Is serum alanine transaminase level a reliable marker of histological disease in chronic hepatitis C infection?

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Abstract

Background: Approximately 20–30% of patients chronically infected with hepatitis C virus (HCV) have persistently normal alanine transaminase (PNALT) levels. These patients are described to have a mild degree of histological liver damage. We aimed to assess the histological liver changes in HCV patients with PNALT.

Patients and methods: Sixty-five patients with HCV and PNALT (group A) underwent a liver biopsy. PNALT was defined as three or more determinations identified to be within the normal range over 6 months or longer. The demographical features and histological changes were compared with 66 consecutive patients with chronic HCV infection and elevated ALT (group B). All patients had a detectable HCV RNA. Histological disease was scored according to the META VIR system.

Results: Females were more likely to have normal ALT levels (65%). The mean ALT level in Group A and B was 30 and 105 IU/L respectively. No patient in either group had normal histology. The mean necro-inflammatory scores in groups A and B (2.0±0.68 vs 2.09±0.67) and the mean fibrosis scores (2.11±0.87 vs 2.24±1.04) were not significantly different. Bridging fibrosis in groups A and B was seen in 24.6 and 37.9% patients, respectively, while cirrhosis was seen in 6.2 and 7.6% patients respectively. Hepatic steatosis in groups A and B (0.94±0.86 vs 1.0±1.02 respectively) was also not significantly different and did not show any association with the fibrosis scores across the two groups. In group A, the necro-inflammatory and fibrosis scores of patients with and without steatosis were not statistically significant. Age was the only predictor of normal ALT levels. However, increasing age did not show a significant increase in histological activity in either group beyond a certain age.

Conclusion: This study demonstrates that ALT is a poor surrogate marker for inflammation and fibrosis in HCV patients. Given the presence of significant necro-inflammation in PNALT patients, the risk/benefit ratio justifies treatment without the need for a liver biopsy.
inflammatory activity and fibrosis (6–8). Patients with normal ALT were found to demonstrate an elevation of ALT in up to 27% of cases when monitored for 5 years (9). Treatment guidelines by different hepatology associations therefore have not advocated treatment in these patients, except in certain conditions such as genotypes 2 and 3. The consensus, otherwise, is of an individualised strategy, a large part of which is defined by liver histological findings, the potential of serious side effects and the likelihood of a response.

In our clinical practice, we have frequently encountered severe liver injury in HCV patients with PNALT. Genotype 4 is the predominant HCV genotype in our region (10–12), and the degree of histological change in this category of patients in relation to the ALT levels has not been studied adequately. Therefore, we conducted this retrospective study so as to assess the value of ALT levels in predicting histological findings in patients with chronic HCV.

**Patients and methods**

**Enrolment of study cohort**

Chronic HCV patients were recruited from the hepatology clinics of three centres in Saudi Arabia (Riyadh Military Hospital, Riyadh; King Khalid University Hospital, Riyadh; and King Abdulaziz University Hospital, Jeddah). These hospitals serve as referral centres for population groups resident in different geographical regions of the country. Patients were also identified through a search of hospital databases for the period extending from June 2001 to June 2006. The diagnosis of HCV was confirmed by both detectable serum anti-HCV (Enzyme-linked immunosorbent assay version 4.0; Murex Biotech S.A, Kyalami, South Africa) and positive HCV RNA by polymerase chain reaction (Amplicor; Roche Diagnostics, Branchburg, NJ, USA). Details of the risk factors for HCV infection and other demographical data were obtained from patients’ notes. A history of alcohol consumption, intravenous drug use and a family history of viral hepatitis were also obtained from the notes. This study was approved by the Medical Ethics Committee in all three centres and all patients signed an informed medical consent before liver histological sampling.

**Study design**

Patients were divided into two groups: group A: patients with chronic hepatitis C and PNALT (≥ 40 IU/L; our laboratory reference range as provided by the manufacturer, and the cut-off level used in our centres for clinical practice and research application) and group B: similar number of consecutive, unselected patients with chronic hepatitis C and elevated ALT (> 40 IU/L). Normal ALT values were defined as those with three or more determinations identified to be within the normal range over a 6-month period or longer (5). The reference ALT used for both groups was the mean value as determined from the last two recorded ALT levels. All patients with PNALT (group A) were offered a liver biopsy (which is a prerequisite for treatment in our centres) as long as they were suitable candidates for antiviral therapy. Patients in group B were included from the clinical practice of two investigators (FMS, AAA) who routinely biopsy all patients with chronic HCV before initiating therapy.

The exclusion criteria were:

(i) Hepatitis B serum antigen serology positive.
(ii) Other causes of chronic liver disease like autoimmune liver disease, haemochromatosis, Wilson's disease and α-1-antitrypsin deficiency.
(iii) A history of hepatotoxic medications in the preceding 3 months of presentation.
(iv) A history of immunosuppressive or antiviral therapy in the past.
(v) Clinically identifiable cirrhosis (ascites, jaundice, hepatic encephalopathy or variceal bleeding), a Child–Pugh score > 6, a platelet count < 100 (10⁹/L) or an international normalised ratio ≥ 1.3.
(vi) Renal insufficiency (serum creatinine > 150 μmol/L).
(vii) Evidence of hepatocellular carcinoma.

**Assessment of histological results**

All patients included in this study had liver biopsies (haematoxylin and eosin for morphological evaluation, Masson’s trichrome stain for assessment of fibrosis and Perls’ Prussian blue stain for assessment of iron loading). All liver biopsy specimens were assessed and scored according to the METAVIR scoring system (13). An inflammatory score of 0–1 was defined as minimal/mild, 2 as moderate and 3 as severe. Fibrosis scores of 0–1 were defined as minimal/mild, 2 as moderate and 3–4 as severe. Patients with evidence of steatosis on liver biopsy were not excluded. The degree of steatosis was assessed on a 0–3 score system, where 0 represented an absence of steatosis, and 1, 2 and 3 represented fatty vacuoles affecting < 10, 10–50 and > 50% of the hepatocytes. The presence or absence of siderosis was also noted. The degree of iron loading was assessed using a Perls’ score 0–4+, based on the scoring system of MacSween.
et al. (14) Biopsy specimens were assessed by a dedicated hepatic pathologist in each centre who was unaware of the clinical, demographical and biochemical details. Histological findings were compared between the two groups. Comparisons were made to assess the differences in the grade and stage of the disease. The grade and stage were also correlated with age, gender, body mass index (BMI) and ALT.

**Statistical analysis**

Differences between the characteristics of patients with elevated ALT and PNALT levels were compared by an unpaired $t$-test for continuous variables and by the $\chi^2$-test for categorical variables. Comparison between more than two groups was performed using the one-way ANOVA method. Numerical variables were expressed as mean ± SD, and $P$ values < 0.05 were considered to be statistically significant. Pearson’s correlation coefficient ($r$) was used to examine the degree of association between ALT level and demographical, biochemical and histological variables. Multiple linear regression analysis was used in both groups to evaluate the best predictors of histological necro-inflammation and fibrosis from demographical (age, gender, alcohol consumption and BMI), biochemical [ALT, aspartate transaminase (AST), $\gamma$-glutamyl transpeptidase, alkaline phosphatase, total bilirubin, albumin], genotypic and histological (siderosis and steatosis) variables. To evaluate the degree of necro-inflammatory activity in response to the ALT level, each group of patients were further subclassified according to the median level of the ALT into two subgroups (lower or higher than the median value of each group’s ALT level). Statistical analysis was performed using the statistical package SPSS 13.0 for Windows (SPSS Inc., Chicago, IL, USA).

**Results**

**Demographical and clinical details**

Initial screening identified 93 patients with PNALT. Of these, seven were excluded because of a lack of histology (refused biopsy) and another 21 were excluded because of study criteria. Overall, 131 patients were included in the analysis; 65 in group A (normal ALT) and 66 in Group B (elevated ALT). The ALT ranged from 2 to 40 IU/L in group A and from 42 to 326 IU/L in group B. The patient characteristics and frequency of risk factors were similar in both groups (Table 1). There was no significant difference between age, gender and BMI across the two groups. By design, the serum ALT and AST levels in the two groups were significantly different. While the males and females were equally distributed in group B, females were more common in group A (42 of 65 patients; 65%). An identifiable risk factor was found for HCV infection in 65% (23 of 35 patients) in group A and 35% (17 of 49 patients) in Group B. An earlier history of alcohol consumption was not found in any patient in group A and in two patients (3%) within group B ($< 20$ g/day). The distribution of genotypes in both groups was not

<table>
<thead>
<tr>
<th>Variables</th>
<th>Normal ALT $n = 65$ (%)</th>
<th>Elevated ALT $n = 66$ (%)</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>49.8 ± 13.5</td>
<td>45.9 ± 11.1</td>
<td>NS</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>23/42</td>
<td>33/33</td>
<td>NS</td>
</tr>
<tr>
<td>BMI</td>
<td>29.6 ± 6.0</td>
<td>29.2 ± 4.8</td>
<td>NS</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>30.04 ± 9.66</td>
<td>105.87 ± 55.75</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>30.2 ± 14.7</td>
<td>80.6 ± 39.1</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>GGT (IU/L)</td>
<td>54.0 ± 55.6</td>
<td>104.2 ± 78.8</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Bilirubin (µmol/L)</td>
<td>11.0 ± 5.5</td>
<td>13.7 ± 8.7</td>
<td>0.046</td>
</tr>
<tr>
<td>Albumin (mg/L)</td>
<td>39.6 ± 3.7</td>
<td>38.9 ± 5.1</td>
<td>NS</td>
</tr>
<tr>
<td>INR</td>
<td>0.98 ± 0.07</td>
<td>1.0 ± 0.14</td>
<td>NS</td>
</tr>
<tr>
<td>Genotype</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>12 (33.3)</td>
<td>9 (20)</td>
<td>NS</td>
</tr>
<tr>
<td>2</td>
<td>1 (2.8)</td>
<td>2 (4.4)</td>
<td>NS</td>
</tr>
<tr>
<td>4</td>
<td>23 (63.9)</td>
<td>34 (75.6)</td>
<td>NS</td>
</tr>
<tr>
<td>Necro-inflammation</td>
<td>2.0 ± 0.68</td>
<td>2.09 ± 0.67</td>
<td>NS</td>
</tr>
<tr>
<td>Fibrosis</td>
<td>2.11 ± 0.87</td>
<td>2.24 ± 1.04</td>
<td>NS</td>
</tr>
<tr>
<td>Steatosis</td>
<td>0.94 ± 0.86</td>
<td>1.0 ± 1.02</td>
<td>NS</td>
</tr>
</tbody>
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ALT, alanine transaminase; AST, aspartate transaminase; BMI, body mass index; F, female; GGT, $\gamma$-glutamyl transpeptidase; INR, international normalised ratio; M, male; NS, not significant.
significantly different \((P = 0.38)\). HCV genotype 4 predominated in both groups \([\text{group A } 23 \text{ patients (63.9\%)} \text{ vs group B } 34 \text{ patients (75.6\%)}]\), followed by genotype 1 \([\text{group A } 12 \text{ patients (33.3\%)} \text{ vs group B } 9 \text{ patients (20\%)}], \text{Table 1}\).

Liver histology

Overall, patients with normal and elevated ALT had similar liver histological disease on biopsy examination. None of the patients in either group had a normal histology. Four patients (6.2\%) in group A had cirrhosis, whereas this was seen in five patients (7.6\%) in group B \((P = \text{NS})\). The distribution of patients across the two groups according to METAVIR activity and fibrosis scores is demonstrated in Table 2. The mean necro-inflammatory scores in groups A and B were \(2.0 \pm 0.68\) and \(2.09 \pm 0.67\), respectively \((P = \text{NS})\). Siderosis in group A was seen in 9\% (three of 32 patients) and in 4\% (one of 26 patients) in group B \((P = \text{NS})\). Exclusion from the analysis of all patients with siderosis did not alter the mean necro-inflammatory and fibrosis scores from either group.

The mean steatosis in groups A \((0.94 \pm 0.86)\) and B \((1.0 \pm 1.02)\) was not significantly different. There was no significant association between hepatic steatosis and the fibrosis scores across the two groups. In addition, in group A, the necro-inflammatory and fibrosis scores of patients with and without steatosis were not significantly different.

In group A, age correlated positively with both necro-inflammatory and fibrosis \((r = 0.265, P = 0.033\) and \(r = 0.503, P < 0.0001\) respectively). On the other hand, in group B, necro-inflammation was only associated with the level of AST \((r = 0.462, P = 0.006)\) while fibrosis showed a positive correlation with ALT \((r = 0.242, P = 0.050)\). In group B, both necro-inflammation and fibrosis showed no correlation with age or other biochemical markers. Genotype was not a predictor of disease severity.

In stepwise regression analysis, age was the only predictor for severity of necro-inflammation and fibrosis in group A patients \((r^2 = 0.136, P = 0.049\) and \(r^2 = 0.351, P = 0.001\) respectively). In group B patients, older age was also a predictor for necro-inflammation \((r^2 = 0.176, P = 0.041)\) along with an elevated level of ALT, which was a predictor of fibrosis stage \((r^2 = 0.317, P = 0.005)\). None of the other variables explained this variability in either the inflammation or the fibrosis. Because we did not know the exact time of HCV infection, we further evaluated the impact of age on histological change by studying the mean necro-inflammatory and fibrosis scores in relation to defined age groups \((< 30, 30–40, 40–50, 50–60, > 60 \text{ years})\), to account for the effect of disease duration. This did not show a significant increase in histological activity in either group (A or B) beyond a certain age (Fig. 1).

Each group was divided into two subgroups according to the median value of ALT in either group. The subgroups of group A were those with ALT values \(\leq 31 \text{ IU/L}\), while group B was divided into those with an ALT value \(\leq 87 \text{ IU/L}\). There was no significant difference in either the necro-inflammatory activity or the fibrosis score between the subgroups of both groups A and B (Fig. 2).

Discussion

An elevated ALT level is indicative of hepatocellular damage and has been used as a surrogate marker of liver injury associated with chronic HCV. The value of ALT as a surrogate marker of liver cell necrosis is well recognised although its utility as a marker of fibrosis is debatable. To this end, a recent systematic review of 66 studies analysed biochemical tests and serological markers towards the predictability of degree of hepatic fibrosis on biopsy in chronic HCV (15). In the 15 studies that evaluated serum ALT, 11 were associated with fibrosis stage, with sensitivity ranging from 61 to

Table 2. The METAVIR activity and fibrosis scores distributed across normal and elevated alanine transaminase levels

<table>
<thead>
<tr>
<th>Groups</th>
<th>Necro-inflammation</th>
<th>Fibrosis</th>
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<tbody>
<tr>
<td>Frequency (%)</td>
<td>Mild</td>
<td>Moderate</td>
</tr>
<tr>
<td>Normal ALT</td>
<td>20</td>
<td>58.5</td>
</tr>
<tr>
<td>(n = 65)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elevated ALT</td>
<td>16.7</td>
<td>59.1</td>
</tr>
<tr>
<td>(n = 66)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(P)-value</td>
<td>NS</td>
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</tbody>
</table>

Necro-inflammation, mild = score 0–1; moderate = score 2; severe = score 3.
Fibrosis, mild = score 0–1; moderate = score 2; severe = score 3–4.
ALT, alanine transaminase.
71% and specificity ranging from 66 to 94%. This study concluded that serum transaminases were of modest value in predicting fibrosis on liver biopsy (15).

The course of chronic HCV with PNALT remains poorly defined. The differences seen in the outcomes of various studies related to PNALT could partly be attributed to the differences in the definition of ALT normality among various studies. ALT levels fluctuate over the course of the disease and it is likely that many patients would have been included based on a snapshot normal ALT result when they could have been excluded had a prolonged period of follow-up and serial ALT results been obtained before their inclusion (9, 16). In this study, we adopted the more stringent criterion recommended by the American Association for the Study of Liver Diseases that calls for three or more determinations identified to be within the normal range over a 6-month period or longer (5).

Our findings were in contrast to the prevailing concepts that PNALT have mild histological abnormalities and that cirrhosis is generally rare (16–19). Firstly, all patients in our study with PNALT levels had evidence of histological disease. Moderate and severe necro-inflammation and fibrosis was seen in 58.5 and 21.5%, and 43 and 31% of our cohort respectively. Secondly, we found that mean hepatic necro-inflammatory activity and fibrosis score did not differ significantly between the groups of normal and elevated ALT. The presence of advanced fibrosis and cirrhosis also did not differ significantly between the two groups. While these findings contradict the mainstream evidence, other studies have reported equivalent liver damage in the two patient populations (11, 20, 21). However, these studies incorporated smaller number of patients and, to our knowledge, this is the

Fig. 1. Mean necro-inflammatory (light bar) and fibrosis (dark bar) scores across different age groups for patients with normal ALT (A) and elevated ALT groups (B). Bars represent means ± SD. ALT, alanine transaminase.

Fig. 2. Mean necro-inflammation (light bar) and fibrosis (dark bar) in different ALT subgroups (less or more than the median ALT value) in both (A) normal ALT or (B) elevated ALT groups (bars represent means ± SD). Neither the inflammation nor the fibrosis differed between the two subgroups. ALT, alanine transaminase.
levels ranging from less than upper limit of normal (ULN) to $1.5 \times$ ULN as ‘normal’ levels. Recent recommendations suggest reducing the ULN for ALT to $\leq 30$ IU/L (22). We analysed the histology in both groups by subdividing the patients along median ALT levels. In the subgroup of patients with (median) ALT levels $< 31$ IU/L, the necro-inflammatory grade and the fibrosis stage did not differ significantly in comparison with those with ALT $> 31$ IU/L. Similarly, Kyrlagkitis et al. (6) found no difference in histological disease between low-normal and high-normal ALT. This demonstrates that lowering of cut-off limit for ULN for ALT in HCV-induced liver disease does not reliably identify patients with more histologically advanced or progressive disease.

Earlier studies, predominantly in genotypes 1–3, showed a lack of significant impact of genotype on disease severity and progression (6, 19, 23–27). Genotype 4 remains the predominant type in our region, seen in 70–94% of HCV patients (10–12), and did not account for disease severity in our cohort as with the findings of earlier non-genotype 4 studies. In a smaller study of predominantly genotype 4 patients, Hasan et al. (11) found that the mean inflammatory and fibrosis scores were similar in patients with normal and elevated ALT. These findings are in concordance to ours, which may further suggest that ALT levels in genotype 4 patients do not reflect the histological changes. Nevertheless, it is worth noting that the previous study by Hasan et al. (11) and our own, dealing with predominantly genotype 4 patients, are both of middle-eastern origin, suggesting that these findings may be peculiar to our geographical region.

In addition, a recent study evaluating hepatic steatosis and histological disease across various HCV genotypes demonstrated that ALT levels in genotype 4 patients were significantly lower than those of genotypes 1–3 (28). Interestingly, despite the lower ALT levels, the necro-inflammation and fibrosis was not significantly different across the studied genotypes. While it is unclear as to why ALT levels are lower, we believe that this report lends further support to our findings that higher ALT levels do not predict histological disease in a genotype 4–predominant population.

Our study is limited by the absence of data on the duration of infection in either group of patients. Disease progression is largely dependent on the duration of viraemia and patients with PNALT progress at a significantly slower rate compared with those with elevated ALT (6, 7, 29, 30). In addition, a European study has shown that age at infection and duration of disease are independently associated with a more severe disease (18). This aspect could not be studied in our patients because we could identify a risk factor in only 30% (40 of 131 patients) of the study group. The mean age in both patient groups was similar in our study and given the slower rate of disease progression in those with PNALT, one would have expected lower fibrosis scores for this group, if anything. We also analysed the histological findings in relation to different age groups (< 30, 30–40, 40–50, 50–60, > 60 years) and this did not reveal any significant difference in the mean inflammatory or fibrosis scores within the age groups of normal or elevated ALT patients. These findings suggest that the disease duration does not account for the lack of a significant difference in the necro-inflammatory and fibrosis scores between the two groups. In addition, there are no prevailing data to suggest that patients with PNALT are infected at a younger age.

Consistent with previous findings, PNALT featured more frequently in women (16) when compared with men in our study population. This further augments the case for lowering the cut-off value for serum ALT levels in females, in line with recent recommendations. However, female gender was not a predictor of significant histological change. In patients with normal ALT, age was the only predictor of histological disease progression. Previous studies have also shown age to be a predictor of increasing necro-inflammatory activity (18, 31). In our patients, while there was a trend towards increased histological activity in both groups A and B after 50 years of age, this did not reach statistical significance (Fig. 1). This contradicts the findings of a previous study, (6) that reported increasing necro-inflammatory activity with increasing age. In the same study, however, the subgroup analysis of patients with normal ALT and significant histological disease was not identified by age.

Finally, the existing evidence suggests that alcohol consumption correlates positively with histological disease in HCV-infected patients (31). This did not serve as a variable in our study because none of our patients were significant alcohol consumers.

There are increasing worldwide calls to lower the ULN for serum ALT on a gender-discriminant basis. However, in our clinical practice, and as evident from other recent regional studies, (32) there is a continuing usage of the previously set value of 40 IU/L as the upper normal level for both genders. It remains to be seen whether lowering the cut-off level will better identify less histologically advanced disease. We believe that our findings further argue in favour of performing
epidemiological studies to identify the normal ALT levels in the middle-eastern population.

In conclusion, we believe that the findings of this study allow us to approach patients with a different perspective. Liver biopsy may not be necessary in middle-eastern patients when ALT is normal, given the high rate of histological abnormality noted in this study. We reiterate that liver biopsy should be reserved for patients with significant comorbid conditions or those at a high risk for adverse reactions to the medication. Also, biopsy is useful in patients who are reluctant to undergo treatment and in whom detection of significant fibrosis may serve as a strong incentive to receive the treatment. The availability of non-invasive means of determining hepatic fibrosis such as the FibroTest and Fibroscan may play a role in optimising the decision-making process.

References