Alcohol has no effect on hepatitis C virus replication: a meta-analysis

B S Anand, J Thornby

Background: Patients with chronic hepatitis C virus (HCV) infection who consume large quantities of alcohol have more severe liver disease compared with HCV patients without a history of alcohol consumption. The mechanism by which alcohol worsens HCV related liver disease is not properly understood. One possibility is that alcohol stimulates HCV replication, and the present meta-analysis was performed to examine this issue.

Methods: The effect of alcohol on viral titres was assessed in three ways: comparison of the heaviest drinkers with non-drinkers; effect of graded doses of alcohol; and effect of abstinence in the same individual.

Results: A total of 14 studies were identified. Comparison of patients with the highest alcohol use with the abstinent group showed a significant association with viral load in three studies, five studies had a positive direction, while the remaining four studies found a negative relationship. Analysis of the combined results showed no association between alcohol consumption and virus levels (p = 0.29). Assessment of graded doses of alcohol also showed no significant difference between non-drinkers and moderate drinkers (p = 0.50), between non-drinkers and heavy drinkers (p = 0.35), or between moderate drinkers and heavy drinkers (p = 0.32). Five studies examined the influence of abstinence on viral titres but none provided sufficient data for statistical analysis.

Conclusions: The present study has failed to show an association between alcohol use and HCV viral titres. These observations raise the possibility that the hepatitis C damage caused by alcohol and HCV may be purely additive, involving different mechanisms and pathways.
time of analysis, methods used to measure HCV titres, and mean values and standard deviations of viral titres. Quantitative HCV assay was performed by two different techniques in the studies: eight studies used the branched DNA (bDNA) methodology while the remaining six studies employed the quantitative polymerase chain reaction (PCR) technique. The bDNA assay expresses the results as genome milliequivalents per ml (mEq/ml) and has two different versions of the test. The initial version (Quantiplex 1.0) had a lower limit of detection of 350 000 viral mEq/ml while the next version of the assay (Quantiplex 2.0) had a lower limit of detection of 200 000 viral mEq/ml. The results of the PCR assay are expressed as log10 copies of RNA/ml of serum.

Data analysis

The effect of alcohol on viral titres was investigated in three different ways. The primary analysis focused on comparing the heaviest drinking group with the non-drinking group in each study, whenever the data provided allowed such a comparison. The second analysis examined the effect of graded doses of alcohol based on studies that divided subjects into non-drinkers, moderate drinkers, and heavy drinkers. Finally, we assessed the effect of abstinence in the same individual. Not all of the requisite data were available in some studies but it was possible to make estimates based on other information, as described in the results section.

To assess the effect of heavy alcohol abuse, confidence intervals and p values for both individual and combined studies were based on our calculations, assuming normal distributions with equal population standard deviations for each group within studies, although such assumptions were not made by the authors in at least some of the studies. It is reasonable to assume an approximate normal distribution for both groups in most studies because of adequate sample sizes, especially when the standard deviations are not excessively large in comparison with the mean values. Whenever direct comparisons could be made to the authors’ confidence intervals and p values, we found quite close agreement. Also, the meta-analysis calculations are based only on the effect size and its variance in each study, and the variance is a function of only the effect size itself and the sample size in each group. Comparisons between groups, as reported by the studies, were based on Wilcoxon rank sum tests, Student’s t tests, or analyses of variance for the linear tests. As our calculations of p values were based on normality assumptions, they were not identical to those reported in studies in which significance was based on the Wilcoxon test. However, there was complete agreement with respect to statistical significance (that is, whether or not p<0.05).

In order to combine the results from the individual studies it was necessary to convert the data to a common metric. This was accomplished by defining an “effect size” for each study by dividing the difference between mean values by the pooled estimate of standard deviation. As the mean values and standard deviations are expressed in the same units of measurement, their ratio is independent of the measurement units. Thus, for example, the studies based on bDNA can be directly assessed along with those based on PCR assay as long as both methods are designed to measure the same phenomenon. A positive effect size indicates that the heavy drinking group had a higher viral load than the non-drinking group.

The variance (Var) of each effect size was calculated by the formula:

$$Var = \frac{n_1 + n_2}{n_1 n_2} + \frac{ES^2}{2 (n_1 + n_2)}$$

where $n_1$ and $n_2$ are the sample sizes of the two groups being examined (for example, non-drinking and heavy drinking groups, respectively) and “ES” represents the effect size. A computer program designed to combine data from separate studies, including input statements, was used, as described by Cooper and Hedges. Data analysis was performed on the SAS statistical software program (version 8; SAS Institute Inc., Cary, North Carolina, USA). Input to the analysis included the individual effect sizes and their variances. Output included the combined estimate of effect size, its standard deviation and confidence limits, and p value based on a “fixed effects” model, along with a test of heterogeneity among the various studies. Heterogeneity would indicate that the studies may not all be estimating the same quantity, and would question the legitimacy of the fixed effects model assumptions. Also included in the output were combined results based on a “random effects” model that is appropriate when the results of individual studies are considered to represent estimates of differing effects.

RESULTS

Study material

A total of 14 studies were identified (13 17 18 21–31) (table 1). Five of these studies (15–20 23 27 31) did not provide actual viral titres. Of these five, two studies did not provide any data at all. The remaining three studies (22 27 31) noted the difference in viral levels between drinkers and abstinent subjects without...
Effect of heavy alcohol abuse

The pertinent data from the individual studies, along with combined results, are shown for both the fixed effects and random effects models in table 2. Three studies found a significant positive association between alcohol consumption and virus load. Another two studies showed a positive effect while the remaining four studies found a statistically significant and the test for heterogeneity among identical characteristics among studies. Based on the random effects model, there was no conclusive evidence that alcohol consumption is associated with increased HCV viral titres (p = 0.29).

In order to further analyse the results, we performed a sensitivity test by deleting the study by Loguercio and colleagues from the combined analysis. This was designed to determine the degree to which the results would change if the study with greatest positive effect was not included. As a result, the fixed effects model was now no longer statistically significant. As before, the random effects model was not statistically significant and the test for heterogeneity among studies was highly significant (p = 0.008). These findings reinforced the interpretation that the nine studies selected for the meta-analysis do not all estimate the same population effect size. Rather, there were unknown differences among the studies accounting for the heterogeneity of results among them.

<table>
<thead>
<tr>
<th>Author</th>
<th>N1</th>
<th>N2</th>
<th>Effect size</th>
<th>Variance</th>
<th>95% CI</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loguercio</td>
<td>17</td>
<td>17</td>
<td>+3.21</td>
<td>0.27</td>
<td>+2.17 to +4.25</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Oshita</td>
<td>37</td>
<td>16</td>
<td>+1.10</td>
<td>0.10</td>
<td>+0.47 to +1.74</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Poynard</td>
<td>105</td>
<td>16</td>
<td>+0.59</td>
<td>0.07</td>
<td>+0.05 to +1.13</td>
<td>0.03</td>
</tr>
<tr>
<td>Anand</td>
<td>18</td>
<td>50</td>
<td>+0.24</td>
<td>0.08</td>
<td>-0.31 to -0.79</td>
<td>0.39</td>
</tr>
<tr>
<td>Sato</td>
<td>16</td>
<td>20</td>
<td>+0.03</td>
<td>0.12</td>
<td>-0.67 to -0.72</td>
<td>0.93</td>
</tr>
<tr>
<td>Wiley</td>
<td>44</td>
<td>28</td>
<td>-0.02</td>
<td>0.06</td>
<td>-0.50 to -0.46</td>
<td>0.92</td>
</tr>
<tr>
<td>Khan</td>
<td>40</td>
<td>24</td>
<td>-0.16</td>
<td>0.07</td>
<td>-0.67 to -0.36</td>
<td>0.54</td>
</tr>
<tr>
<td>Yoshihara</td>
<td>33</td>
<td>14</td>
<td>-0.31</td>
<td>0.10</td>
<td>-0.96 to -0.33</td>
<td>0.33</td>
</tr>
<tr>
<td>Ohsishi</td>
<td>35</td>
<td>10</td>
<td>-0.48</td>
<td>0.13</td>
<td>-1.20 to -0.24</td>
<td>0.19</td>
</tr>
<tr>
<td>Combined studies</td>
<td></td>
<td></td>
<td>+0.24</td>
<td>0.010</td>
<td>+0.05 to +0.45</td>
<td>0.01</td>
</tr>
<tr>
<td>Random effects</td>
<td></td>
<td></td>
<td>+0.43</td>
<td>0.143</td>
<td>-0.31 to +1.17</td>
<td>0.29</td>
</tr>
</tbody>
</table>

N1, non-drinkers; N2, subjects consuming alcohol at the highest level in each study.

Effect of graded doses of alcohol

Only four studies divided drinking subjects into moderate drinking and heavy drinking groups. The results are shown on table 3. The cut off for moderate and heavy drinking varied in the four studies. In one study, alcohol intake was divided into three categories: <40 g/day, 41–80 g/day, and >80 g/day. HCV RNA levels were comparable for the two lower categories which were combined. In another study, moderate and heavy drinking were defined as <50 g/day and >50 g/day, respectively. In the third study, the categories were <46 g/day and >46 g/day, respectively. The fourth study divided the drinking groups into <80 g/day and >80 g/day. Within each study and for the combined analysis we performed pairwise comparisons of effects between the non-drinking subjects and the two drinking groups. In two studies, there was an increasing effect on viral titres with higher doses of alcohol. The third study showed the same effect for non-drinkers and moderate drinkers with a decreasing effect for heavy drinkers. The fourth study showed the same decreased effect for moderate and heavy drinkers. Basing our choice of model on the test for heterogeneity, there was no significant difference between non-drinkers and moderate drinkers (p = 0.50; fixed effects model), between non-drinkers and heavy drinkers (p = 0.35; random effects model), or between moderate drinkers and heavy drinkers (p = 0.32; random effects model). For this analysis the requisite data for study by Loguercio and colleagues was
estimated from a graph as the actual values were not provided.

**Effect of abstinence**

Five studies assessed the same individuals before and after a period of abstinence.\(^{13, 21, 23, 24, 28}\) The results are shown in table 4. In all of the studies the number of subjects included were small. In three studies viral levels were not provided: in one of these studies,\(^{13}\) HCV markers disappeared in four of eight patients (50%) after abstinence; in the second\(^{21}\) viral titres fell in five of 11 (45%) and remained the same or increased in the remaining six patients; while in the third study abstinence was associated with a significant fall in viral load.\(^{23}\) There were discordant findings in the two studies where actual titres were available: one study\(^{24}\) showed a significant fall in viral load after abstinence while the other found no difference.\(^{28}\) Statistical analysis of the combined studies was not possible because none of the studies provided the necessary mean and SD of change in viral load before and after abstinence.

**Effect of HCV quantitative assays**

In order to assess whether the differences in HCV quantitative methodologies did not account for the lack of significance, we performed separate analyses for the six bDNA and three PCR studies and found that both failed the heterogeneity test, their effect sizes under the random model were non-significant (\(p = 0.30\) and \(p = 0.82\), respectively), and the difference between the two random models was also not significant (\(p = 0.50\)).

**DISCUSSION**

The present study was carried out to determine the influence of alcohol consumption on HCV viral levels. Several problems were encountered in conducting the meta-analysis. Firstly, there was little uniformity in different studies in grading the severity of alcohol consumption. The division between modest and high alcohol use, based on daily consumption, showed wide variation: 10 g,\(^{22}\) 40 g,\(^{24}\) 46 g,\(^{26}\) 50 g,\(^{27}\) and 80 g.\(^{29, 26}\) In one study, subjects were divided into drinkers and abstinent groups, but the amount of daily consumption was not noted. However, the primary consideration in assessing the effect of alcohol was to compare a group of heavy alcohol consumers with complete abstainers or minor users of alcohol. Fortunately, in all of the studies included in the meta-analysis such a division was possible. There is no question in all the studies that the heaviest user group qualified to be thereby defined, and if there was a relationship between alcohol consumption and viral titre it should emerge. Another area of difficulty was the use of two different methodologies for measurement of serum viral titres, which express results differently: mEq/ml with the bDNA technique and log copies of RNA/ml with the PCR assay. Although the values provided by the two assays cannot be exactly equated, they represent a similar spectrum in serum viral titres. The advantage of meta-analysis is that it takes into account the disparities in different studies. Moreover, by defining the “effect size” for each study (obtained by dividing the difference between mean values by the pooled estimate of standard deviation), data obtained by the bDNA assay could be directly compared with the HCV assay as the effect size is then independent of the measurement units and both methods were designed to measure the same phenomenon. Finally, it should also be emphasised that studies based on patient recall of alcohol consumption have an inherent fundamental problem and a meta-analysis cannot completely exclude the possibility that a true positive relationship between alcohol use and viral titres does or does not exist. The only way to conclusively assess the effect of graded doses of alcohol on HCV viral replication is to perform a controlled prospective study using validated questionnaires of life time alcohol consumption. However, we believe that in the absence of such data, the next best approach is collective analysis of different studies by meta-analysis.

Our findings indicate that alcohol consumption does not influence serum hepatitis C virus levels. We analysed the influence of alcohol use in three different ways. Firstly, we compared hepatitis C viral titres between the group categorised as the heaviest drinkers in each study and abstinent subjects (table 2). The combined analysis based on the random effects model showed no conclusive evidence (\(p = 0.29\)) to support the fact that alcohol consumption is associated with increased viral titres. Assessment of graded doses of alcohol was performed by dividing subjects into three groups: complete abstainers, moderate alcohol users, and heavy alcohol users, based on the authors’ assessments. Meta-analysis could be performed in only four studies where such data were available (table 3). Again, based on the test for heterogeneity, there was no significant difference between non-drinkers and moderate drinkers (\(p = 0.50\); fixed effects model), between non-drinkers and heavy drinkers (\(p = 0.35\); random effects model), or between moderate drinkers and heavy drinkers (\(p = 0.32\); random effects model). We also examined the effect of abstinence on HCV levels in the same individual. The number of subjects enrolled in all of the studies was small. There were discordant findings among the studies and statistical analysis of the combined data could not be performed because none of the studies provided the mean and standard deviation for change of viral titres before and after abstinence.

The question arises as to why uniform results were not obtained in different studies. One possibility is the methodology (bDNA or PCR) used to quantitate HCV viral load. However, this factor is unlikely to be important because we

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**Table 4** Hepatitis C virus (HCV) titres in the same individuals at baseline and after a period of abstinence

<table>
<thead>
<tr>
<th>Author</th>
<th>Subject No.</th>
<th>Method</th>
<th>Duration of abstinence</th>
<th>Viral titres</th>
<th>Baseline</th>
<th>Abstinence</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Takase(^{13})</td>
<td>9</td>
<td>PCR</td>
<td>Unclear</td>
<td>NA</td>
<td>NA</td>
<td>HCV –ve in 4 of 8</td>
<td></td>
</tr>
<tr>
<td>Sowenda(^{24})</td>
<td>11</td>
<td>bDNA</td>
<td>4 weeks</td>
<td>NA</td>
<td>NA</td>
<td>HCV fell in 5 of 11</td>
<td></td>
</tr>
<tr>
<td>Cronie(^{22})</td>
<td>12</td>
<td>PCR</td>
<td>4.4 (0.2) mo</td>
<td>NA</td>
<td>NA</td>
<td>0.035</td>
<td></td>
</tr>
<tr>
<td>Sato(^{26})</td>
<td>24</td>
<td>bDNA</td>
<td>30–90 days</td>
<td>16.5±10</td>
<td>2.84±10</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Anand(^{27})</td>
<td>3</td>
<td>bDNA</td>
<td>6 mo</td>
<td>108 (111.3)</td>
<td>98 (78.7)</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

Statistical analysis of the combined studies was not possible because none of the studies provided the mean and SD of change in viral load for individual subjects at baseline and after abstinence.

bDNA, branched DNA technique; bDNA results expressed as viral mEq/ml.

PCR, polymerase chain reaction assay; NA, not available; mo, months.
performed separate analyses for the bDNA and PCR studies and found that both failed the heterogeneity test, their effect sizes under the random model were not significant, and the difference between the two random models was also not significant. Moreover, one of the PCR based studies found the second to largest positive effect size while the other two found the largest negative effect sizes. Another potential cause for lack of uniformity could be the timing of the HCV assay with respect to alcohol consumption. Fortunately, most studies included in the meta-analysis comprised subjects who were “current” alcohol users, implying recent or active consumption as opposed to past alcohol use. We also considered the possibility of publication bias, which again failed to indicate any explanation for the differences among studies. We believe the only plausible explanation is the relatively small sample size employed in the different studies, a defect that is overcome to some extent by the use of meta-analysis.

In conclusion, the present study fails to support the theory that alcohol consumption has a stimulatory effect on HCV levels in serum. It is possible that alcohol may influence intrahepatic viral kinetics. However, in the absence of such data, it is tempting to postulate that the damaging effect of ethanol and HCV is simply additive and may involve different mechanisms and signalling pathways.

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