Author's response to reviews

Title: Methylene tetrahydrofolate Reductase (MTHFR) C677T polymorphism and high plasma homocysteine in Chronic Hepatitis C (CHC) infected patients from the Northeast of Brazil.

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Author's response to reviews: see over
Dear Prof.

We are resubmitting the Manuscript : 1601730666548107-R1 entitled: “Methylenetetrahydrofolate Reductase (MTHFR) C677T polymorphism and high plasma homocysteine in Chronic Hepatitis C (CHC) infected patients from the Northeast of Brazil”, by Siqueira ERF, Oliveira CPMS, Muniz MTC, Silva F, Pereira LMHB, Carrilho FJ. The manuscript has been improved according to the suggestions of the reviewers. Please, find bellow our considerations to the reviewer’s comments.

Reviewer #1: Manuscript Number: 1601730666548107-R1

The manuscript entitled on MTHFR polymorphism and high plasma homocysteine in CHC infected patients from the Northeast Brazil was reviewed. The primary aim of the study was investigated the interaction between MTHFR genotype, plasma homocysteine (Hcy), HCV genotype and histopathology of liver, especially steatosis and fibrosis of the liver. The structure of the manuscript was so complicated, and therefore, the authors failed to show the relationship between these parameters mentioned above efficiently. The manuscript included several criticisms, which should be addressed properly.

1) The structure of the manuscript was not proper to address the inter-relationship among CHC genotype, pathological finding of liver, homocysteine concentration and MTHRF genotype. The typical missing ring was in the Table 3. Why didn’t you present plasma Hcy level between the genotypes of MTHFR ? Did your population bearing T allele of MTHFR show higher level of Hcy?

Comments

As suggested by the Reviewer 1, we added some paragraphs in the sections “Results and Discussion” that were underline in the text, explaining better the inter-relationship among CHC genotype, pathological finding of liver, homocysteine concentration and MTHRF genotype. We also added these data in Table 3.
2) The same non adequate presentation was seen in the Table1. Why didn’t you present plasma Hcy level in the groups of patients infected by HCV genotype 1 and 2. Because plasma Hcy level seemed to be key substance to connect MTHFR polymorphism, HCV genotype and steatosis in liver. If plasma Hcy is higher in the patients with HCV genotype 1 and the patients bearing T allele of MTHFR than the other genotypes, the authors can say the interaction among the 4 elements plays an important role to have steatosis in the patients with HCV.

Comments
As suggested by the Reviewer 1, we added some paragraphs in the sections “Results and Discussion” that were underline in the text.

3) In page 5, the last paragraph of the introduction, the authors stated the aim of the present study, but the statement should be changed more definitely.

Comments
As suggested by the Reviewer 1, we added this paragraph in the section Introduction “The aim of the present study was to investigate whether MTHFR C677T polymorphism might play a role in progression of fibrosis and steatosis in hepatitis C patients from Northeast of Brazil and correlate with homocysteine levels according to histological grades of fibrosis and steatosis.”

4) The subject’s break down was unclear, because around 20% patients were missing and 138 patients were adapted to the analysis. The remaining 36 patients were missing by the unknown reason. Please make sure whether or not the final group had selection bias.

Comments
The MTHFR polymorphism was analyzed in all 174 patients, however only in 138 of these patients the serum samples were collected at the time of liver biopsy. Thus, we used 138 serum samples to determine total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglycerides (Tg), alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (γGT), alkaline phosphatase (AP), fasting glucose, fasting insulin.

5) The reason(s) why the patients with T allele showed higher susceptibility of Genotype non-I HCV. Please discuss more.
Comments
As suggested by the Reviewer 1, we added some paragraphs in the section “Discussion” that were underline in the text.

6) The statistical method in the Table 7 and 8 were not suitable to show the evidence, which you want to indicate. Logistic analysis only indicated the OR between two variables, however, we would like to know what element is the mostly contributed variable to have steatosis. Therefore, the authors must utilize multi-regression analysis, in which MTHFR polymorphism, plasma Hcy level and HCV genotype as a independent variables for steatosis as a dependent variable.

Comments
As suggested by the Reviewer 1, we did a statistic review and added this paragraph below in the section “Results” and modified table 7 and 8. “In multi regression analysis no relation were observed among MTHFR polymorphism, Hcy level, HCV genotype and lipid profile as a independent variables for steatosis and fibrosis (Table 6 and 7).”

Reviewer #2: Manuscript Number: 1601730666548107-R1

In the present study, the authors evaluated the interaction of one polymorphism of MTHFR with chronic hepatitis C, in what concerns genotype, degree of steatosis and fibrosis. They found an higher prevalence of TT in genotype non-1 and an association between the MTHFR genotype TT x CT/CC polymorphism and the degree of steatosis and fibrosis in both hepatitis C genotype. This is a well written manuscript, having however some problems, as detailed below.

Criticisms and suggestions:
1) My main criticism regards the finding that is more emphasized, that is the higher prevalence of TT in genotype non-1. Since it is difficult to find an explanation for that fact, the issue should be fully discussed in Discussion.

Comments
As suggested by the Reviewer 1, we added some paragraphs in the section “Discussion” that were underline in the text.

2) I would suggest the authors to focus more on the effect of the polymorphism on fibrosis and steatosis and how it correlates with homocysteine levels. In fact, it seems that if
the polymorphism has an effect, it should be on the severity of chronic hepatitis, on the progression, or on the response to treatment. It would be of much interest having that kind of information (progression, response to treatment), according to the presence of the polymorphism for this group of patients.

**Comments**

As suggested by the Reviewer 1, we added some paragraphs in the section “Discussion” that were underline in the text.

3) The abstract is lacking some important information. In Methods there is no mention to the determination of plasma homocysteine levels. Furthermore, in Conclusions there are statements regarding the higher prevalence of hyperhomocysteinemia in CHC, that have not been mentioned in Results, where we only have information regarding the higher levels of homocystein in the presence of steatosis.

**Comments**

As suggested by the Reviewer 1 and 2, we added some paragraphs in the abstract that were underline in the text and we did a statistic review and added this paragraph below in the section “Results” and modified table 7 and 8. “In multi regression analysis no relation were observed among MTHFR polymorphism, Hcy level, HCV genotype and lipid profile as an independent variables for steatosis and fibrosis (Table 6 and 7).”

Thank you again for your kind consideration of the revised manuscript.

Best regards,

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