Moderate alcohol consumption is associated with advanced fibrosis in non-alcoholic fatty liver disease and shows a synergistic effect with type 2 diabetes mellitus

Julia Blomdahl, Patrik Nasr, Mattias Ekstedt, Stergios Kechagias

Department of Gastroenterology and Hepatology, Department of Health, Medicine, and Caring Sciences, Linköping University, SE-581 83 Linköping, Sweden

ABSTRACT

Background: Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease worldwide. Whether moderate alcohol consumption plays a role for progression of NAFLD is disputed. Moreover, it is not known which tool is ideal for assessment of alcohol consumption in NAFLD. This study aimed to evaluate if moderate alcohol consumption assessed with different methods, including the biological marker phosphatidylethanol (PEth), is associated with advanced fibrosis in NAFLD.

Methods: We conducted a cross-sectional study of patients with biopsy-proven NAFLD. All participants were clinically evaluated with medical history, blood tests, and anthropometric measurements. Alcohol consumption was assessed using PEth in blood, the questionnaire AUDIT-C, and clinical interview.

Findings: 86 patients were included of which 17% had advanced fibrosis. All participants reported alcohol consumption < 140 g/week. Average weekly alcohol consumption was higher in the group with advanced fibrosis. Moderate alcohol consumption, independently of the method of assessment, was associated with increased probability of advanced fibrosis (adjusted OR 5.5–9.7, 95% CI 1.05–69.6). Patients with type 2 diabetes mellitus (T2DM) consuming moderate amounts of alcohol had a significantly higher rate of advanced fibrosis compared with those consuming low amounts (50.0–60.0% vs. 3.3–21.6%, p < 0.05).

Conclusions: Moderate alcohol consumption, irrespective of assessment method (clinical interview, AUDIT-C, and PEth), was associated with advanced fibrosis. PEth in blood ≥ 50 ng/mL may be a biological marker indicating increased risk for advanced fibrosis in NAFLD. Patients with T2DM consuming moderate amounts of alcohol had the highest risk of advanced fibrosis, indicating a synergistic effect of insulin resistance and alcohol on the histopathological progression of NAFLD.

© 2020 The Author(s). Published by Elsevier Inc. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

1. Introduction

According to European guidelines, weekly alcohol consumption in non-alcoholic fatty liver disease (NAFLD) is set at a maximum of 30 g/day for males and 20 g/day for females [1]. However, guidelines do not state how the assessment of alcohol consumption should be performed. Commonly, clinical interview and/or information from a self-reported questionnaire are used. One of the most widespread and validated tools for excluding excessive alcohol consumption in NAFLD is AUDIT [2]. A shorter version of AUDIT (AUDIT-C), consisting of only three questions, has been developed and more largely used [3]. However, people with alcohol misuse will often inaccurately report that they do not have a problem, creating a need for more objective methods to investigate a person’s drinking habits.

Analysis of phosphatidylethanol in blood (PEth) has emerged as a sensitive and specific method to investigate a person’s drinking habits, particularly for social drinkers and risk drinkers [4]. PEth comprises a group of homologous phospholipids found in cell membranes [5], which are formed in the presence of ethanol. No false-positive PEth-values have been reported [6] and it can be detected in blood between 2 and 4 weeks after ingestion [7].

A potentially important factor for clinical and histopathological progression of NAFLD is the impact of moderate alcohol consumption. There is evidence for beneficial effects of modest alcohol consumption on the risk of metabolic syndrome and insulin resistance [8], which
are important components of the NAFLD disease process. However, the impact of moderate alcohol consumption on histopathology and clinical outcome in NAFLD is disputed as studies show conflicting results, stating both improvement and worsening of histological parameters in moderate drinkers [9].

The purpose of this study was to evaluate if moderate alcohol consumption is associated with the presence of advanced fibrosis in NAFLD patients. Moreover, we evaluated the assessment of alcohol consumption in NAFLD with three different methods: PEth, clinical interview, and AUDIT-C.

2. Methods

In this cross-sectional study of prospectively enrolled patients with biopsy-confirmed NAFLD, alcohol consumption was assessed using three different methods, AUDIT-C, clinical interview, and measurement of the direct biomarker PEth in blood. More details on Methods are available in the online-only Supplementary data.

3. Results

3.1. Participant characteristics and advanced fibrosis

Baseline characteristics of patients are presented in Table 1. Participants were also divided into two groups based on fibrosis stage (F0–2 and F3–4). Patient characteristics in the two groups are presented in Table 1. Weekly alcohol consumption was higher in the advanced fibrosis group, both when assessed with AUDIT-C and interview (47.5 and 56.3 g/week compared to 27.6 and 31.2 g/week). Assessment with interview attained statistical significance, however, only a trend was seen when assessed with AUDIT-C (p = 0.10). Average PEth was 66.5 ng/mL in the advanced fibrosis group and 38.4 ng/mL in participants with F0–2, but it did not reach statistical significance (p = 0.25).

Correlations between the three methods used for assessment of alcohol consumption were all significant. The strongest correlation was seen between consumption assessed with AUDIT-C and clinical interview (r = 0.89, p < 0.01). The correlation coefficient was 0.44 (p < 0.01) between AUDIT-C and PEth and 0.56 (p < 0.01) between interview and PEth.

3.2. Alcohol consumption and diabetes mellitus type 2

To determine if the presence of T2DM was associated with advanced fibrosis, patients were divided into four groups (Fig. 1), 1) low alcohol consumption and no T2DM, 2) low alcohol consumption and T2DM, 3) moderate alcohol consumption and no T2DM, and 4) moderate alcohol consumption and T2DM. Moderate alcohol consumption was defined using three different cut-offs, A) >66 g/week (AUDIT-C), B) >96 g/week (clinical interview), and C) PEth ≥ 50 ng/mL. The different cut-offs were attained from multivariate logistic regression models. Similar results were seen with respect to all three assessments of alcohol consumption. The group with moderate alcohol consumption and T2DM

Table 1. Weekly alcohol consumption was higher in the advanced fibrosis group, both when assessed with AUDIT-C and interview (47.5 and 56.3 g/week compared to 27.6 and 31.2 g/week). Assessment with interview attained statistical significance, however, only a trend was seen when assessed with AUDIT-C (p = 0.10). Average PEth was 66.5 ng/mL in the advanced fibrosis group and 38.4 ng/mL in participants with F0–2, but it did not reach statistical significance (p = 0.25).

Correlations between the three methods used for assessment of alcohol consumption were all significant. The strongest correlation was seen between consumption assessed with AUDIT-C and clinical interview (r = 0.89, p < 0.01). The correlation coefficient was 0.44 (p < 0.01) between AUDIT-C and PEth and 0.56 (p < 0.01) between interview and PEth.

3.2. Alcohol consumption and diabetes mellitus type 2

To determine if the presence of T2DM was associated with advanced fibrosis, patients were divided into four groups (Fig. 1), 1) low alcohol consumption and no T2DM, 2) low alcohol consumption and T2DM, 3) moderate alcohol consumption and no T2DM, and 4) moderate alcohol consumption and T2DM. Moderate alcohol consumption was defined using three different cut-offs, A) >66 g/week (AUDIT-C), B) >96 g/week (clinical interview), and C) PEth ≥ 50 ng/mL. The different cut-offs were attained from multivariate logistic regression models. Similar results were seen with respect to all three assessments of alcohol consumption. The group with moderate alcohol consumption and T2DM

Table 1. Weekly alcohol consumption was higher in the advanced fibrosis group, both when assessed with AUDIT-C and interview (47.5 and 56.3 g/week compared to 27.6 and 31.2 g/week). Assessment with interview attained statistical significance, however, only a trend was seen when assessed with AUDIT-C (p = 0.10). Average PEth was 66.5 ng/mL in the advanced fibrosis group and 38.4 ng/mL in participants with F0–2, but it did not reach statistical significance (p = 0.25).

Correlations between the three methods used for assessment of alcohol consumption were all significant. The strongest correlation was seen between consumption assessed with AUDIT-C and clinical interview (r = 0.89, p < 0.01). The correlation coefficient was 0.44 (p < 0.01) between AUDIT-C and PEth and 0.56 (p < 0.01) between interview and PEth.

3.2. Alcohol consumption and diabetes mellitus type 2

To determine if the presence of T2DM was associated with advanced fibrosis, patients were divided into four groups (Fig. 1), 1) low alcohol consumption and no T2DM, 2) low alcohol consumption and T2DM, 3) moderate alcohol consumption and no T2DM, and 4) moderate alcohol consumption and T2DM. Moderate alcohol consumption was defined using three different cut-offs, A) >66 g/week (AUDIT-C), B) >96 g/week (clinical interview), and C) PEth ≥ 50 ng/mL. The different cut-offs were attained from multivariate logistic regression models. Similar results were seen with respect to all three assessments of alcohol consumption. The group with moderate alcohol consumption and T2DM

Table 1. Weekly alcohol consumption was higher in the advanced fibrosis group, both when assessed with AUDIT-C and interview (47.5 and 56.3 g/week compared to 27.6 and 31.2 g/week). Assessment with interview attained statistical significance, however, only a trend was seen when assessed with AUDIT-C (p = 0.10). Average PEth was 66.5 ng/mL in the advanced fibrosis group and 38.4 ng/mL in participants with F0–2, but it did not reach statistical significance (p = 0.25).

Correlations between the three methods used for assessment of alcohol consumption were all significant. The strongest correlation was seen between consumption assessed with AUDIT-C and clinical interview (r = 0.89, p < 0.01). The correlation coefficient was 0.44 (p < 0.01) between AUDIT-C and PEth and 0.56 (p < 0.01) between interview and PEth.
had significantly higher presence of advanced fibrosis compared to the groups with low alcohol consumption (Fig. 1), regardless of the presence of T2DM.

3.3. Multivariate models

Weekly alcohol consumption assessed with AUDIT-C, interview, and PEth was used in a bivariate, and a multivariate logistic regression model, comparing the risk for advanced fibrosis (Supplementary Table 1). In the bivariate unadjusted model, the risk of having advanced fibrosis increased by 1.4–1.6% for each gram of ethanol per week (assessed with interview and AUDIT-C). When adjusted for age, sex, BMI, and diagnosis of T2DM, the risk was even higher, 1.8–1.9%. PEth did not reach statistical significance in the bivariate model. In all three bivariate models, T2DM was significantly associated with increased risk for advanced fibrosis.

In the multivariate logistic regression model, alcohol consumption assessed with AUDIT-C showed a crude OR for advanced fibrosis of 6.3 in those consuming >66 g/week as compared to 0–2.99 g/week. After adjusting for age, sex, BMI, and T2DM the OR was even higher (9.7, 95% CI 1.4–68.9). Alcohol consumption of 3–66 g/week was not associated with significantly increased risk of advanced fibrosis compared to consumption of 0–2.99 g/week. Using the same cut-off level in the model of alcohol consumption assessed with clinical interview, statistical significance was not reached. Instead, alcohol consumption of more than 96 g/week was statistically significant compared with consumption of 0–2.99 g/week. After adjusting for age, sex, BMI, and T2DM, the OR of advanced fibrosis compared with consumption of 0–2.99 g/week, with an OR of 7.0 (95% CI 1.1–44.1). Alcohol consumption of 3–96 g/week was not associated with significantly increased risk of advanced fibrosis compared with consumption of 0–2.99 g/week. After adjusting for age, sex, BMI, and T2DM, the OR of advanced fibrosis in participants drinking >96 g/week, was even higher (8.5, 95% CI 1.05–69.6). In this model, a PEth-value of ≥ 50 ng/mL was significantly associated with advanced fibrosis (OR 3.3, 95% CI 1.02–10.5; adjusted OR 5.5, 95% CI 1.4–22.1). In the adjusted multivariate model for AUDIT-C and PEth, T2DM was significantly associated with advanced fibrosis.

4. Discussion

The main finding of this study was that moderate alcohol consumption, irrespective of assessment method (clinical interview, AUDIT-C,
and NAFLD, was associated with advanced fibrosis. Moreover, we showed that NAFLD patients with T2DM consuming moderate amounts of alcohol had the highest risk of advanced fibrosis, indicating a synergistic effect of insulin resistance and alcohol on NAFLD histopathology.

Accurate assessment of alcohol consumption is mandatory for a correct diagnosis of NAFLD. Questionnaires used to assess alcohol consumption have been developed for detecting alcohol abuse, not to evaluate moderate alcohol intake. AUDIT-C, consisting of only three questions, has a good specificity for heavy drinking and dependence [3]. Yet, its effectiveness to diagnose moderate alcohol drinking has never been thoroughly investigated. In the present study, we show an excellent correlation (r = 0.89) between AUDIT-C and the more time-consuming clinical interview in individuals with moderate alcohol consumption. Moreover, we show that consumption > 66 g/week according to AUDIT-C was associated with advanced fibrosis. The corresponding figure for assessment with clinical interview was slightly higher (>96 g/week). The most probable explanation for this discrepancy is the construction of the AUDIT-C questionnaire in which the patient specifies intervals of the number of drinks rather than the actual numbers consumed.

The effect of moderate alcohol consumption on NAFLD seems to be multifaceted. Most studies, including a meta-analysis of over 40,000 individuals [10], indicate that modest alcohol consumption is associated with decreased risk for the development of fatty liver disease [11,12]. In a recent cross-sectional study, Vilar-Gomez et al. [13] examined the association between the gene variant ADH1B*2 and moderate alcohol consumption and histologic severity of NAFLD. The variant gene, ADH1B*2, results in a higher metabolising activity. It was shown that consuming moderate amounts of alcohol and having the variant gene, resulted in less NASH and fibrosis, compared with those without the variant gene. Moreover, consuming moderate amounts of alcohol compared with abstention resulted in less NASH and fibrosis, regardless of having the variant gene or not. Studies evaluating ultrasonographical findings or levels of serum aminotransferases as surrogate markers for NAFLD have mainly shown positive effects of moderate consumption, especially for hepatic steatosis [12,14–18]. However, recent studies [19] have shown that fibrosis stage, but no other histopathological parameters, determines the future risk of mortality and liver-related morbidity in NAFLD.

In recent years, new non-invasive techniques for diagnosing fibrosis have been developed [20,21], but liver biopsy remains the gold standard for the diagnosis of NAFLD and assessing the severity of fibrosis. Hitherto, 12 studies have assessed the impact of alcohol on histopathology in NAFLD [9]. Robust conclusions cannot be drawn because study design varies and particularly since the results are divergent. In this study, we show an association of moderate alcohol consumption and advanced fibrosis in NAFLD.

A major strength of our study is the use of an objective marker of alcohol consumption. Analysis of PEth has hitherto been used only in few NAFLD studies, making it hard to assess its utility. For the first time, we show that PEth as low as ≤50 ng/mL is associated with advanced fibrosis in NAFLD. Our results can be used to aid the interpretation of PEth in NAFLD patients.

5. Conclusions

Although limited by a small sample size, this study indicates that moderate alcohol consumption in NAFLD may aggravate fibrosis and increase the risk for future development of end-stage liver disease. NAFLD patients with T2DM consuming moderate amounts of alcohol seem to be at the highest risk for advanced fibrosis. Further prospective studies assessing alcohol consumption using sensitive direct biomarkers, such as PEth, are needed to confirm our results.

CRediT authorship contribution statement

Julia Blomdahl: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Validation, Visualization, Writing - original draft. Writing - review & editing. Patrik Nasr: Conceptualization, Formal analysis, Investigation, Methodology, Project administration, Writing - review & editing. Mattias Ekstedt: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing - review & editing. Stergios Kechagias: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing - review & editing.

Declaration of competing interest

None.

Acknowledgements

We would like to thank the Clinical Pharmacology Laboratory, Region Östergötland, for the analysis of phosphatidylethanol in blood. We would also like to thank the Medical Research Council of Southeast Sweden for providing funding for our research.

Financial support (funding)

ALF Grants, Region Östergötland, Medical Research Council of Southeast Sweden (grant no. 75287). The funding sources had no role in the conduction of the research or the preparation of the article.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.metabol.2020.154439.

References

[13] Vilar-Gomez E, Sookoian S, Pirola CJ, Liang T, Cawrieh S, Cummings O, et al. ADH1B *2 is associated with reduced severity of nonalcoholic fatty liver disease in adults,


