, a significant correlation was observed between the ALT and GU activity (r=0.872, p<0.0001). When analyzed by disease, a significant (p<0.0001) correlation between the serum ALT and GU activity was observed in the CH group (r=0.756), the LC group (r=0.662), the HCC group (r=0.944) and the "other disease" group (r=0.918). Favorable correlation were observed among the HCC group and the "other disease" group. When analyzed by the type of virus, the correlation between the ALT and GU activity was significantly stronger (p<0.05) in the HCV group (r=0.891) than in the HBV group (r=0.756). Among patients with CH, the correlation was less strong in the HCV group (r=0.747) than in the HBV group (r=0.854), while among patients with HCC, the correlation tended to be stronger in the HCV group (r=0.946) than in the HBV group (r=0.816). However, none of these differences between the HBV and the HCV groups was statistically significant.

2) Analysis of cases not showing any correlation between the serum ALT and serum GU activity

Fig. 2 shows the percentage of cases not showing any correlation between the serum ALT and serum GU activity in each virus-type group. The overall percentage of cases with normal serum ALT activity but elevated serum GU activity was 11.4%. This percentage was higher in the HCV group (12.2%) than in the HBV group (4.4%), although this difference was not significant. The overall percentage of cases with elevated serum ALT activity but normal serum GU activity was 30.4%. This percentage was higher in the HBV group (34.4%) than in the HCV group (29.2%), although the difference was not statistically significant. In both the HBV and the HCV groups, the percentage of cases with normal serum ALT activity but elevated serum GU activity was significantly lower than the percentage of cases with elevated serum ALT activity and normal serum GU activity.

We then conducted a disease-wise analysis of the percentage of cases with elevated serum ALT activity but normal serum GU activity (Table 3). There was no significant difference in this percentage between the HBV and the HCV groups. In the HCV group, this percentage was significantly higher in patients with CH (38.4%) than in those with LC or HCC. In the HBV group also, this percentage was lower in patients with LC or HCC than in patients

Table 2. The coefficient of correlation between the serum ALT and serum GU activity and the p value for all samples (n=3744)

	HBV group (n=414)	HCV group (n=2474)	B+C+ (n=8)	NBNC group (n=848)	Total (n=3744)
CH (n=2198)	r=0.854 p<0.0001	r=0.747 p<0.0001	r=1.000 p=0.0183	r=0.899 p<0.0001	r=0.756 p<0.0001
LC (n=720)	r=0.663 p<0.0001	r=0.648 p<0.0001	r=0.945 p<0.0001	r=0.758 p<0.0001	r=0.662 p<0.0001
HCC (n=340)	r=0.816 p<0.0001	r=0.946 p<0.0001	r=0.715 p=0.0008	r=0.887 p<0.0001	r=0.944 p<0.0001
Another (n=486)	r=0.965 p<0.0001	r=0.950 p<0.0001		r=0.916 p<0.0001	r=0.918 p<0.0001
Total (n=3744)	r=0.756 p<0.0001	* r=0.891 p<0.0001	r=0.253 p=0.1943	r=0.914 p<0.0001	r=0.872 p<0.0001

* : p<0.05

with CH, similar to the relationship observed in the HCV group ; the difference between the two groups was not statistically significant.

The percentage of cases with normal serum ALT activity but elevated serum GU activity was also analyzed disease-wise (Table 4). Among patients with CH, this percentage was significantly higher in the HCV group (9.7%) than in the HBV group (0.0%). In both the HBV and the HCV groups, this percentage tended to increase as the underlying disease ad-

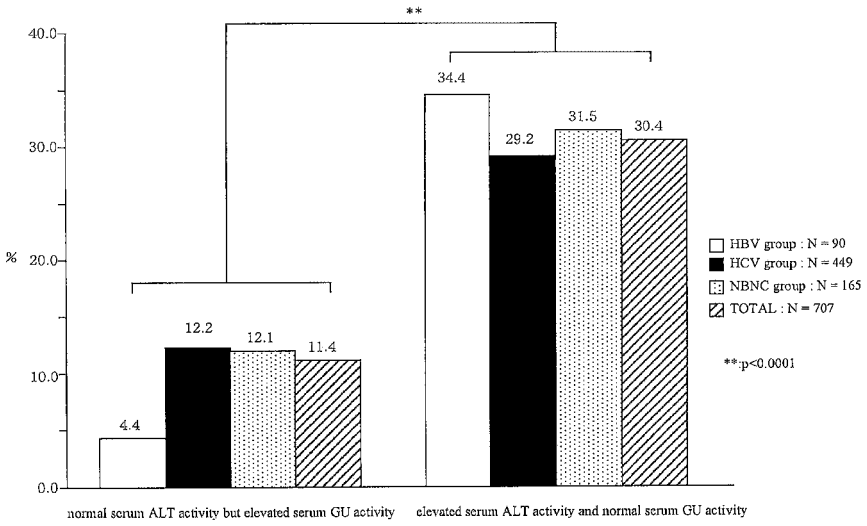

Fig. 2. The percentage of cases not showing any correlation between the serum ALT and serum GU activity in each virus-type group

The overall percentage of cases with normal serum ALT activity but elevated serum GU activity was 11.4%. The overall percentage of cases with elevated serum ALT activity but normal serum GU activity was 30.4%. In both the HBV and the HCV groups, the percentage of cases with normal serum ALT activity but elevated serum GU activity was significantly lower than the percentage of cases with elevated serum ALT activity and normal serum GU activity.
(** : $p<0.0001$)

Table 3. A disease-wise analysis of the percentage of cases with elevated serum ALT activity but normal serum GU activity

	ASC	CH	LC	HCC	other disease	mean
		NS				
		NS				
HBV group	0.0% (0/11)	49.2% (29/58)	10.0% (1/10)	12.5% (1/8)	0.0% (0/3)	34.4% (31/90)
		*				
		*				
HCV group	0.0% (0/25)	38.4% (119/310)	9.2% (7/76)	11.4% (4/35)	3.6% (1/3)	29.2% (131/449)

NS : not significant
* : $p<0.05$
(counted in duplicate)

Table 4. The percentage of each cases
(cases with normal serum ALT activity but elevated serum GU activity analyzed disease-wise in HBV and HCV groups, cases with normal serum ALT activity and cases with normal ALT but elevated serum GU activity in HCV group)

	ASC	CH		LC	HCC	other disease	mean
HBV group	0.0% (0/11)	0.0% (0/58)	*	18.2% (2/10)	25.0% (2/8)	0.0% (0/3)	4.4% (4/90)
HCV group	4.0% (1/25)	9.7% (30/310)		18.4% (14/76)	28.6% (10/35)	0.0% (0/3)	12.2% (55/449)
normal ALT activity in HCV group	100.0% (25/25)	61.6% (191/310)		46.1% (35/76)	54.3% (19/35)	33.3% (1/3)	60.4% (271/449)
normal ALT but elevated GU activity in HCV group	4.0% (1/25)	15.7% (30/191)		40.0% (14/35)	52.6% (10/19)	0.0% (0/1)	20.3% (55/271)

*$p<0.01$ (counted in duplicate)

vanced from CH to LC or from LC to HCC, but the change was not statistically significant.

3) Analysis of cases with normal serum ALT activity

In the HCV group, we analyzed the percentage of cases with normal serum ALT activity and the percentage of cases with normal ALT but elevated serum GU activity (Table 4). Of the 449 HCV-positive cases, 60.4% had normal ALT activity. Of these cases, 20.3% had elevated serum GU activity, while the serum ALT activity was normal. This percentage tended to increase as the disease advanced, however, the change was not statistically significant.

DISCUSSION

Guanase is an enzyme that was first detected in rabbit liver homogenates by Schmidt in 1932. In 1963, Passananti found that the serum guanase activity rose in patients with liver disease. Since then, guanase has been utilized in liver function testing, and its relationship to post-transfusion hepatitis has recently attracted close attention. In 1989, HCV antibody was detected (12), and some relationship between hepatitis C and serum GU activity was suggested. Thereafter, however, no report on this relationship has been published.

Under these circumstances, the present study was undertaken to examine the relationship between the serum GU and serum ALT activity in patients with hepatitis, and to examine the clinical significance of measuring the serum GU activity when dealing with hepatitis C. On the whole, a good correlation was found between the serum ALT and serum GU activity, but no correlation was seen in some cases. When analyzed by disease, the correlation was more marked in patients with HCC or "other disease" than in patients with CH or LC. When analyzed by virus type, the correlation tended to be higher in the HCV group than in the HBV group. Among patients with LC, the degree of correlation differed little between the HBV and the HCV groups. Among patients with CH, the correlation was more marked in the HBV group, while among patients with HCC, the correlation was more marked in the HCV group. In the cases of "other disease" and NBNC group, they include several different etiologies. So, the meanings of the differences concerned them are unknown. Thus, no definitive tendency was observed in this analysis. This suggests that the serum ALT and serum GU activities are not directly related to the underlying pathology or the pathogenic virus type, but rather to differences in the quantum of release of these enzymes from the liver depending on the underlying pathophysiological condition.

Takino *et al.* (16) and Noma *et al.* (17) have published some reports previously regarding the correlation between the serum ALT and serum GU activity in patients with liver disease. The coefficient of correlation reported by them is considerably lower than that obtained in our study. In the study conducted by Takino *et al.*, a correlation between the serum ALT and GU activities was observed in patients with acute hepatitis and CH, but they noted no correlation between the two in the LC group ($r=0.290$). In the study conducted by Noma *et al.*, a correlation was seen in patients with acute hepatitis, but not in patients with CH or LC. The low coefficient of correlation noted in the studies of Takino *et al.* and Noma *et al.* could probably be attributed to the conventional method with low sensitivity that they employed in these studies to determine the enzyme activities in the serum. In our study, the coefficient of correlation was higher in the HCC group and the "other disease" group than in the CH and the LC groups. These findings suggest that whether or not the serum ALT activity is correlated with the serum GU activity depends on the underlying pathophysiological condition in each patient.

In our analysis of the cases in whom no correlation was detected between the serum ALT and serum GU activity, the percentage of cases with normal serum ALT activity but abnormally elevated serum GU activity was significantly lower than the percentage of cases in whom the reverse was true. This finding could be associated with the differences in tissue distribution between ALT and GU, differences in the half-lives in the blood of the two enzymes, or differences in their release from the hepatocytes depending on the underlying pathophysiological condition. The percentage of cases with normal serum ALT activity but elevated serum GU activity was significantly higher in HCV-positive patients with CH. In both the HBV and the HCV groups, this percentage tended to increase as CH advanced to LC or LC advanced to HCC. One factor that could be responsible for this result is that as liver disease advances, the serum ALT activity returns towards normal more frequently than serum GU activity. In the HCV group, the percentage of cases with abnormally high serum ALT activity but nor-

mal serum GU activity was significantly higher in patients with CH than in patients with LC or HCC. This could be because the release of ALT and GU into the blood varies depending on the underlying liver disease.

Of the HCV-positive patients with normal serum ALT activity, 20.3% had abnormally high GU activity. Patients with hepatitis C are often asymptomatic and their serum levels are rarely elevated. It is highly probable that asymptomatic HCV-positive patients who were ASC, or had CH or LC, donated blood before the HCV antibody was discovered in this study. The serum ALT activity was probably normal in many of these patients, and it would seem that 20% of these patients could have been detected by serum GU activity measurement. Therefore, the results obtained by us endorses the view that serum GU measurement could serve as a useful means for screening blood donated for transfusion.

Overall, a significant correlation was observed between the serum GU and serum ALT activity in patients with liver disease. However, because the release of these enzymes into the blood varies depending on the pathogenic virus type and the underlying liver condition, there were cases in which the serum ALT activity was normal while the serum GU activity was elevated. The percentage of these cases was high among patients with chronic hepatitis C. Patients with chronic hepatitis C are often asymptomatic, and their condition is difficult to diagnose unless a test for HCV antibody is performed. Before 1989, no method to check for HCV antibody in the serum was available. It is highly probable that blood donated by HCV-positive individuals with normal serum ALT activity were used for transfusion before 1989. Serum GU activity measurement thus can probably serve as a very useful means for the screening of blood donated by HCV-positive individuals with normal ALT activity. The fact that the incidence of post-transfusion hepatitis decreased following the introduction of screening of donated blood for high GU activity implies that this screening test made it possible to reject blood donated by patients of hepatitis C with normal serum ALT activity but elevated serum GU activity.

Conclusion

Serum ALT and GU activities were analyzed in relation to the pathogenic virus type and the underlying liver disease. A significant correlation was observed in all the groups examined between the serum ALT and serum GU activity. However, the degree of this correlation varied, probably because the release of these enzymes into the blood varies depending on the type of the pathogenic virus and the underlying liver disease. The percentage of cases with normal serum ALT activity but elevated serum GU activity was high in the HCV group. Before 1989, no test to check donated blood for HCV antibody was available. However, screening of donated blood for high GU activity was associated with a reduced incidence of post-transfusion hepatitis. This is probably because following the screening, blood donated by patients with hepatitis C who had normal serum ALT activity but elevated serum GU activity was rejected.

After the introduction of HCV antibody measurement, GU measurement is still useful to reveal the pathophysiological condition in patients with chronic hepatitis type C.

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