Format for ANSWERING REVIEWERS

February 22, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 8977-revised.doc).

Title: Association between serum alpha-fetoprotein levels and fatty liver disease: a cross-sectional study
Author: Ping Xu, Chengfu Xu, Xiongyong Wan, Chaohui Yu, Chao Shen, Peng Chen, Genyun Xu, Youming Li

Name of Journal: *World Journal of Gastroenterology*

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Replies to Reviewer 1:

Comment 1: The scientific innovation of this study is limited since similar studies have been published in many Journals (Hepatol Int. 2009; 3: 551–555 and Eur Rev Med Pharmacol 2013; 17 (11): 1536-1541.). However, this study has been done in Chinese Population and may have some clinical significance.

Reply: Thanks very much for this comment!

It is true that the relation between AFP and FLD has been investigated by previous studies. However, as mentioned in the Introduction section of our manuscript, the conclusions of previous studies were contradictory. The inconsistency among these studies may be explained by differences in study population, sample size, and diagnostic methods for FLD. Therefore, we performed this large sample, cross-sectional survey to analysis the relation between AFP and FLD in a Chinese population. We have highlighted this information in the revised manuscript.

Page 3, line 17-24: The potential association between AFP and FLD has been examined in recent studies. Babali *et al.* [10] observed that patients with fatty liver had a significantly increased serum AFP level, and the level was positively correlated with the grade of hepatic steatosis. Kara *et al.* [11] compared serum AFP levels in 130 male NAFLD patients with those in 57 health male controls; however, they did not observe any significant association between AFP and histopathological findings in NAFLD patients. Polyzos *et al.* [12] did not observe any positive association between AFP and NAFLD. The inconsistency of these studies may arise from differences in study population, sample size, and diagnostic methods for FLD.

Comment 2: The authors should add more data onto the manuscript to clarify what are the differences between NAFLD and alcoholic fatty liver disease and the difference in different stages of disease?

Reply: Thanks very much for this comment and suggestion!

We agree that it would be better to clarify whether AFP is differentially associated with NAFLD and ALD, and whether AFP is associated with different stages of disease. In this study, FLD was diagnosed by hepatic ultrasonography. We could not determine steatohepatitis or fibrosis by ultrasonography, neither have we recorded the alcohol consumption information from all subjects. The association of serum AFP levels with different etiologies and histopathological

findings of FLD cannot be analyzed as a result. We will take this into consideration in our future studies. We have described this limitation in the Discussion section of revised manuscript.

Page 8, line 10-13: FLD was diagnosed based on hepatic ultrasonography, which is not sensitive for mild hepatic steatosis; neither is it sensitive for diagnosing steatohepatitis or fibrosis. Therefore, the association of serum AFP levels with histopathological findings in FLD could not be analyzed in this study.

Page 8, line 16-19: FLD can be caused through both alcoholic and nonalcoholic etiologies. In this study, we did not record alcohol consumption information in all subjects. Whether AFP is differentially associated with alcoholic and nonalcoholic fatty liver disease remains to be determined.

Comment 3: Is there any association between pathological changes and AFP level?

Reply: Thanks very much for this comment!

In this study, we could not determine steatohepatitis or fibrosis by hepatic ultrasonography. The association of serum AFP levels with histopathological findings of FLD could not be analyzed as a result. We will take this into consideration in future studies. We have added this shortage as study limitations in the Discussion section of revised manuscript.

Page 8, line 10-13: FLD was diagnosed based on hepatic ultrasonography, which is not sensitive for mild hepatic steatosis; neither is it sensitive for diagnosing steatohepatitis or fibrosis. Therefore, the association of serum AFP levels with histopathological findings in FLD could not be analyzed in this study.

Comment 4: The discussion is too simple. Author should discuss and explore the possible mechanisms and clinical implications.

Reply: Thanks very much for this comment! The potential mechanisms and clinical implications are discussed in more detail in the revised manuscript.

Page 8, line 1-3: Hepatocyte proliferation during liver regeneration is also observed to be associated with dedifferentiation of mature hepatocytes and temporarily increased expression of AFP in the liver [22].

Page 8, line 4-9: Although the precise mechanisms remain unclear, our results still have significant clinical implications. Based on the fact that FLD may progress to HCC and that serum AFP levels are elevated in subjects with FLD, monitoring serum AFP levels to screen for HCC in FLD patients has significant clinical importance. Indeed, a study reported that AFP combined with prothrombin induced by a lack of vitamin K or a vitamin K antagonist-II (PIVKA-II) may be considerably valuable for surveillance of HCC in FLD [24].

Replies to Reviewer 2

Comment 1: Page 2, line 14-15, “Our results suggested that serum AFP levels are significantly associated with FLD, and AFP acts as a cofactor but not as an independent factor for FLD.” should be “Our results suggested that serum AFP levels were significantly associated with FLD, and AFP acted as a cofactor but not as an independent factor for FLD.”

Reply: Thanks very much for your positive comments and valuable suggestions. We have made the correction in the revised manuscript.

Page 2, line 20-21: Our results suggest that serum AFP levels are significantly associated with FLD and that AFP acts as a cofactor but not as an independent factor for FLD.

Comment 2: Page 6, line 19-20, “Subjects with FLD had a higher serum AFP levels…” should be “Subjects with FLD had higher serum AFP levels…”

Reply: Thanks very much for this suggestions. We have made the correction in the revised manuscript.

Page 6, line 27-28: The subjects with FLD exhibited higher serum AFP levels than those without FLD

Comment 3: Page 6, line 20-21, “A significantly association between serum AFP levels with metabolic syndrome” should be “A significantly association between serum AFP levels and metabolic syndrome”.

Reply: Thanks very much for this suggestions. We have made the correction in the revised manuscript.

Page 6, line 28-29: A significant association between serum AFP levels and metabolic syndrome…

Comment 4: Page 6, line 22, “elevated serum AFP levels was associated with an increased risk for FLD.” should be “elevated serum AFP levels were associated with an increased risk for FLD.”

Reply: Thanks very much for this suggestions. We have made the correction in the revised manuscript.

Page 6, line 30: elevated serum AFP levels are associated with an increased risk of FLD.

Replies to Reviewer 3

Comment 1: Hepatocyte proliferation during liver regeneration is associated with dedifferentiation of mature hepatocytes and temporarily increased expression of AFP in the liver. In NAFLD/NASH, the degree of apoptosis of hepatocytes is known to be elevated. Thus, it would be valuable to correlate AFP levels with some markers of hepatocyte cell death (e. g. 30 kDa fragments of cytokeratin 18).

Reply: Thanks very much for this valuable comment. We have added this information in Discussion section of our revised manuscript.

Page 8, line 1-3: Hepatocyte proliferation during liver regeneration is also observed to be associated with dedifferentiation of mature hepatocytes and temporarily increased expression of AFP in the liver [22].

Page 8, line 24-26: Moreover, the degree of apoptosis in hepatocytes is known to be elevated in steatotic livers [27, 28]. Thus, it would be valuable to correlate AFP levels with markers of hepatocyte cell death, such as cytokeratin 18 fragments. Further studies are needed to clarify these issues.

Comment 2: Liver biopsy is the gold standard for histological diagnosis of NAFLD/FLD. Were any patients also examined by liver biopsy? If so is there any correlation between the level of AFP and the histological severity of the disease?

Reply: Thank you very much for this comment.

FLD was diagnosed by hepatic ultrasonography in this study. The association of serum AFP levels with different histopathological findings of FLD cannot be analyzed due to lacking of hepatic histology information. We will take hepatic histology into consideration in our future studies. We have described this limitation in the Discussion section of revised manuscript.

Page 8, line 10-13: FLD was diagnosed based on hepatic ultrasonography, which is not sensitive for mild hepatic steatosis; neither is it sensitive for diagnosing steatohepatitis or fibrosis.

Therefore, the association of serum AFP levels with histopathological findings in FLD could not be analyzed in this study.

Comment 3: In some parts of the manuscript, it is not clear whether you discuss alcoholic, non-alcoholic or both etiologies of FLD. Dividing FLD group into ALD and NAFLD would give the reader more information.

Reply: We apologize for the confusion!

In this study, we discuss the association of AFP with both etiologies of FLD. We agree that it would be better to divide FLD group into ALD and NAFLD, and investigate whether AFP is differentially associated with NAFLD and ALD. However, we did not record alcohol consumption information from all subjects in this study. The association of serum AFP levels with different etiologies of FLD cannot be analyzed as a result. We will take this into consideration in our future studies. We have described this limitation in the Discussion section of revised manuscript.

Page 7, line 5-6: both alcoholic and nonalcoholic,

Page 8, line 16-19: FLD can be caused through both alcoholic and nonalcoholic etiologies. In this study, we did not record alcohol consumption information in all subjects. Whether AFP is differentially associated with alcoholic and nonalcoholic fatty liver disease remains to be determined.

Comment 4: In the discussion, you state that "FLD is a common liver disease that affects more than one third of world's population." Do you mean NAFLD or FLD in general? Do you really mean general world's population? Or adult population of some countries? In this case authors should identify the source the information quoted.

Reply: We apologize for the confusion!

We mean FLD in general in this study. We have revised this sentence in our revised manuscript as follow: FLD is a common liver disease that affects about 27% urban adult population in China, and affects even higher proportions proportions of adults in Western countries.

Page 7, line 3-4: FLD is a common liver disease that affects approximately 27% of the urban adult population in China [17] and an even higher proportion of adults in developed countries [18].

Comment 5: Did all subjects give informed consent for participation in the study?

Reply: We apologize for the confusion.

All participants were informed verbally about the purpose and design of the study. Written informed consent was not required because of the observational nature of the study. The participant personal information was anonymized at collection and anonymized prior to analysis. We have added this information in our revised manuscript.

Page 4, line 5-9: All participants were informed verbally about the purpose and design of the study. Written informed consent was not required due to the observational nature of the study. The personal information of each participant was anonymized at collection and anonymized prior to analysis. The study was approved by the Ethics Committee of the First Affiliated Hospital, College of Medicine, Zhejiang University.

Comment 6: All abbreviations should be explained in the text (HDL-C, PIVKAII) and in each table/figure (IQR).

Reply: Thank you very much for this suggestion!

We have explained the abbreviations in each table/figure in the revised manuscript.

Comment 7: I would recommend using the median value instead of the mean for the age.

Reply: Thank you very much for this suggestion! We used median value for the age in the revised manuscript.

Page 4, line 4-5: with a median (interquartile range) age of 46.0 (39.0–53.0) years…

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,

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