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## Serum Alanine Aminotransferase Levels in Hepatitis-C Patients of District Rahim Yar Khan, Pakistan

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This study was designed to assess the frequency of raised serum alanine aminotransferase (ALT) levels in males and females with chronic hepatitis-C virus infection (HCV) and to determine factors associated with serum ALT. The sample size of 300 patients comprising 157 male and 143 female subjects was used. The age range of the patients was found to be between 25-45 years. After the recruitment of patients, blood samples were collected and tested for HCV-RNA. The HCV positive samples were further analyzed for their ALT levels using a spectrophotometer. The results showed that 46% (138 out of 300) patients were found with raised ALT levels categorized as the group I, while 54% (n=162) patients had normal ALT levels categorized as the group II. Further division of the group I into males and females on gender basis indicated that 57.3% (n=79) males and 42.8% (n=59) females were found with raised ALT levels, while 48.1% (n=78) males and 51.9% (n=84) females had levels within the normal reference range. There was a significant positive association found between serum ALT level and age factor. It is concluded on the basis of frequency of raised and normal ALT levels in hepatitis C patients of Rahim Yar Khan District that ALT levels are not essentially be increased in all hepatitis C cases even when compared gender-wise. It shows that ALT levels cannot be used as a diagnostic marker specifically in the progression of hepatitis C.



SCAN ME



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## Introduction

Alanine aminotransferase (ALT) is used to screen or monitor liver diseases. Basically, it catalyzes the transfer of amino groups to form the hepatic metabolite oxaloacetate [1]. The ALT enzyme is found in serum and organ tissues, especially in the liver, kidney, skeletal muscle, and myocardium [2]. The ALT is also present at very low levels in the pancreas, spleen, lung muscles, adipose tissues, intestines, colon, prostate, and brain [3]. Alanine aminotransferase increases in serum in case of significant cellular necrosis and uses as an indicator of liver function. Levels of ALT may be elevated in case of hepatitis, congestive heart failure, liver or bile duct damage, and myopathy. ALT concentration in the cytosol of the hepatocyte and its activity in the liver is about 3000 times than that of serum activity [4]. Thus, in the case of hepatocellular injury or death, the release of ALT from damaged liver cells is higher than normal. It is found that within a given day there is a significant diurnal variation with ALT activities being up to 45% higher in the afternoon than in the early morning [5, 6]. In chronic hepatocellular injury, particularly if ongoing damage occurs, ALT is more commonly elevated than AST within 24 to 48 hours; because of its longer plasma half-life. However, as fibrosis progresses, ALT activities typically decline, and the ratio of AST to ALT gradually increases, so that by the time, cirrhosis is present, AST is often higher than ALT [7, 8].

ALT analysis is a valuable screening test to detect asymptomatic viral hepatitis and non-alcoholic fatty liver disease. The normal range of values for ALT (SGPT) is about 0-56 units per liter of serum [9, 10]. ALT elevated levels greater than 15 times the normal range indicate severe acute liver cell injury [11]. Hepatitis C is an infectious disease caused by the hepatitis C virus (HCV) that primarily affects the liver [2]. During the initial infection, people often have mild or no symptoms, but later on fever, dark urine, abdominal pain, and yellow tinged skin occurs [1, 11]. The virus persists in the liver and often leads to liver disease (cirrhosis) after many years. Since HCV infection is frequently asymptomatic, ALT elevations on routine blood testing may lead to the diagnosis of HCV later on. According to one study, 69% of 248 asymptomatic blood donors tested positive for HCV antibody had elevated ALT activity [13]. In another study, 29% of HCV infected patients with, initially normal ALT

values, when followed, developed persistently elevated ALT levels, and 57% developed transient elevation in ALT activities within 5 years [14, 15]. It was shown that ALT levels fluctuate in HCV and values may occasionally fall into the normal range [11, 12].

On the basis of various studies that showed conflicting levels of ALT in hepatitis C patients, the current study was performed to explore the significance of ALT as a diagnostic marker of hepatitis C by determining the frequency of raised levels of ALT in patients with hepatitis C in Rahim Yar Khan district and to investigate the factors associated with it.

## Materials and Methods

### Study site and selection of participants

This study was conducted in the Pathology Department, Sheikh Zayed Medical College / Hospital Rahim Yar Khan, Pakistan from May 2018 to June 2019 and approved by the Ethical Committee of Sheikh Zayed Medical College/Hospital. Three hundred (157 males and 143 females) hepatitis C patients, aged between 25 – 45 years, who visited the hospital were recruited in this study. The criteria used in this study for non-cirrhotic chronic hepatitis C diagnosis was positive immunochromatographic assay (ICT) by lateral flow dipstick immunoassay or simply strip tests, positive HCV RNA by polymerase chain reaction (PCR) and serum ALT analysis (>42 IU/L). The participants were included after obtaining written informed consent. In addition, a questionnaire was completed by a physician interviewer with an emphasis on psychosocial, and medical history, including disease history, dietary pattern, history of blood transfusions, surgical intervention, infectious hepatitis C, injection drug use and sexually transmitted diseases.

### Immunochromatographic assay

Blood samples were collected under aseptic conditions and centrifuged within 30 minutes of collection. The immunochromatographic test was performed using a kit (Cortez Diagnostics, Inc. One Step Tests) as per manufacturer's instructions for the qualitative detection of antibodies to HCV in human serum. Each sample had three replicates.

### Hepatitis C virus RNA detection assay

After the immunochromatographic test, the patients undergo confirmatory tests for the presence of

HCV-RNA. For that, The HCV RNA was extracted from 140 µl blood plasma using QIAamp R Viral RNA Minikit (QIAGEN Inc., Germany) as per manufacturer's instructions and stored at -70°C. Later, HCV-RNA was quantified using real-time Taqman based AmpliSens HCV-FRT kit (InterLabService Ltd., Russia). A 15 µL master mix containing RT-G-mix-2, RT-PCR-mix-1, RT-PCR-mix-2, polymerase (TaqF), and TM-Revertase (MMIv) was added to 10 µL of each eluted RNA sample. RT-qPCR was completed on Rotor-Gene Q 6000-5 Plex-HRM using the following cycling parameters: an initial cDNA synthesis by holding at 50 and 95°C for 15 min each, followed by 45 cycles of denaturation (95°C for 2 s), annealing (60°C for 5 s), and extension (72°C for 15 s). Mathematical analysis was performed using Rotor-Gene Q software. The primers used to target the NS5B region of the HCV genome. Each sample had three replicates.

**Alanine aminotransferase (ALT) analysis**

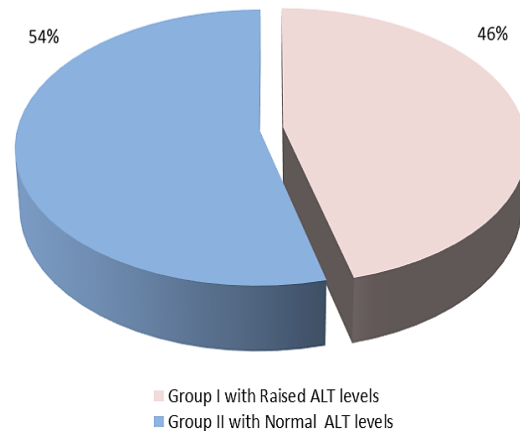
The blood ALT analysis was performed using the ALT assay kit (Biobase Biodustry Co., Ltd, Jinan, China) as per manufacturers' instructions. The ALT assay kit used the method of the International Federation of Clinical Chemistry (IFCC). Each sample had three replicates.

**Statistical analysis**

Statistical Analysis was carried out with Statistical Package for Social Sciences software (SPSS) version 23 (SPSS Inc, Chicago, IL, USA). Categorical data were expressed as percentages and continuous data were presented as mean and standard deviation for descriptive variables. The univariate associations between factors and ALT levels among the study variables were assessed by the Student's t-test and Pearson's correlation.

**Results**

A total of 315 patients were screened for the study. Out of those, 15 patients were excluded from the study. A total of 300 patients that meet the inclusion criteria were included in the study. They were tested for hepatitis C specific antibodies by Immunochromatographic test (ICT). This test was positive in 300 patients as performed by the PCR method. Fig. 1 shows a comparison of normal and raised ALT levels in two groups I and II. Out of 300 patients, 46% (n=138) were found with raised ALT/SGPT levels, while in 54% (n=162) was within normal reference range.



**Fig. 1** Comparison of frequency of raised and normal alanine aminotransferase (ALT) levels in two groups of patients of hepatitis C.

Table I shows baseline parameters of total hepatitis C patients included in a study. The mean age was found to be 34.76 years of patients participated in the study with serum ALT levels of 54.92 U/L, and a significant correlation was found between ALT levels and the age of hepatitis C patients ( $R^2= 0.40, P<0.01$ ). The comparison of the frequency of raised ALT levels between males and females in groups I and II is shown in Table 2. It is obvious that 57.25%

**Table 1** Baseline parameters of total participants of the study.

Parameters	Mean value ±SD
Age	34.74 ± 6.16
Body mass index (kg/m <sup>2</sup> )	32.60 ± 2.58
Serum alanine aminotransferase (U/L)	59.92 ± 4.12*

Total no. of subjects (n) = 300

**Table 2** Comparison of frequency of raised alanine aminotransferase (ALT) levels between males and females in groups I and II.

Gender	Patients with raised ALT levels (group I)	Patient with normal ALT levels (group II)
Males	57.25%, n = 79	48.14%, n = 78
Females	42.75%, n = 59	51.86%, n = 84

n = no. of cases

**Table 3** Age-wise distribution of hepatitis cases among total patients included in the study.

Age (years)	Percentage (no. of cases)
25-29	27% (79)
30-34	28% (83)
35-39	24% (72)
40-45	22% (66)

(n=79) males and 42.75% (n=59) female cases were found with raised ALT/SGPT levels while 48.14% (n=78) males 51.86% (n=84) females were within normal reference range (no patient was found having advanced liver cirrhosis with less than the lower normal limit of ALT/SGPT level, *i.e.*, <9 U/L). Table 3 shows the percentage comparison between the age of all patients. It indicates that 27% patients laid between 25-29 years of age, 28% were between 30-34 years, 24% laid between the age of 35-39 and there were 22% patients between the age of 40-45.

## Discussion

Hepatitis C is a widely spreading disease in our community. Its high prevalence rate needs more effective evaluation regarding its mode of transmission. Chronic hepatitis C is defined as infection with the hepatitis C virus persisting for more than six months based on the presence of its RNA [16]. Chronic infections are typically asymptomatic during the first few decades and thus are most commonly discovered following the investigation of elevated liver enzyme levels or during a routine screening of high-risk individuals. Testing is not able to distinguish between acute and chronic infections [17]. Liver enzymes are variable during the initial part of the infection and on average begin to rise seven weeks after infection. The elevation of liver enzymes does not closely follow disease severity [18]. The criteria for a persistently normal ALT level in patients with chronic hepatitis C varies from one study to the other, with no universally accepted definition [19, 20]. Those who are exposed to HCV, about 40% recovered fully but the remaining, whether they had symptoms or not, become chronic carriers. Amongst these carriers, about 20% developed cirrhosis of the liver [21, 22]. There are reports of negative plasma ALT levels with positive PCR for the viral genome within peripheral blood monocytes of liver cells [23]. This condition has been termed occult HCV infection and it was first recognized in 2004 [24, 25]. Our study supported the previous study showing that the majority of hepatitis patients (46%) are found with normal serum ALT levels despite having a positive PCR for hepatitis C. The same results were obtained in this study again when cases were observed in male and female patients. 48.1% of males and 51.9% of female patients were shown to have normal ALT levels reflecting almost the same ratio of the frequency of raised and normal ALT levels among hepatitis C patients in this study.

It is clear that ALT levels can't be used as a direct indicator to predict the hepatitis C severity in patients. Moreover, management of ALT levels specifically can't be helpful as a direct target for the treatment of hepatitis C. It is also interesting to note that serum ALT levels are moderately increased in males as compared to females indicated by comparison of the frequency of ALT levels on gender basis (Table 2). Similar results were obtained in another study by Bilal et al. [26] that showed a 42% elevation in the ALT in males in relation to females. It can be suggested that ALT levels may be associated with increased BMI in males as compared to females. In addition to this, it was assumed that non-alcoholic fatty liver disease is linked closely with central obesity and a higher waist to hip ratio (WHR), which is greater in males as compared to females. Moreover, it may be due to the fact that the liver receives a rich supply of potentially injurious fatty acids from the visceral adipose tissue, which seems to be linked with WHR [27, 28].

## Conclusions

On the basis of the high frequency of normal ALT levels among hepatitis C patients, it is concluded that ALT levels are not necessarily raised in all hepatitis C patients. Hence, ALT levels are not directly involved in the progression of hepatitis C and also showed that the liver was functioning well in the initial stages of PCR positive cases.

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## Conflict of interest

The authors have no conflict of interest.

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