Reviewer comments (specific comments in bold) and our responses

Round 1

Reviewer #1:

Specific Comments to Authors: The ms could be of potential interest, but has severe limitations.

1. **We cannot find why these patients assumed cannabis, which type, and how (orally, inhalant, etc.). Patients are typically not routinely tested for cannabis use upon admission, and the diagnosis of cannabis use is often made from patient reports. Is cannabis prescribed or freely consumed?**

   Thank you for the inquiry. The types and methods of cannabis use are not recorded in the National Inpatient Sample (NIS) database. We agree that the type, method of use as well as whether prescribed or freely consumed would be of interest. We are aware that the metabolism and metabolic products of cannabis vary with mode of use.

   A very different study design would be needed. As the mode of cannabis use is not systematically recorded in records, a retrospective review of medical records would be unlikely to reliably capture mode data. A large prospective study in which mode would be ascertained and monitored would be of interest. Our approach utilizes currently available information. We have added references to relevant literature about the modes of use in some studies in response to the reviewer's inquiry, but these do not necessarily reflect the mode of use in these patients.

2. **The authors made some interesting conclusions, but do not give them in a logical and rational presentation, rather in a chaotic way. The authors should give some hypothesis on their results, not simply wrote them down, like the laundry list**

   We had a broad list of outcomes, and therefore we focused on clinical outcomes. We have re-written the sections to improve clarity, and added a hypothesis in the

introduction, and we clarify that we have focused on liver-related clinical outcomes as our primary outcomes.

We hypothesize that cannabis is associated with less liver-related outcomes in patients with NAFLD after adjusted for risk factors for NAFLD.

Reviewer #2:

Scientific Quality: Grade D (Fair)

Language Quality: Grade A (Priority publishing)

Conclusion: Major revision

Remarks to the author: Increased incidence of and excess weight lead to an increased incidence of Non-alcoholic fatty liver disease (NAFLD) has been demonstrated to has highly correlation with obesity. The evidence of weight gain and related metabolic alterations caused by cannabis consumption have been proved in psychosis patients, which may be at greater risk of presenting fatty diseases, such as NAFLD. Therefore, the meta-analysis of observational studies of cannabis use and the risk of NAFLD is a critical issue. In this manuscript, the authors report the cannabis is associated with liver-related complications of NAFLD, however, the previous studies on the likely effect of cannabis on liver steatosis has been investigated, which aimed to explore if cannabis consumption had an effect on hepatic steatosis (doi: 10.1016/j.pnpbp.2019.109677). Although previous investigation reduces the novelty of this study, this analysis was performed with a large population database to measure clinical outcomes at the national level. This cohort-analysis has several limitations that need to be addressed before being re-evaluated for its publication.

Major comments 1. The purpose of this article focuses on the effect of cannabis on clinical outcomes and in-patient mortality in patients with NAFLD, therefore the time effect of the period and dose-dependent analysis with an increase of the cumulative defined daily doses (cDDD) compared with nonusers will be necessary in this study.

We agree that time and dose-dependent issues are important. As reviewer 1 also pointed out, the mode of use and dosage are important metrics to evaluate the effects of cannabis. Unfortunately, the specific time and dosage were not available with the NIS database. The NIS database is based on ICD-9 codes, and there are no specific codes for long-term vs short-term use. For further subcategorization of cannabis abuse, there are codes that we could divide into dependent vs non-dependent uses. However, 94.1% of the cannabis users were coded under non-dependent use, and due to the limited sample size, we could not provide dose-dependent data. A possible explanation for the low baseline percentage of dependent cannabis use is a lack of available data, limited current routine clinician assessment, or truly low number of patients who abuse cannabis.

Our study has strengths, especially with large database study, and we can evaluate the clinical effects of cannabis at national level. These limitations are listed under discussion. A strength of our manuscript is to alert clinicians of the possible relevance of ascertainment of cannabis use, which in turn might alter future routine assessments to further probe about cannabis use, especially in light of trends showing recent increases in use in the U.S.

2. What kind of strategies was applied in this article for the study quality examination? The random-effect model need to be used to calculate the overall risk ratio (RR) with a 95% confidence interval (CI), and the heterogeneity among the studies need to be assessed.

The NIS dataset is released as a public use dataset, which we have cited. The quality controls used in assembling it are included in the public use materials. We have not independently attempted to examine its quality. It is used in many studies examining medical care in the U.S., and its limitations have been discussed in review articles.

3. To detect population bias and heterogeneity, the pooled analysis or subgroup analyses are required, and all the included cases need to be adjusted with the same and potential confounding factors

In order to better address the potential confounding factors and bias, we took the following steps.

1) In order to achieve a more specific control group with NAFLD, we used ICD-9 code with 571.8, and we excluded other types of hepatitis including alcoholic liver disease, hemochromatosis, viral hepatitis B and C, primary biliary cirrhosis, autoimmune hepatitis and toxic liver disease.

2) We used case control matching analysis, where the groups are matched on age, sex, race, comorbidity, and the difference between the group is the exposure to cannabis. Therefore, we tried to control the potential confounding factors by case-control matching analysis.

3) As the writer pointed out, in order to address potential confounding factors, we examined diabetes, hyperlipidemia, obesity as co-variates in our multi-variate analysis. Also, we added these variables in Table 1 to compare baseline characteristics.

Diabetes, obesity, and hyperlipemia were more common in non-cannabis group although these conditions were part of elixhauser comorbidity score. These three factors were also used in the univariate screen in the process of a regression model building.

4) The cannabis user and non-user groups are sufficiently large for us to conduct multivariate analysis of each. The risk factor patterns differ in these two groups. These differences are masked in an overall analysis. We have replaced our prior multivariate model with separate analyses for the 2 groups, in our new Table 2, and present both univariate and multivariate analysis.

Minor comments 1. The structure of introduction are very weak, the authors need to provide more information about the cannabis and NAFLD.

Thank you for pointing out the weakness. We have extended our treatment in the introduction and discussion, and provide more extensive references.

2.Table 1: The total number of patients in each variable is different and needs to be confirmed. For instance, cannabis use group N=9,735, but total number 9,225 in race, 9,715 in primary payer; non-cannabis N=18,440, total number 18,530 in elixhauser comorbidity index.

We have re-analyzed the data, and provided more detailed annotation in the Table 1 footnotes on the statistical analyses.

3.Table 1: The item and number should be aligned in order to facilitate reading. For example, household median income data of two groups are not aligned.

Thank you for the comment. In order to add clarity to our study, a hypothesis was addressed and we focused on clinical outcomes instead of a broad list of compared items based on the review.

We have improved the formatting and the alignments.

4.Discussion : "A likely explanation for the variations is the differential effects of active cannabinoids extracted from various types of cannabis strains with different concentrations." This description to convince readers that further data is needed. In addition, is it related to the activation and inhibition of cannabinoid receptors?

We agree that this is a possible explanation. We now cite some of the literature supporting activation of cannabinoid receptors, such as CB1 and CB2, although there can also be antagonist effects. It is increasingly becoming understood that the effects may be quite complex. We primarily focused on the activation of different cannabinoid receptors.

5. References mark should be consistent, such as ref 2 and 4 are inconsistent. Please overall recheck each of them.

Thank you for catching the discrepancy, the references were further edited.

<u>Step 6: Editorial Office's comments</u>

The author must revise the manuscript according to the Editorial Office's comments and suggestions, which listed below:

(1) Science Editor: 1 Scientific quality: The manuscript describes a retrospective cohort study of the cannabis is associated with liver-related complications of nonalcoholic fatty liver disease. The topic is within the scope of the WJH. (1) Classification: Grade D and Grade D; (2) Summary of the Peer-Review Report: The authors report the cannabis is associated with liver-related complications of NAFLD, which is of potential interest. However, it has several limitations that need to be addressed. The questions raised by the reviewers should be answered; and

(3) Format: There are 4 tables and 1 figure. A total of 18 references are cited, including 8 references published in the last 3 years. There are no self-citations. 2 Language evaluation: Classification: Grade C and Grade A. The authors are from United States. 3 Academic norms and rules: The authors provided the Biostatistics Review Certificate, the signed Conflict-of-Interest Disclosure Form and Copyright License Agreement. The Institutional Review Board Approval Form, and the Written informed consent are waived. The STROBE Statement lacks of the page number.

The page number was added to the STROKE statement. We have reduced the number of tables.

No academic misconduct was found in the CrossCheck detection and Bing search.

(4) Supplementary comments: This is an unsolicited manuscript. The topic has not previously been published in the WJH.

(5) (Specific) Issues raised: (1) The language classification is Grade C. Please visit the following website for the professional English language editing companies we recommend: https://www.wjgnet.com/bpg/gerinfo/240; (2) The authors did not provide original pictures. Please provide the original figure documents. Please prepare and arrange the figures using PowerPoint to ensure that all graphs or arrows or text portions can be reprocessed by the editor

We removed the figure, instead provided detailed explanation to the method.

; and (3) The "Article Highlights" section is missing. Please add the "Article Highlights" section at the end of the main text.

An "Article Highlights" section has been added at the end of the main manuscript text.

(6) Re-Review: Required.

(7) Recommendation: Conditional acceptance.

Round 2

The revised manuscript has ameliorated in general quality of presentation and in the logical terms of sequence. The findings are not irrelevant, even if authors failure to detect a possible motivation of their relevant data on ascites.

Thank you for noticing the changes we made based on the reviewers' suggestions.

It should be expanded any hypothetical reason for the fact that Cannabis was associated with higher rates of ascites, but there was no statistical difference in the prevalence of portal hypertension, varices and variceal bleeding, and cirrhosis.

Thank you for your comment. Possible explanations for the isolated finding of more prevalent ascites in the cannabis group are hypoalbuminemia and a lack of long-term follow-up data. Hypoalbuminemia was observed as one of the spectrums of toxicity from intravenous cannabis use by Payne et al. Therefore, hypoalbuminemia from severe cannabis use may explain the higher rates of ascites in cannabis users. Furthermore, lower body mass from illicit drug use including cannabis use was previously reported, and therefore the toxicity from cannabis along with poor nutritional status from cannabis use can lead to hypoalbuminemia which can contribute to the development of ascites. However, it only partly explains the isolated finding without a significant difference in the prevalence of portal hypertension, varices and variceal bleeding and cirrhosis. It can also be related to the nature of the NIS, which only captures hospitalized patients, and therefore manifestations of decompensated cirrhosis may not

be captured if occurred outside the hospital setting. This was added to our discussion in updated manuscript as below, highlighted in yellow

"The patients who used cannabis had higher prevalence of ascites compared to non-cannabis group (OR 1.45, 95% CI (1.24-1.51)). Possible explanations for this finding can be related to 1) hypoalbuminemia due to the toxicity of cannabis use or 2) lower body mass, which is a potential indicator for inadequate nutritional status. Hypoalbuminemia was described as a spectrum of toxic reactions from intravenous cannabis use along with fulminant gastroenteritis, toxic hepatitis, acute renal failure, electrolyte disturbances, leukocytosis, anemia, and a relative thrombocytopenia from a study of 4 cases of intravenous cannabis use by Payne et al in 1975.[27] Serum albumin is the most abundant plasma protein and therefore binding to albumin is a key determinant of the drug efficacy, distribution and possible toxicity of cannabinoids.[28] The increase in plasma albumin may reduce the unbound fraction of cannabinoids, which further reduces the efficacy of the drug.[28] Along the same reasoning, it is conceivable to hypothesize more cannabinoid toxicity in patients with hypoalbuminemia due to more unbound cannabinoids. A study by Bluml et al[29] showed an inverse relationship between body mass index (BMI) and illicit drug use including cannabis use among young males, and therefore along with the toxicity of high-dose cannabis use, hypoalbuminemia from poor nutritional status may explain higher rate of ascites in cannabis users due to the low oncotic pressure from hypoalbuminemia. The forementioned explanation for hypoalbuminemia can partly explain the isolated finding of higher rates of ascites in cannabis users without higher rates of portal hypertension, varices and variceal bleeding and cirrhosis. Another explanation of such finding can be related to a lack of long-term follow-up. As the NIS is limited to hospitalized data, a spectrum of clinical presentations of decompensated cirrhosis such as varices and variceal bleeding and actual clinical diagnosis of cirrhosis may not be captured in our study design. Further studies with a long-term follow-up

investigating cannabis use in patients with NAFLD are warranted to further evaluate the rates of clinical manifestations of decompensated cirrhosis."