March 12, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 9278-manuscript.doc).

Title: Circulating MicroRNAs in Patients with Non-Alcoholic Fatty Liver Disease

Author: Mehmet Celikbilek, Mevlut Baskol, Serpil Taheri, Kemal Deniz, Serkan Dogan, Gokmen Zararsız, Sebnem Gursoy, Kadri Guven, Omer Ozbakır, Munis Dundar, Mehmet Yucesoy

Name of Journal: *World Journal of Hepatology*

ESPS Manuscript NO: 9278

We lacked the inclusion of the simple steatosis patients due to the limited source of funding. Despite the certain limitations, we consider this study as a pioneer that may guide the following researchs on this occult issue. Among our future projects, we are planning to do additional research on miRNA measurements and the inclusion of the steatosis patients with a larger cohort, all of which we lacked due to the limited source of funding. In this sense, we appreciate if you could consider our revised manuscript for publication in World Journal of Hepatology. The manuscript has been improved according to the suggestions of reviewers showed all changes in black bold.

1 Format has been updated
2-Revision has been made according to the suggestions of the reviewers;
Reviewer 1:
Comment 1: This excellent human study showed a possibility that biopsy could be omitted in diagnostic procedures for NAFLD. However, clinicians' largest interest is how to differentiate between NASH and simple steatosis without biopsies. The authors excluded patients with simple steatosis from the present study. I think that simple steatosis should have been created as the third group, in addition to NASH and normal controls.
Answer 1: The reviewer is right. We lacked the inclusion of the simple steatosis patients due to the limited source of funding. We have mentioned this constraint in the limitations at discussion section.

Reviewer 2:
Comment 1: The article of Celikbilek et al. "Circulating MicroRNAs in Patients with Non-Alcoholic Fatty Liver Disease" is interesting with a remarkable presentation. The search of new non-invasive methods which can clinicians used as alternative to liver biopsy has emerged in recent years. Authors give interesting data which related to diagnostic accuracy assessment of miRNAs for NAFLD. But there are some minor revisions in manuscript which should be corrected beforehand. Author should carefully read list of statistical procedures. For example your didn't used multivariate logistic regression analyses, one-way analysis of variance or the Kruskal-Wallis test but give the descriptions of this tests in sections 'Materials and methods'. Please give the clarification for method which you used for ROC-curve comparison analysis. Also will be grateful if you give for journal readers one more table with correlation analysis between miRNAs and NAS-scale parameters (etc. steatosis, fibrosis).
Answer 1: Thanks for your instructive comments. Materials and methods section was re-evaluated and corrected. The method for ROC-curve analysis was included in *Statistical analysis section.* Correlation analysis between miRNAs and NAS-scale parameters were mentioned at table 3.

Reviewer 3:

Comment 1: The manuscript entitled Circulating microRNA in NAFLD patients treats a very interesting subject in current clinical practice, such as non invasive diagnosis of NAFLD. It has a very thorough explanation of clinical relevance of each microRNA, and very detailed methods description. The conclusions are well stated and clear as to the utility of results. As minor revisions, the paper states NAFLD is compared with controls, however this is not accurate, since only NASH patients were included. Since the most important feature in NAFLD diagnosis is to differentiate between fatty liver and NASH, both this groups should be represented. A curious fact is that despite all patients had NASH diagnosis; they had normal blood pressure, fasting blood glucose and HOMAIR. Since this is rare in NASH patients, perhaps some information regarding group selection would be useful.

Answer 1: The reviewer is right. Since NASH patients were included, we have changed the term 'NAFLD' as 'NASH' in manuscript where necessary. We are sorry but, we lacked the inclusion of the simple steatosis patients due to the limited source of funding. We have mentioned this constraint in the limitations at discussion section. Our NASH population had normal blood pressure as controls but they significantly differ from controls among fasting blood glucose and HOMA-IR. As we mentioned in study population section, we excluded hypertensive and diabetic patients.

Reviewer 4:

Comment 1: In this paper, Celikbilek et al. analyzed the serum expression profile of some miRNAs in NAFLD, concluding that NAFLD is associated with altered serum miRNA expression pattern. The search of new non-invasive methods which can clinicians used as alternative to liver biopsy has emerged in recent years. Given the goal of achieving a non-invasive method to diagnose NAFLD is a growing concern, the analysis is justified and the aim of the study is clinically relevant. However, the design presents some problems that enhance doubts about conclusion. The most important feature in NAFLD patients is to be able to distinguish those patients showing NASH, because the presence of liver inflammation is related to a worse prognostic, so both groups should be represented.

Answer 1: The reviewer is right. Since NASH patients were included, we have changed the term 'NAFLD' as 'NASH' in manuscript where necessary. We are sorry but, we lacked the inclusion of the simple steatosis patients due to the limited source of funding. We have mentioned this constraint in the limitations at discussion section.

Reviewer 5:

Comment 1: In this study, Celikbilek et al address that Circulating MicroRNAs in Patients with Non-Alcoholic Fatty Liver Disease. The authors demonstrated that serum levels of miR-181d, miR-99a, miR-197 and miR-146b were significantly lower in biopsy proven NAFLD patients than in the healthy controls. Serum levels of miR-197 and miR-10b were inversely correlated with degree of inflammation and miR-181d and miR-99a were inversely correlated with serum gamma glutamyl transferase levels in NAFLD patients. NAFLD is associated with altered serum miRNA expression pattern. This study gives clues to define non-invasive diagnosis of NAFLD. COMMENTS Although the study is of potential interest and relevance, there is some space for improvement. In particular, as presented the study looks too descriptive in nature with little mechanistic insights for the observations made. 1. The results are a little few and the author should describe and analysis more data and results. 2. AUROC values of miR-197, mir-146b, mir-181d and mir-99a miRNA's were around 0.75- 0.86. What is the best marker with NAFLD in your study? What value is cut off? 3. What is associated with DM, Chole, TG, NAFLD and mi-RNA? 4. Several studies showed that mi-RNA is a biomarker for NAFLD. What is difference between previous studies and your study. 5. There is minor language polishing.

Answer 1. Results section was modified and prolonged.

Answer 2. Using Youden index, best cut-off values and the related diagnostic measures are given in Table 5.

Answer 3. Correlation analysis between miRNAs and NAS-scale parameters were mentioned in a new table.

Answer 4. The serum expression profile of miR-10b, miR-29a, miR-146b, miR-181d, miR-99a and miR-197 and their relation with histopathology were studied for the first time in the literature in

NAFLD.

Answer 5. The correction of the English content of manuscript has been done by a native English speaker at English editing service of our institution.

3 References and typesetting were corrected

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

Sincerely yours,

Mehmet Celikbilek, MD
Erciyes University Faculty of Medicine
Department of Gastroenterology and Hepatology
Kayseri, TURKEY
drcelikbilek@yahoo.com