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Biomarkers of Liver Status in Heavy Drinkers, Moderate Drinkers and Abstainers

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Abstract — Aims: Although a wide variety of biomarkers reflecting liver status are known to be influenced by excessive ethanol consumption, the dose–response relationships between ethanol intake and marker changes have remained less understood. Methods: Serum gamma-glutamyltransferase (GGT), aspartate aminotransferase (AST), alanine aminotransferase (ALT) activities, and ferritin and albumin protein concentrations were compared in a large population of heavy drinkers (105 men, 28 women), moderate drinkers (781 men, 723 women) and abstainers (252 men, 433 women), who were devoid of apparent liver disease. Results: In heavy drinkers, serum GGT, AST, ALT, ferritin and albumin were all significantly higher than in moderate drinkers or abstainers (P < 0.001 for all comparisons). The highest incidences of elevated values were found for GGT (62%) followed by AST (53%), ALT (39%), ferritin (34%) and albumin (20%). Serum GGT (P < 0.001), ALT (P < 0.01) and ferritin (P < 0.05) in moderate drinkers were also higher than the levels observed in abstainers. When the study population was further divided into subgroups according to gender, significant differences between moderate drinkers and abstainers in GGT and ALT were noted in men whereas not in women. Conclusions: The data demonstrate that biomarkers of alcohol abuse and liver function may respond to even rather low levels of ethanol intake in a gender-dependent manner, which should be implicated in studies on the early-phase interactions of ethanol and the liver and in the definition of normal ranges for such biomarkers.

INTRODUCTION

Ethanol consumption and associated medical disorders continue to grow in most Western countries (Lieber, 1995; Room et al., 2005; Leon and McCambridge, 2006). Therefore, the need for more effective diagnostic procedures for detecting problem drinking in its early phase has been widely acknowledged (Conigrave et al., 2002). Because nearly all ethanol consumed is metabolized by the liver, it is also a primary target of ethanol-induced adverse health effects. Previous studies in alcoholic patients have demonstrated several liver-derived biomarkers, which are associated with excessive ethanol intake and alcoholic liver disease (Rosman and Lieber, 1994; Sharpe, 2001; Niemelä, 2007). Heavy drinkers typically show increased activities of serum GGT and transaminases (ALT, AST), whereas on progression of alcoholic liver disease, there may be elevations in liver enzymes together with abnormally low serum concentrations of hepatic proteins.

Recent studies have indicated a gradual effect of alcohol on GGT enzyme induction, which may be initiated at rather low levels of ethanol intake (Hietala et al., 2005). The status of oxidative stress has also been closely linked with serum GGT activities (Whitfield, 2001; Lee and Jacobs Jr, 2004; Puukka et al., 2006). However, the dose–response relationships between various liver enzymes, proteins and ethanol intake have, however, continued to be poorly known. Comparisons of the different variables between abstainers and moderate drinkers have also been limited.

In order to gain further insight on the interpretation of the interactions between ethanol intake and biomarkers of liver status, we compared here the serum levels of liver-derived enzymes and proteins in individuals with a wide range of ethanol consumption including (i) abstainers, (ii) moderate drinkers and (iii) heavy drinkers, who were all devoid of apparent liver disease.

METHODS

Study protocol

The sample of heavy drinkers consisted of 133 patients, 105 men (mean age 45 ± 10 years) and 28 women (mean age 43 ± 11 years), who had been admitted for detoxification in a consecutive manner. All patients underwent detailed clinical examinations and personal interviews on the amounts and patterns of ethanol consumption using a time-line follow-back technique, which indicated a history of continuous ethanol consumption or binge drinking, the mean consumption being 110 g (range 40–540 g) of ethanol per day from the period of 4 weeks prior to sampling. The percentage of patients reporting a mean consumption <60 g/day or 80 g/day was 5% and 11%, respectively. In addition to the above, the patients were also advised to sum up their intake from the last 1 week and 24 h, which indicated considerably higher levels of daily ethanol intake from the past 1 week prior to admission. All patients were devoid of any clinical signs of liver dysfunction (collateral circulation, oedema, ascites, encephalopathy, spider nevi, anorexia or weakness).

In addition, samples were collected from 1504 moderate drinkers, 781 men (mean age 46 ± 17 years) and 723 women (mean age 45 ± 16 years), and from 685 abstainers, 252 men (mean age 49 ± 20 years) and 433 women (mean age 49 ± 19 years). All of these subjects were apparently healthy volunteers who participated in a Nordic survey for establishing reference intervals for common laboratory tests (Puukka et al., 2006). The mean recent alcohol consumption in the population of moderate drinkers had been <40 g/day per day, the maximum amount during the past 24 h prior to sampling being two standard drinks (each containing 12 g of alcohol). The survey excluded individuals who had clinical or laboratory evidence of current or recent illnesses or infections, were pregnant, had donated blood during the past 5 months or had used any prescription drugs during the...
Table 1. Liver enzyme and protein levels in heavy drinkers, moderate drinkers and abstainers

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Heavy drinkers</th>
<th>Moderate drinkers</th>
<th>Abstainers</th>
</tr>
</thead>
<tbody>
<tr>
<td>GGT (U/l)</td>
<td>177 ± 317 (578–1612)</td>
<td>29 ± 23 (70–97)***</td>
<td>24 ± 15 (50–64)*** †† †††</td>
</tr>
<tr>
<td>AST (U/l)</td>
<td>63 ± 51 (171–208)</td>
<td>24 ± 8 (38–43)***</td>
<td>24 ± 7 (36–41)***</td>
</tr>
<tr>
<td>Men</td>
<td>65 ± 49 (160–204)</td>
<td>26 ± 8 (40–45)***</td>
<td>25 ± 7 (35–45)***</td>
</tr>
<tr>
<td>Women</td>
<td>55 ± 57 (235–263)</td>
<td>21 ± 7 (33–37)††</td>
<td>23 ± 7 (36–41)†</td>
</tr>
<tr>
<td>ALT (U/l)</td>
<td>67 ± 65 (211–273)</td>
<td>25 ± 15 (51–64)***</td>
<td>23 ± 12 (46–52)***</td>
</tr>
<tr>
<td>Men</td>
<td>71 ± 68 (220–282)</td>
<td>29 ± 17 (59–73)***</td>
<td>26 ± 12 (49–58)**, †</td>
</tr>
<tr>
<td>Women</td>
<td>51 ± 48 (195–214)</td>
<td>20 ± 11 (40–48)*</td>
<td>21 ± 11 (40–49)*</td>
</tr>
<tr>
<td>Ferritin (µg/l)</td>
<td>212 ± 207 (687–777)</td>
<td>100 ± 83 (246–333)***</td>
<td>70 ± 63 (245–257)***</td>
</tr>
<tr>
<td>Men</td>
<td>258 ± 222 (750–853)</td>
<td>136 ± 90 (332–378)***</td>
<td>101 ± 72 (257–257)***</td>
</tr>
<tr>
<td>Women</td>
<td>105 ± 112 (396–465)</td>
<td>65 ± 55 (188–231)</td>
<td>48 ± 45 (184–225)*</td>
</tr>
<tr>
<td>Albumin (g/l)</td>
<td>44 ± 4 (50–51)</td>
<td>41 ± 3 (46–47)***</td>
<td>41 ± 3 (46–47)***</td>
</tr>
<tr>
<td>Men</td>
<td>44 ± 4 (50–50)</td>
<td>42 ± 3 (47–48)***</td>
<td>42 ± 3 (47–47)***</td>
</tr>
<tr>
<td>Women</td>
<td>44 ± 4 (51–53)</td>
<td>41 ± 3 (45–46)***</td>
<td>40 ± 3 (45–46)***</td>
</tr>
</tbody>
</table>

*Mean ± SD (95th–97.5th percentile) (all such values).
†P < 0.05, **P < 0.01, ***P < 0.001 when compared to heavy drinkers.
‡P < 0.05, ††P < 0.01, †††P < 0.001 when compared to moderate drinkers.
GGT, gamma-glutamyltransferase; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

RESULTS

Table 1 summarizes the values of the various biomarkers of liver status in the groups of heavy drinkers, moderate drinkers and abstainers. Among heavy drinkers, serum GGT, AST, ALT activities and ferritin and albumin concentrations were all significantly higher than those observed in either abstainers or moderate drinkers (P < 0.001 for all comparisons). Interestingly, in moderate drinkers the activities of GGT (P < 0.001) and ALT (P < 0.01) as well as ferritin (P < 0.05) levels were also significantly higher than those in abstainers (Table 1). When the study population was further divided into subgroups according to gender, the alcoholic men showed significantly higher values than both moderate drinkers and abstainers in all of the study variables (Table 1). Male moderate drinkers and abstainers also differed in their serum GGT (P < 0.001) and ALT (P < 0.05) activities. In women, the heavy drinkers also showed the highest values, whereas in comparisons between moderate drinkers and abstainers, no significant differences were noted (Table 1).

The amount of self-reported ethanol intake correlated significantly with serum GGT (r = 0.50, P < 0.001), AST (r = 0.45, P < 0.001), ALT (r = 0.39, P < 0.001) and ferritin (r = 0.46, P < 0.001). Among the different biomarkers, GGT was found to correlate strongly with ALT (r = 0.42), ALT (r = 0.53) and ferritin (r = 0.49); AST with ALT (r = 0.62) and ferritin (r = 0.61); and ALT with ferritin (r = 0.46) (P < 0.001 for all) (Table 2).

In order to explore the interpretation of these variables as possible biomarkers of alcohol abuse, we also determined their upper normal limits based on the present population of abstainers or moderate drinkers (Table 3). Notable differences in the cutoffs based either on the use of the database obtained from moderate drinkers or abstainers were found for serum GGT, AST, ALT and ferritin. The incidences of observations, which

Table 2. Correlations between study variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>GGT</th>
<th>AST</th>
<th>ALT</th>
<th>Ferritin</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST</td>
<td>0.42***</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALT</td>
<td>0.53***</td>
<td>0.62***</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ferritin</td>
<td>0.49***</td>
<td>0.61***</td>
<td>0.46***</td>
<td></td>
</tr>
<tr>
<td>Albumin</td>
<td>0.10***</td>
<td>0.11***</td>
<td>0.15***</td>
<td>0.12*</td>
</tr>
</tbody>
</table>

*P < 0.05, ***P < 0.001.
For abbreviations, see Table 1.
Alcohol and Liver Markers

Table 3. Upper normal limits for the study parameters, as based either on the data from abstainers or moderate drinkers

<table>
<thead>
<tr>
<th></th>
<th>Upper normal limit</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Abstainers Moderate drinkers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GGT (U/l)a</td>
<td>63 106 (+68%)</td>
<td>63 79 (+25%)</td>
<td></td>
</tr>
<tr>
<td>AST (U/l)a</td>
<td>39 45 (+15%)</td>
<td>41 37 (-9%)</td>
<td></td>
</tr>
<tr>
<td>ALT (U/l)b</td>
<td>58 73 (+26%)</td>
<td>49 48 (-2%)</td>
<td></td>
</tr>
<tr>
<td>Ferritin (µg/l)b</td>
<td>274 342 (+25%)</td>
<td>141 191 (+35%)</td>
<td></td>
</tr>
<tr>
<td>Albumin (g/l)b</td>
<td>47 48 (+2%)</td>
<td>46 46 (0%)</td>
<td></td>
</tr>
</tbody>
</table>

For abbreviations, see Table 1.

The percentages in parentheses indicate the relative change in the upper normal limits in moderate drinkers as compared to abstainers, calculated upper normal limits calculated as 97.5th percentilea or as mean + 2SDb, as required.

exceeded the abstainer-based upper normal limits among heavy drinkers and moderate drinkers, are summarized in Figure 1. The highest incidences of elevated values in heavy drinkers were found for GGT (62%) followed by AST (53%), ALT (39%), ferritin (34%) and albumin (20%).

DISCUSSION

The present study in a large population of subjects with a wide range of alcohol consumption indicates that excessive drinking even in individuals without apparent liver disease induces the activities of several liver-derived enzymes and elevates the concentrations of hepatic proteins, which have recently been linked with defence mechanisms towards oxidative stress (Whitfield, 2001; Lee and Jacobs Jr, 2004; Faure et al., 2008). Moderate drinkers also show higher enzyme activities than abstainers underscoring an early occurrence of the biochemical responses in response to ethanol intake. While GGT and transaminase enzymes are also known to specifically increase as a result of obesity (Lawlor et al., 2005; Puukka et al., 2006; Alatalo et al., 2008), the presence or absence of overweight should not account for such differences here, since the groups of abstainers and moderate drinkers showed essentially similar BMIs (24.3 ± 3.5 and 24.0 ± 2.9, respectively). Obviously, overweight when occurring together with alcohol drinking could, however, aggravate the metabolic burden and hepatic enzyme responses, as recently observed for both GGT (Puukka et al., 2006) and ALT (Ruhl and Everhart, 2005a; Alatalo et al., 2008). Current BMIs (24.5 ± 3.8) of the heavy drinkers also exhibited similar mean levels. Although the relatively small number of observations in the heavy drinker group here does not provide enough statistical power for assessing independent effects of ethanol drinking and overweight on hepatic enzymes and proteins within this subgroup, it should be noted that obesity has been previously found to be a risk factor for cirrhosis in the alcoholics (Naveau et al., 1997).

When compared to abstainers, the group of moderate drinkers also showed elevated levels of serum ferritin, a marker of stored body iron. Thus, ethanol-related biochemical consequences in iron homeostasis may also be expected to occur at rather low levels of ethanol consumption. Previously, heavy drinking has been shown to increase ferritin levels, and secondary hepatic iron overload is a typical characteristic of alcoholic patients (Fletcher, 1996; Whitfield et al., 2001). Recent studies have indicated that even consumption of more than two alcoholic drinks/day may be associated with a significant elevation in the risk of iron overload (Ioannou et al., 2004). Deposition of excess iron in hepatic tissue is in turn an important secondary risk factor for the development of alcoholic liver disease. In experimental animals, iron and alcohol have been shown to act in a synergistic manner to enhance lipid peroxidation and liver injury (Bacon and Britton, 1990; Tsukamoto et al., 1995;
Cederbaum, 2003; Harrison-Findik, 2007). Alcohol consumption also increases the risk of liver injury in human patients with iron overload (Fletcher et al., 2002). It has recently been hypothesized that serum ferritin may be produced in order to sequester catalytically active free iron and increases in serum ferritin could actually reflect a defence mechanism, which occurs in response to ethanol-induced oxidative stress (Lee and Jacobs Jr, 2004). Increased ferritin levels could thereby protect from oxidative stress and consequence pathology due to free iron. In a similar manner, the responses in serum GGT, which is responsible for extracellular metabolism of glutathione, the main antioxidant in mammalian cells, could be linked to protection from reactive oxygen species (Whitfield, 2001; Puukka et al., 2006). On the other hand, acetaldehyde, the main oxidative metabolite of ethanol, has been previously shown to alter the gene expression of several proteins, such as collagen (Parés et al., 1994; Moirand et al., 1995; Niemelä, 2001; Thiele et al., 2005; Purohit and Brenner, 2006). Since the ferritin concentration is also under tight genetic control, it is also possible that its concentration may be affected in a similar way rather than through the cellular iron concentration.

The present data also show increased levels of serum albumin among heavy drinkers suggesting increased rates of albumin protein synthesis in response to regular ethanol intake prior to the development of liver dysfunction, whereas in patients with advanced liver disease the rates of hepatic protein synthesis are obviously decreased. Although the mechanisms underlying this observation remain unclear, it should be noted that previous studies in cell cultures have shown elevated hepatic protein synthesis rates as a result of chronic ethanol administration (Potter et al., 1985; Ohtake et al., 1986; Rothschild et al., 1988). Recently, Tyulina and co-workers (2006) found elevated plasma albumin levels in alcoholics, which correlated with elevated protein carbonyls, suggesting that covalent modifications of proteins by acetaldehyde could also be associated with albumin protein expression among heavy drinkers. Serum albumin is also an important antioxidant agent, whereas any structural modification of albumin induced by ethanol metabolites, glucose or free radicals has been suggested to impair its antioxidant properties (Faure et al., 2008).

The ethanol-induced biochemical changes in hepatic tissue appear to occur in a gender-dependent manner. It remains to be established whether such findings would also correlate with the differences in the individual susceptibility to tissue damage, which is known to be not equal between men and women (Schenker, 1997). It should also be noted that previous studies have indicated effects of smoking (Whitehead et al., 1996; Steffensen et al., 1997) and coffee consumption (Nakanishi et al., 2000; Ruhl and Everhart, 2005b) on the activities of hepatic enzymes. Unfortunately, in this study, the possible confounding effects of smoking or coffee consumption could not be addressed. Subjects who drink heavily usually also smoke a lot, we cannot rule out the possibility of additional increasing effects of smoking on hepatic enzymes among the heavy drinkers. However, in comparisons between the present population of moderate drinkers and abstainers, the enzyme activities or protein levels were not found to be significantly different between smokers and non-smokers (data not shown). In light of recent evidence indicating that coffee drinking could protect against liver injury and lead to reduced activities of liver enzymes (Nakanishi et al., 2000; Ruhl and Everhart, 2005b; Hu et al., 2008), we feel that the interactions between ethanol intake, smoking and coffee consumption clearly warrant further studies in large populations.

Our data also emphasize the view that due to the possible effects of even rather low ethanol doses on hepatic enzymes and proteins, the clinical interpretations of any ethanol-sensitive biomarkers as diagnostic tests in health care need further attention. Current surveys indicate a trend towards permanent increases in GGT activities at the population level (Hietala et al., 2005; Lee et al., 2006; Niemelä, 2007). On the other hand, serum activities of hepatic enzymes have recently been suggested to be useful as general indicators of health and disease and long-term survival (Kazemi-Shirazi et al., 2007; Kim et al., 2008). Therefore, in order to improve the discriminative power of any biomarker reflecting liver status, the normal ranges should perhaps be re-defined based on databases of healthy individuals who abstain from ethanol. Moreover, the possible roles of a wide variety of biochemical markers as players in defence mechanisms towards oxidative stress warrant further studies.

REFERENCES


