

Dr. Jin-Zhou Tang
Science Editor,
World Journal of Hepatology

Dear Dr. Jin-Zhou Tang,

We are pleased to submit our manuscript, entitled: **"Interleukin-6-174G/C polymorphism is associated with a decreased risk of type 2 diabetes in patients with chronic HCV"** for consideration and if approved to be published in the World Journal of Hepatology.

The study was initially submitted to the World Journal of Gastroenterology (Ref 53641). The Editor-in-Chief, Professor Dr. Lian-Sheng Ma, suggested us to submit our paper to the World Journal of Hepatology after we answer and modify the manuscript according to the reviewer's suggestions.

Please, find below the answers to the reviewer's comments. We are also sending the new version of the manuscript. All changes made in the text are underlined.

Best regards,

Luciana Diniz Silva, MD
Corresponding author

Responses to the Reviewer's comments

We are grateful to you for your comments and suggestions.

1. It is already known that IL-6 is associated with any type of inflammation.

R- We agree with you that IL-6 is increased with inflammation. However, there are few and controversial studies evaluating associations between IL-6 polymorphisms and type II diabetes mellitus and we are not aware of studies evaluating the role of *IL6*-174G/C polymorphism in increasing IL-6 concentrations in the risk of type 2 diabetes mellitus in patients with chronic hepatitis C.

2. Explain for the selection of chronic hepatitis patients.

R- This is a prospective study. From March 2017 to July 2019, we consecutively included 245 adult patients who attended, Brazil. All included patients signed the informed consent form. The diagnosis of hepatitis C was performed by the presence of serum antibodies to HCV and confirmed by the HCV–RNA test.

3. Why the controls number is less than cases?

R - During the inclusion of the patients, we recruited asymptomatic blood donors who agreed to participate in the study. Blood donors were screened for viral hepatitis, HIV, syphilis, Chagas' disease and only those (n=179) who neither had infectious diseases nor hepatic enzymes above the reference values were included in this study. It has to be emphasised that we adjusted the data for confounding variables in logistic regression models.

4. Is this study included children?

R- In the present study, we included only adult patients. We added the information in the first paragraph of the session "Participants", page 10 of the manuscript.

5. What is the name of the instrument used to analyse liver function tests.

R- The liver function tests were evaluated by using Vitros® 5600 (Ortho Clinical Diagnosis, Raritan, NJ). We added the information to the first line, page 13 of the manuscript.

6. There are some articles which showed the relationship of diabetes and inflammatory markers (Nayak BS et al). Include it as reference.

R- We agree with you and now we included the study of Nayak & Roberts (2006) in the manuscript (Please, see reference 30).

Regarding the Cross-check report, in the cases of high similarity index between this manuscript and the published ones, the studies are from our research group, which includes master's and doctoral students and collaborators. Our group has been actively studying the relationship of Hepatitis C virus with the host, including immune response, gene polymorphism and extra-hepatic manifestations of the disease such as nutritional status, quality of life, sarcopenia and bone changes. Thus, the criteria of inclusion of the patients, the methods used to diagnosis the infection and its complications are similar. We modified the manuscript in order to diminish the cross-check report.

Previous Cover Letter:

Professor Subrata Ghosh

Professor Andrzej S Tarnawski

Editors - in - Chief,

World Journal of Gastroenterology

Dear Professors,

We are sending the manuscript entitled "Interleukin-6-174G/C polymorphism is associated with a decreased risk of type 2 diabetes in patients with chronic HCV" for consideration and for publication in World Journal of Gastroenterology as an original case control study manuscript. Although, chronic hepatitis C (CHC) is associated with type 2 diabetes mellitus, the mechanisms underlying the modified

glucose metabolism in hepatitis C virus (HCV) infection have not been fully elucidated. Globally, an estimated 422 million adults have diabetes mellitus and around 71 million people are living with HCV infection. Given the high prevalence and the worldwide distribution of these two comorbidities, their association have an utmost impact on public health. Various factors can be involved in this interaction including direct viral effects, and host factors, such as overweight and pro-inflammatory cytokines. Concerning the last factor, increased serum concentrations of IL-6 have been associated with insulin resistance, type 2 diabetes mellitus as well as advanced forms of liver disease in chronic hepatitis C infection. Given the paucity of studies that aimed to evaluate the role played by the pro-inflammatory cytokines genes polymorphisms in this two-way interface, i.e., the relationship linking HCV and type 2 diabetes mellitus, we investigated the frequency of IL6-174G/C (rs1800795) single nucleotide polymorphism (SNP) in CHC patients and in healthy subjects of the same ethnicity. In addition, we also evaluated, by using models of logistic regression, the association between demographic, clinical, nutritional, virological and IL6 genotyping data with type 2 diabetes mellitus in CHC patients. We found that the frequency of type 2 diabetes mellitus, blood hypertension and liver cirrhosis was 20.8% (51/245), 40.0% (98/245) and 38.4% (94/245) in CHC patients, respectively. To the best of our knowledge, our report is the first to demonstrate that GC and GG genotypes of IL6-174 are inversely associated with type 2 diabetes mellitus in patients chronically infected with HCV. In the current study, CHC patients with type 2 diabetes mellitus had lower frequency of IL6-174 genotypes GC and CC compared to those without. In addition, our current data show that neither different HCV genotypes nor viral load was significantly associated with type 2 diabetes mellitus. Therefore, the results of the current study pointed to the role of IL-6 pathway in mediating both liver injury and disturbed glucose metabolism in CHC patients. Furthermore, even in the era of direct antiviral agents (DAAs) that has been causing dramatic changes in the treatment of CHC, the evidences of the current investigation should not be disregarded. Although most of the studies have demonstrated that HCV clearance by DAA treatment reverses or improves the insulin resistance, in a more recent investigation, the authors observed that successful hepatitis C virus treatment among patients with type 2 diabetes significantly reduces glycated haemoglobin (HbA1c) shortly after treatment, but

not for a long time. These evidences aligned with our results reinforce the role played by the immune-mediated mechanisms in the pathogenesis of insulin resistance and diabetes in HCV chronic hepatitis. The challenge remains for forthcoming research to identify potential inflammatory mediators involved in the crosstalk between HCV and the pancreas-liver axis. Moreover, better comprehension of these processes may positively influence the management strategies for decreasing the extra-hepatic manifestations and their negative impact on health status in patients with CHC.

The manuscript has not been submitted elsewhere and not published in any form. All authors have read the manuscript and approved the submission to World Journal of Gastroenterology. All authors contributed significantly to the work. Authors declare no conflict of interests. ID (02860590) - invitation to contribute an article to the World Journal of Gastroenterology.

Best regards,

Luciana Diniz Silva, M.D.

Associate Professor,

Faculdade de Medicina, UFMG - Belo Horizonte, Brazil