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The Analysis of Polymorphism of *Alcohol Dehydrogenase 3 (ADH3)* Gene and Influence of Liver Function Status in Indonesia

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Indonesian culture actually has no historical record of behaviors in consuming alcohol, but there are many recent reports of alcohol abuse among Asian people involving their traditional drink. In genotype studies, the damage of the liver caused by consuming alcohol is influenced by the presence of the polymorphism enzyme gene. The lack of study regarding such topic is a signal to further investigate *ADH3* gene distribution and its effect on liver function status.

The total of 197 research subjects of Javanese descent received *alcohol dehydrogenase 3 (ADH3)* genetic polymorphism and liver status tests in the city of Yogyakarta, Indonesian. An analytical study with a cross-sectional design was then conducted on the subjects, with the resulting isolated DNAs amplified through polymerase chain reaction (PCR). The genotype of *ADH3* was determined by means of restriction fragment length polymorphism (RFLP) using *Ssp1* restricting enzyme.

Liver function status was assessed by measuring serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvate transaminase (SGPT) and gamma glutamyl transferase (GGT) using a photometric system. Gene types of *ADH3*1* (2.1%), *ADH3*2* (82.7%) and *ADH3*1/3*2* (15.2%) on the subjects were concluded, finding that there is no difference between the gender. In conclusion most of the *ADH3* gene polymorphism of the subjects were *ADH3*2* (82.7%). The influence of genetic polymorphisms on the status of liver function in the subjects showed significant difference according to GGT measurement, but the same cannot be said on the other two values measuring SGOT and SGPT.

INTRODUCTION

The abuse of alcohol leads to the damage of many organs: the liver, pancreas, gastric mucous membrane, and cerebral tissue resulting in the loss of behavior control.[1] Alcohol drinking is generally not a culture of Indonesians, but reality shows that several ethnics have, to date, uphold a tradition in which there is local beverage with a hint of alcohol involved in it. According to the Basic Health Institute (Riskesmas) 2007, 4.9% of men and 0.3% of women among individuals of age 15 and older have the prevalence of drinking alcohol within the last month. Moreover, 55.4% of total liquors being consumed are that of traditional brand or local-made. While 11.7% of them drink almost every day, 24.4% almost every week, and 35.8% nearly every month, based on Indonesia Demographic and Health Survey 2012, men are much more likely than women to drink alcohol. Overall, around 4 in 10 men had ever consumed alcohol, 23 percent of men are ex-drinkers, 16 percent consume alcohol occasionally, and less than 1 percent drink alcohol on a daily basis. Men age 20-24 and men with secondary or higher education are more likely than other men to have ever drunk alcohol.[2] Alcohol consumption may produce effects throughout the body, especially in the liver, because 80% of the alcohol is metabolized in the liver. However, individuals have varying responses to alcohol exposure, which is caused by the polymorphism of liver enzymes that metabolize alcohol. This enzyme polymorphism affects metabolism of alcohol in the body and influences an individual's susceptibility for becoming addicted to alcohol.[3]

Genetic polymorphism of the *alcohol dehydrogenase3*2 (ADH3*2)* enzyme is associated with a condition that slowly oxidizes alcohol and is reported to result in an inability to tolerate alcohol, have higher levels of high-density lipoprotein (HDL) and a decrease the risk of myocardial infarction.[4] Other studies have reported that genetic polymorphism of the *ADH3*2* enzyme allele increases the risk of alcoholism in Mexican-American men and protects against the negative consequences of chronic pancreatitis.[1,5]. The *ADH2*2* allele decreases the risk for alcoholism, whereas the *ADH2*2* and *ADH3*1* alleles are found to be associated in the European population [6]. In addition, further studies have reported that genetic polymorphism of

*ADH2*2* and *ADH3*1* enzymes decreases the risk of alcoholism and that polymorphism of *ADH3*1/*1* enzyme augments the risk of colorectal adenoma.[7,8]

Alcohol is widely misused in Indonesia; however no studies have been published to date on polymorphism of the alcohol-metabolizing enzymes in relation to liver function status of any Indonesians. Alcohol causes more than 60 known diseases and has other negative impacts. Hence, there is an urgent need to further study the influence of genetic polymorphism of the *ADH3* enzyme on liver function status among people of Javanese ethnicity in Indonesia.

MATERIALS AND METHODS

Study Population

A cross-sectional analytic study was conducted between November 2014 and July 2015 in Yogyakarta, Indonesian. Participants included 197 subjects who were adults of Javanese ethnicity from Yogyakarta, Indonesian. Individuals were considered Javanese with the criteria that they have 3 and more older generations (grandfather/grandmother, father/mother) of Javanese descent. Based on in-depth interviews of the subjects, all of them stated that they are in a normal health condition without any sign of liver disease or infection. All subjects had signed an informed consent form, and the study received approval from the ethics committee of Faculty of Medicine, Universitas Gadjah Mada.

Identification of genotypes

Blood samples of 6 ml were collected from each participant. The assessment of *ADH3* gene polymorphism in leucocytes DNA used the Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) method. Recognition sequences for amplification were *ADH3*321 (5'-GCTTTAAGAGTAAATATTCTGTCCCC-3') and *ADH3* 351 (5'-AATCTACCTCTTTCCAGAGC-3'). The PCR total reaction mixture of 25 µl contained: 0.5 µl recognition sequences, 1.0 µl of DNA, 0.2 µl of dNTP, 2.5 µl of PCR buffer (KAPA2G Fast ReadyMix PCR Kit), 0.25 µl of Tag polymerase, 1.25 µl of 50 mM MgCl₂, 18.80 µl of H₂O. The PCR method used for pre denaturation of DNA was 2 min at 96 °C, followed by 20 cycles at 94 °C for 1 min, 64 °C for 1 min, 70 °C for 1.5 min, and then 10 cycles at 92 °C for 1 min, 64 °C for 1 min, and 70 °C for 1.5 min. To perform RFLP for *ADH3* allele detection, aliquots of the amplified DNA products were digested with *SspI* enzyme (Thermo Fisher USA) at 37 °C for 16 h (overnight). Digestion products were subsequently run through electrophoresis. The *SspI* recognition sites (cutting sites) were the forward primer 5'...AAT*ATT...3'...and reverse primer 3'...TTA*TAA...5'. [9], the results of the pieces can be seen in Figure 1.

The levels of SGPT and SGOT were measured by Diasys ALAT glutamic pyruvate transaminase (GPT) FS reagent and Diasys ALAT glutamic oxaloacetic transaminase (GOT) FS reagent (Diasys Diagnostic Systeme), respectively, which are diagnostic reagents for in vitro quantitative measurement of SGPT and SGOT in serum or plasma using the photometric system. Similarly, GGT was measured by Diasys Gamma-GT FS reagent to determine the level of GGT in serum or plasma using the spectrophotometric system. We performed optimized UV tests that were in accordance with the International Federation of Clinical Chemistry and Laboratory Medicine guidelines.[10]

Statistical analysis

For statistical analysis, we used Chi-Square Tests

RESULTS

Below is the provided data about the characteristics of research participants to be seen in Table I. Most of the subjects are male (62.9%), adults (80.2%), and *ADH3*2* (82.7) .

Table I.
Characteristics of Javanese
population in Yogyakarta,
Indonesia

No	Variable	Subject n(%)
1.	Sex	
	Male	124(62.9)
	Female	73(37.1)
2.	Age groups according to criteria Hurlock	
	Adolescents (14-21 years)	6 (3.0)
	Adults (22-60 years)	158 (80,2)
	Elderly (> 60 years)	33 (16,8)
3.	<i>ADH3</i> type	
	<i>ADH3*1</i> (wild type)	4 (2,1)
	<i>ADH3*2</i> (polymorphic type)	158 (82,7)
	<i>ADH3*1/3*2</i> (heterozygote)	29 (15,2)

ANALYSIS OF ADH3 GENE POLYMORPHISM AND LIVER STATUS

Type of gene polymorphism *ADH3* pieces using enzymes *Ssp1* results can be seen in Figure 1, 2 and 3.

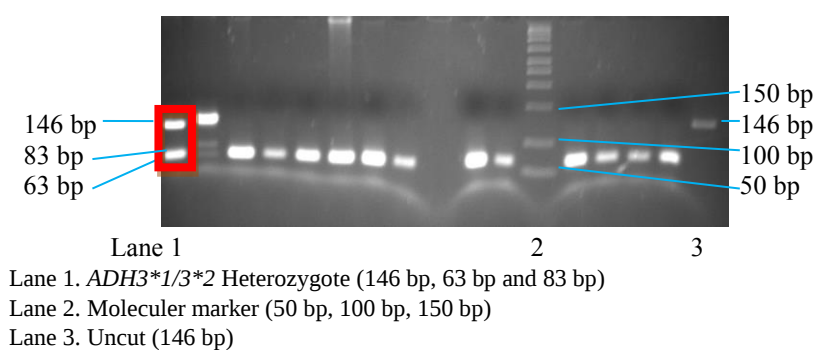


Figure 1. Schematic determination of allele gene *ADH3*1/3*2*

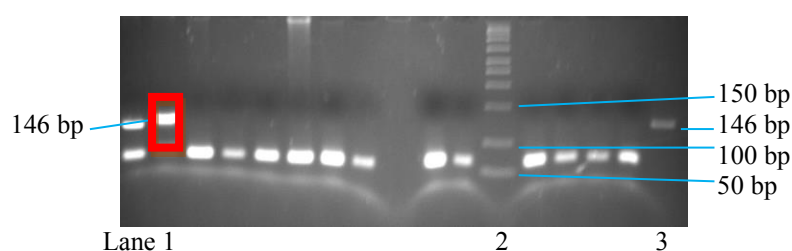


Figure 2. Schematic determination of allele gene *ADH3*1*

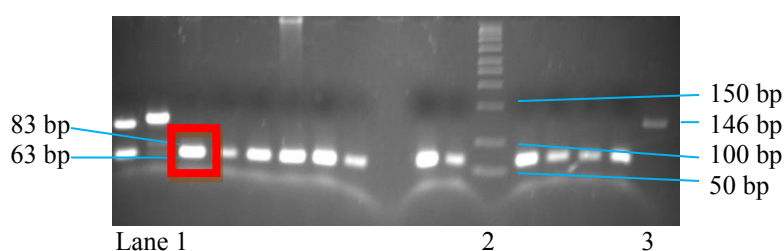


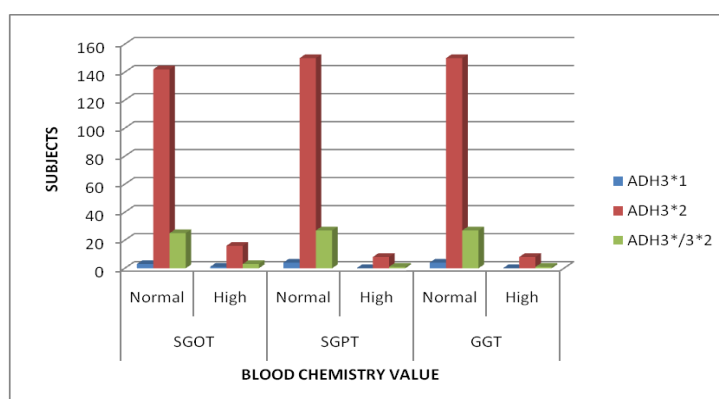
Figure 3. Schematic determination of allele gene *ADH3*2*

There were no significant differences in *ADH3* gene polymorphism between males and females, and gene types of *ADH3*2* were found in both genders, $p = 0,157$. Table II provides data about any gender-based differences in test results. Cut-off level is needed to determine the normal blood chemistry parameter. The numbers are as follows: normal SGOT level, male: 10-40 u/l, female: 7-35 u/l, normal SGPT level, male: 15-40 u/l, female: 13-35 u/l. normal GGT level, male: 9-49 u/l, female: 9-45 u/l. Otherwise, the sample is then considered to be 'high' or 'low' in relevance to the cut-off level. The analysis of blood chemistry for SGOT, and SGPT in relation to *ADH3* type among the subject showed no significant statistical differences, while GGT showed significant statistical differences. The p -values for SGOT, SGPT and GGT were 0,162, 0,171, 0,001, respectively. Table II below provides data about participants' blood chemistry and *ADH3* type.

Table II. Data regarding ADH3 type according of Javanese ethnicity in Yogyakarta, Indonesian.

Variable	ADH3 type.			p-value
	ADH3*1 (wild type) n (%)	ADH3*2 (homozygote polymorphic type) n (%)	ADH3*1/3*2 (heterozygote polymorphic type) n (%)	
Sex				
Male	4 (100,0)	99 (62,7)	18 (62,1)	>0,05
Female	0 (0,0)	59 (37,3)	11 (37,9)	
SGOT				
Normal	3(75,0)	142(89,9)	25(89,3)	>0,05
High	1(25,0)	16(10,1)	3 (10,7)	
SGPT				
Normal	4 (100,0)	150 (94,9)	27 (96,4)	>0,05
High	0(0,0)	8(5,1)	1 (3,6)	
GGT				
Normal	4 (100,0)	150 (94,9)	27 (96,4)	<0,05
High	0(0,0)	8(5,1)	1 (3,6)	

The distribution of data values from blood chemistry and polymorphism of *ADH3* gene can be seen in Figure 4. Most of blood chemistry values within normal limits and types of genes are largely *ADH3*2*.

Figure 4. The distribution of data values blood chemistry and polymorphism of *ADH3* gene

DISCUSSION

The subjects of the research were predominantly male participants (Table I), which may be associated with the cultural practice of men more often taking part in activities away from the home compared to women. Most were adult subjects although there were more males than females, but results did not show any significant difference. Analysis concluded to be no significant difference between age and gender among the subjects. The relationship between age with mean of liver function status on the subject shows no difference between age and the average liver function status. This pattern is reflected in the results of the participants' questionnaire stating that no subjects ever had any history of liver disease. The endemicity of hepatitis surface antigen in Indonesia is intermediate to high with a geographical difference. The risk of HBV infection is high in hemodialysis (HD) patients, men having sex with men, and health care workers. Occult HBV infection has been detected in various groups such as blood donors, HD patients, and HIV-infected individuals and children. The most common HBV subgenotype in Indonesia is B3 followed by C1. Various novel subgenotypes of HBV have been identified throughout Indonesia, with the novel HBV subgenotypes C6-C16 and D6 being successfully isolated. Although the number of studies on HBV in Indonesia has been increasing, adequate databases on HBV infection are limited [11].

In Table II, subjects were found to possess the *ADH3*2* (polymorphic type) genotype allele, showing that the majority types gen *ADH3*2* in males and females. Determination of the appropriate gene polymorphism *ADH3* was conducted as described by Vidal et al[9] Genetic polymorphisms of the enzyme alcohol dehydrogenase and aldehyde dehydrogenase in humans are also linked to alcohol consumption and the incident of alcohol abuse. Research conducted by Quertemont has concluded that the accumulation of blood acetaldehyde causes unpleasant effects that prevent further drinking of alcohol, which is also related to alcohol drinking habits and the incidence of alcohol abuse [12]. According to the Basic Health Institute (Riskesdas) in 2007, the prevalence

ANALYSIS OF ADH3 GENE POLYMORPHISM AND LIVER STATUS

of drinking alcohol in at least within the last month among samples were 4.9% of male's and 0.3% of female's; Based on the domicile of homes among the male sample, 4.5% are that of urban areas and 5.2% of rural areas. There are 13 out of 33 provinces, all of them outside Java, which have higher prevalence among their male populations. Types of beverage consumed among urban males were 33.6% of beer, 14.4% of liquor, 27.1% of wine and 25.0% of traditional/local alcohol; while among rural males were 18.6% of beer, 6.6% of liquor, 19.4% of wine and 55.4% of traditional/local alcohol. The proportions of traditional alcohol predominantly consumed in provinces with high prevalence varied in urban areas from 42.8% in North Sulawesi to 66.2% in North Sumatera; while in rural areas from 41.2% in Papua to 90.7% in West Kalimantan [13].

There are many fatalities in Indonesia that result from high alcohol intake and the adulteration of alcoholic beverages with other harmful substances, such as glue and methanol. Alcohol drinking is not only practiced by adults, but also by adolescents; a previous study reported that 4.6% of adolescents aged between 10 and 18 years have drank alcohol. Yogyakarta is a popular city visited by large numbers of tourists. This tourism traffic increases the possibility of alcoholic beverage being distributed among and consumed by the residents of Yogyakarta, who are mainly of Javanese ethnicity. The most commonly consumed alcoholic beverage among the Indonesian adult population is beer, while the second-most consumed alcoholic drink is wine, which comprises less than 1 percent of alcohol consumed [14].

Alcoholism is a maladaptive pattern of alcohol use that causes clinical problems as a result of developing a tolerance for alcohol, withdrawal symptoms and an inability to cease consumption. Alcohol tolerance is a decreased biological or behavioral response resulting from repeated alcohol use that causes drinkers to need increasing amounts of alcohol to achieve the same effects. Withdrawal symptoms are a group of physical and psychological symptoms that arise upon ceasing the continuous use of alcohol.[15] There are also individual factors such as genetics that effect how the body reacts to alcohol. Distribution of *ADH3* gene polymorphism among subjects of Javanese ethnic in Yogyakarta indicated that the *ADH3**2 genotype allele is more dominant compared with other genotypes among males and females (Table II). However, there were no statistically significant differences ($p>0.05$). Polymorphism of the *ADH3**2 allele gene has been reported to protect against the negative consequences of chronic pancreatitis.[1] Therefore, the prevalent allele type of *ADH3**2 that was found among subject in Yogyakarta may play a role in preventing the negative impact of chronic pancreatitis and in reducing the risk of myocardial infarction.

Differences between class I and II *ADH* are shown with nucleotides in exon 8, isoleucine on *ADH3**1 and valine at *ADH3**2. There are two loci *ADH3* variants: *ADH3**1 and *ADH3**2 that encode a subunit γ_1 and γ_2 sequentially. *ADH3**1 metabolizes alcohol with a maximum speed of 88 $\mu\text{M}/\text{min}$, while *ADH3* * 2 with speed 35 $\mu\text{M}/\text{min}$. This difference makes *ADH3**2 relatively slower to *ADH3**1 and is a slow metabolizing enzyme. Alcohol metabolism produces acetaldehyde as a toxic substance in the body. Among the *ADH3* enzymes, *ADH3**1 type is faster at metabolizing ethanol into acetaldehyde [16]. There were only four male subjects (2,1% of all participants) that possessed the *ADH3**1 type (Table I). Interestingly, these participants exceeded the upper normal limit of the SGOT, SGPT, and GGT levels. Further studies about the genetic subtypes of *ALDH*, as an enzyme that metabolizes acetaldehyde to acetic acid are needed to confirm our findings. Research conducted by Wanandi (2002), demonstrating that 70 subjects (70%) have the *ALDH2* wild-type allele, while 29 (29%) subjects were with atypical heterozygous *ALDH2* alleles and only 1(1%) were homozygous atypical. This pattern may be related to the ethnic diversity of the population found in Indonesia [17].

Harmful changes to the body related to alcohol metabolites include increased formation of acetaldehyde-adduct, creation of reactive oxygen species (ROS) and the increased ratio of Nicotinamide Adenine Dinucleotide Hydrogenase:Nicotinamide Adenine Dinucleotide (NADH:NAD). Formation of acetaldehyde and ROS triggers inflammation and the inflammatory mediators that contribute to alcoholic hepatitis and hepatic cirrhosis. In addition, an increased level of NADH initiates activation of intrahepatocellular lipid peroxidase, which results in fatty liver disease. Impaired function of the liver increases the level of urea, a toxic substance that affects the central nervous system [18].

Liver diseases are the most prevalent health complications that occur with alcohol abuse; and approximately 15–30% of chronic heavy drinkers develop severe liver diseases [19]. Spirits, including vodka, rum, and whiskey, usually contain between 40 to 50 percent of alcohol. A standard beverage served in most bars contains 0.5–0.7 fluid ounce of absolute alcohol. (One ounce equals approximately 30 ml.) Thus, a 1.5-ounce (45-ml) shot of vodka, a 5-ounce (150-ml) glass of wine, and a 12-ounce (355-ml) bottle of beer are equally intoxicating [20]. Alcohol-induced liver damage can be classified into three categories: fatty liver, alcoholic hepatitis and alcoholic cirrhosis. Genetic polymorphism of *ADH2**2 and *ADH3**1 enzymes decreases the risk of alcoholism.[6] The results of our study are not notably different from other investigations of the *ADH3* genotype in various Asian ethnicities; *ADH3**1 allele was found to have 83% prevalence among Han Chinese and Taiwanese, 88% prevalence among Chinese and Taiwanese aborigines and 86% prevalence among Korean [21].

In our study, we also found *ADH3**1/3*2 (heterozygote) was more common in male than in female participants (Table II). This result 18 (9,1% from all subject) males is slightly higher than the results of a study on men of Korean ethnicity, in which *ADH3**1/3*2 type was 8.9% in participants with alcoholic cirrhosis [22]. However, our finding was considerably lower than the result obtained by Vidal *et al.*, among Spanish men, in which *ADH3**1/3*2 type was found in 43.94% of participants.[9] A literature review on *ADH3* genetic polymorphism in Asia showed that *ADH3**1/3*2 type had prevalence rates of 14% among Han Chinese and Taiwanese, 1% among Taiwanese aborigines and 13% among Korean. Another study was conducted on males of Korean ethnicity and it was shown that the frequency of genotypes *ADH3**1/3*2, are as follows: Alcoholic cirrhosis (n = 56) = 5 (8.9 %), Alcoholic (n = 52) = 2 (3.8 %); and Nondrinkers (n = 64) = 7 (10.9 %) [21]. The predominant gene type found in the literature was *ADH3**1, whereas our study only found this genotype in 3.3% of males and none in female subjects (Table II).

To find out the status of liver function, this study observed and analyzed showed no statistically significant differences liver enzyme levels that are present in the serum. Liver enzymes were observed among others Serum Glutamic oxaloacetic transaminase (SGOT), Serum Glutamic Pyruvate Transaminase (SGPT) and Gamma glutamyl transferase (GGT). Measurement of SGOT and SGPT becomes very important because these liver enzyme are the most important liver enzymes to represent groups or transaminase aminotransferase enzyme, which catalyze the keto acids into amino acids by transfer of amino groups. As a specific liver enzyme, alanine amino transferase levels that were significant were only found in the case of hepatobiliary disease, while increased levels of SGOT can also be caused by damage of the heart muscle or skeletal muscle (not specific to the liver). Because of that difference, parallel measurements in SGOT and SGPT were done to differentiate liver damage from damage to the heart muscle and skeletal muscle. Ratio SGOT/SGPT was used to determine the differential diagnosis of liver disease. Ratio SGOT/SGPT <1 indicated mild liver damage, whereas when the ratio of SGOT / SGPT > 1 indicate severity, often found in chronic liver disease [9]. Gamma glutamyl transferase (GGT) is an enzyme found in the liver and bile ducts. Measurement of GGT levels is important because GGT is also the most sensitive indicator of hepatobiliary disease as stated by Stephen (2008). GGT which were removed from the biliary system and into the blood stream is a sensitive marker of damage within the bile ducts and is therefore useful for the evaluation of liver function [23]. Together with SGOT and SGPT, all three of these enzymes are very important to determine the differential diagnosis of liver disease [9].

The rareness of the *ADH3**1 genotype in our study is relevant to the fact that liver function status (blood chemistry of SGOT, SGPT and GGT) was found to be within normal limits (Table II). Our study found that the predominant gene type among subject was *ADH3**2. The majority of the liver function concentrations in the subjects appeared to be within the normal limits (Figure 4). Based on the blood chemistry value of SGOT and SGPT, there is no significant difference observed, suggesting that *ADH3* gene polymorphism has no influence on liver function status. Since GGT blood chemistry value results were at lower than 0.05, this result suggests that there is significant difference (Table II). This pattern indicates that the GGT analysis is more sensitive than that of SGOT and SGPT. Further studies about *ADH3* gene polymorphism and its influence on liver cirrhosis patients in Indonesia are encouraged to use the GGT analysis.

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Competing Interests: bio molecular, forensic toxicology

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Key Messages: The genetic type *ADH3**2 (polymorphic type) is more common among Javanese males than females and may mitigate or slow liver disease and cirrhosis due to chronic alcoholism. Study results on the influence of this polymorphism gene on liver function status indicate that the GGT analysis is more sensitive than SGOT and SGPT.

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