Author's response to reviews

Title: Measurement of the total antioxidant response using a novel automated method in subjects with nonalcoholic steatohepatitis

Authors:

Mehmet Horoz (mehmethoroz@yahoo.com)
Cengiz Bolukbas (dcengizbolukbas@hotmail.com)
Fusun F Bolukbas (fusunbol@yahoo.ca)
Tevfik Sabuncu (tsabuncu@harran.edu.tr)
Mehmet Aslan (m.aslan301@mynet.com)
Serpil Sarifakiogullari (serpilsarfaki@yahoo.com)
Necla Gunaydin (g.necla@mynet.com)
Ozcan Erel (erelozcan@harran.edu.tr)

Version: 7 Date: 15 August 2005

Dear Editor, August 15, 2005

Thanks for your attention that you have paid for our manuscript entitled as "Measurement of the total antioxidant response using a novel automated method in subjects with nonalcoholic steatohepatitis (MS: 3288188686365540)". We revised the manuscript according to suggestions of reviewer. The revisions that made are listed below by providing point-by-point response.

Reviewer #1 (Luis A. A Videla)

Major Compulsory Revisions

1. Page 8 (lines 11-18): FFA oxidation is not the only and most important cause of Oxidative stress in the liver of NASH patients. Alternate mechanisms should be included in this paragraph, namely, (i) CYP2E1 induction, (ii) leukocyte infiltration and activation of NADPH oxidase, and (iii) mitochondrial dysfunction involving electron transfer inhibition in the respiratory chain.

Alternate mechanisms that may play role in oxidative stress in the liver of NASH subjects were included at page 8 (line 20-24) of revised version of manuscript, as follows:

* FFA oxidation, CYP2E1 induction, leukocyte infiltration and activation of NADPH oxidase, and mitochondrial dysfunction involving electron transfer inhibition in the respiratory chain increase the production of reactive oxygen species (ROS) such as singlet oxygen, superoxide anion, hydrogen peroxide, and hydroxyl radical.

2. Page 8 (lines 19-22): This paragraph should be re-written to discuss recent data by Videla L.A. et al., Cli Sci 2004;106:261-268.

*This paragraph re-written as follows:

- In a recent study, which was conducted by Videla et al. (29), it has been shown that NAFLD patients with steatosis exhibit a substantial pro-oxidant condition in the liver at early stages of steatosis. This prooxidant condition was observed to occur concomitantly with a significant decrease in hepatic SOD activity, changes involving an overall derangement in the antioxidant status of the liver, with the consequent diminution in the antioxidant capacity of plasma. They also observed that further exacerbation in oxidative stress was associated with CYP2E1 induction in pations with steatohepatitis.

Reference 35 is a book that gives a general view of oxidative stress: replace by Gawrieh S. et al., J Investig Med 2004;52:506-514, which refers to oxidative stress in NASH.

*Reference 35 was removed.

3. Page 9 (lines 5-9): This statement should be shortened and added to the Methods in page 5 (exclusion criteria), and references 36 and 37 must be re-numbered.

*Done.

4. Page 9 (lines 18-19): Authors should update the references on oxidative stress realated parameters in the liver of NASH patients, now available.

*Done.

5. Page 10 (line 6): Reference 41 is not required.

*Removed.

6. Page 10 (lines 7-18): Authors should compare the TAR values reported with the published data using the FRAP (Ferric Reducing Ability of Plasma) index.

*It was done as follows:
- Randox- total antioxidant status (TAS) assay and ferric reducing ability of plasma (FRAP) assay are the widely used colorimetric TAR measurement methods. In the FRAP assay, the reference range of serum TAR is lowest because this assay practically measures nonprotein total antioxidant capacity. However, proteins constitute the main antioxidant component of serum (plasma). The Randox-TAS assay can determine the antioxidative effects of bilirubin, vitamin C, uric acid, polyphenols, and proteins; hence, the reference range for serum TAR is higher than that for the FRAP assay because the antioxidative effect of proteins is accounted for. The novel method is more sensitive for determining the antioxidative effects of bilirubin, uric acid, vitamin C, polyphenols, and proteins. Hence, serum TAC measured by the novel method is higher than those of the Randox-TAS and FRAP assays (20).

Minor Essential Revisions
1- In general, the english needs to be improved and some spelling errors should be corrected. *Done.
2- There are several cited references that are not directly related to the statements made, and they should be replaced: (i) references No. 1, 2, 3, 4, 5, and 7 do not refer to the general features of NASH; replace them by Angulo P, New Engl J Med 2002;346:1221-1231 and Neuschwander-Tetri B.A. et al. Hepatology 2003;37:1202-1219. (ii) The discussion of oxidative stress as a concomitant of NASH is found in Videla L.A. et al., Free Radic. Biol. Med. 2004;37:1499-1507, which can replace Reference No. 14. (iii) Finally, references 15, 19, 35, and 41 are not essential for the work and should be eliminated. *Done.
3- Page 6 (line 5): FOX2 should be defined. In this respect, reference No. 33 does not seem to correspond to the FOX2 assay, whereas reference No. 32 is not cited in the text. *Done.

Discretionary Revisions
1- Correlation coefficients and the significance values may be added to the figures. *Done.

Reviewer#2 (Giulio Marchesini)
1- Previous data on hepatic oxidative stress have already been published (Videla LA, Rodrigo R, Orellana M et al. Oxidative stress-related parameters in the liver of non-alcoholic fatty liver disease patients. Clin Sci (Lond) 2004; 106:261-8.) and also data on circulating oxidative stress are known (Chalasani N, Deeg MA, Crabb DW. Systemic levels of lipid peroxidation and its metabolic and dietary correlates in patients with nonalcoholic steatohepatitis. Am J Gastroenterol 2004; 99:1497-502.) and should be discussed and quoted. *Done as stated above.
2- The Pearson test cannot be used to demonstrate a correlation between a parametric variable (TAR, OSI, or TBARS) and a nominal variable (histology). This correlation should be tested by Spearman correlation analysis. Better, the association should be tested by means of logistic regression, having histology as dependent variable and oxidative stress as independent, corrected for covariates. The association should also be tested the other way round, having oxidative stress above a definite cut-off as dependent variable. *The correlation of fibrosis scores and necro-inflammatory grades with total preroxide level, OSI or TAR were re-analyzed using Spearman correlation test.

Minor Essential Revisions
1- The clinical, demographic and histological data of NAFLD cases are not part of the results. *Done.
2- The discussion should be reduced by 50% without any loss of information. *Discussion section was re-arranged according to suggestions of reviewer#1.
*Manuscript were re-checked for the conformity with the manuscript formatting checklist and re-arranged.

With Best Regards,
Mehmet Horoz, MD.