

Reviewer's report

Title: Higher Plasma Homocysteine in Non-Alcoholic Fatty Liver Disease (NAFLD)

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Reviewer: Anna Remkova

Reviewer's report:

The results of this work indicated that, despite the small number of north-eastern Brazilian patients with NAFLD in this sample, NAFLD is associated with elevated plasma homocysteine (Hcy). The MTHFR C677T and A1298C polymorphisms, which can influence a metabolism of Hcy, did not differ significantly between NAFLD and control groups. Thus, it seems that the genetic factors do not impair the Hcy metabolism in these patients by a crucial way.

Minor Essential Revisions:

Abstract (also Discussion and Conclusion): What is the (possible) explanation for higher plasma Hcy in NAFLD than in controls?

Background:

- It has been reported that Hyperhomocysteinemia (HHcy) alters intracellular lipid metabolism [13]. In this relation, it is stated by the authors, that “these data support the view that increased serum levels of Hcy may be associated with hepatic fat accumulation”. But maybe it is more probable that the impaired hepatic metabolism in NAFLD and other chronic liver diseases can lead to the increase of Hcy (Remkova A, Remko M. Eur J Int Med 2009, 20: 482–486).
- NAFLD – NALFD (not identical abbreviation is used in Background and Results)
- Mutations in MTHFR gene (C677T and A1298C) result in aminoacids substitutions that lead to a decreased enzyme activity, reducing the 5mTHF (explain, please, what is it) availability [16]. The MTHFR C677T and A1298C polymorphisms have been shown to be associated with higher levels of homocysteine, when plasm folate levels are (...not clear) [16,17].

Methods:

- According to Abstract, thirty-five patients diagnosed with NAFLD by means of liver biopsy and forty-five healthy controls were investigated. According to Methods, this study comprised 35 patients with a diagnosis of NAFLD based on liver biopsy findings and 51 healthy subjects, without NAFLD. What is a correct number of controls?

Laboratory Assays:

- “Blood samples were collected after fasting overnight and centrifuged within 60 min to separate plasma, serum and leukocyte cells and stored at – 80°C. The Hcy levels were determined by HPLC with fluorimetric detection [20]”. In this

context, it is not clear, what samples were used (plasma or serum?). What about the way of blood collection and pre-analytical sample treatment?

- Index (BMI) is defined as the individual's body mass divided by the square of his or her height. Which units?

- Statistical analysis (and Table 2): which type of t-test was used?

Results:

- Table 1: Explanation of all abbreviations is absent (in legend).

- In text description, that "Table 2 shows the results of B12 vitamin levels demonstrating a significant difference between patients and controls", p value is useful to be added (p=...). It is useful to describe also the results for folate here.

- The correlation between Hcy and vitamin B12 / folate should be added in Results - with a Figure when correlation between Hcy and vitamin B12 was found.

Discussion:

- The authors state, that "these data support the view that increased serum levels of Hcy may be associated with hepatic fat accumulation". Is there a direct evidence for this statement?

- It is surprising (in relation to a correlation between Hcy and vitamin B12), that increased level of vitamin B12 in NAFLD patients against control was found (see Table 2). What is the explanation for this "paradox" result?

References:

- References are not cited in a uniform form. The older papers are listed.

Quality of written English: Acceptable

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests:

I declare no conflict of interest.