January 08, 2015

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 15361-review.doc).

Title: Interferon-λ polymorphisms and response to pegylated interferon in Iranian hepatitis C patients.

Author: Arghavan Haj-sheykholeslami, Maryam Keshvari, Heidar Sharafi, Ali Pouryasin, Khalil Hemmati, Fatemeh Mohammadzadehparjikolaei

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 15361

The manuscript has been improved according to the suggestions of reviewers:

1. Format has been updated.

2. Revision has been made according to the suggestions of the reviewers

(1) Reviewer No. 12216:

- Material and methods are properly described but they should state the histological score they used to categorize liver fibrosis.

It has been added to the text accordingly (Page: 7, Line: 4).

(2) Reviewer No. 53556:

- In the paragraph "Laboratory assessment" the definition of EVR is incomplete/not clear (12 weeks…?).

Twelve weeks was mistakenly omitted from the sentence but it was corrected (Page: 8, Line: 18).

- A more accurate description of fibrosis level is necessary (how many patients were F0, F1, F2…?)

It was changed accordingly (Page: 9, Line: 19).

- In genotype 1 patients, BMI is unexpectedly higher in responders vs non responders: it should be explained and discussed.

I think it is due to the presence of 3 obese individuals with BMI >34 among the responders in HCV genotype 1 group versus no one with BMI >31 among the non-responders. After omitting these 3 from the analysis, the difference lost its significance.

- It is reported that CC genotype had more than 2 times higher viral load than non CC ones. It is also reported that CC genotype is significantly associated to SVR. So that it is not possible that responders had higher viral load than non-responders as reported in Table 2.

This paradox has been reported in other studies some of which were mentioned in this manuscript (Page: 13, Line: 15). In our study though, the baseline HCV RNA level was not associated with the treatment outcome in the multivariate analysis therefore such conflict cannot be reported.

(3) Reviewer No.54275:

TITLE:

- It does not reflect the major contents of the article. The authors were mainly discussing the frequency of the rs12979860 CC and non CC and the frequency of the rs8099917 TT and non TT neglecting the alleles; CT and TT genotypes for rs12979860 and GT and GG genotypes for rs8099917.

According to the journal's instructions the title should be no more than 12 words therefore there was no room for more explanation. Given that this was a clinical study, the focus was on rs12979860 CC and rs8099917 TT genotypes because of their clinical significance in treatment of patients and other genotypes were all referred to as rs12979860 non-CC and rs8099917 non-TT genotypes because of their similar clinical

value.

ABSTRACT

- Interferon-λ (IL28B) is better to be mentioned as the former name is still in use

It is explained in the core tip part and also in the introduction section that INFL polymorphisms are also known as IL28B genotypes since they are located near IL28B gene (page5 and page: 6 line: 18).

- P value is missing and is better to be added.

P value was accordingly added for the only significant relationship (page 4, last line).

- Results were incomplete regarding Interferon-λ polymorphisms.

Appropriate results were added to the abstract and result section (page 4, lines 20, 21 and 24) and (page9, line 15-17).

INTRODUCTION

- The authors have to emphasize the role of rs12979860 C and rs8099917 T alleles and their value in predicting SVR in chronic HCV patients. Some important references were missed and are better to be added.

Eight new references were added. Reference no.4 is a meta-analysis that includes 34 studies and 2 of the newly added references (no.11 and 12) are also meta-analysis studies covering most of the important references in this field.

MATERIALS AND METHODS

- Criteria for chronic HCV infection have to be specified.

It was corrected accordingly (Page: 6, Line: 29).

- Liver biopsy and the scoring system for assessment of the stage of fibrosis is lacking although were mentioned in the results section.

It was mentioned in the material & methods section too (Page: 7, Line: 4).

- "HCV viral load and other blood tests". The authors have to clarify the blood tests that were ordered for the study population.

It was corrected (Page: 7, Line: 16-20).

- Quantitative PCR tests: The authors have to mention that procedures were following the manufacturer's instructions. Also, they did not specify the time interval for conducting such tests.

It was accordingly corrected (Time intervals are given in Page: 7, Line: 18 and the suggested sentence was added in page 7, line26 and first line of page 8).

- IFNL single nucleotide polymorphism: PCR-RFLP is not described in a reproducible way; some important data were missing and has to be added; the type of sample (whole blood, buffy coat,…), method used for DNA extraction,..,, the primers used and the cycling condition in PCR, the restriction endonuclease for each genotype.

The detailed methods for genotyping of rs12979860 and rs8099917 were added (Page: 7, line 30 and page: 8, Line: 1-13).

- Definitions: RVR, EVR, SVR breakthrough and relapse lack their corresponding references.

Corresponding references were added (Page: 8, Line: 26).

- The frequency of the rs12979860 CC, CT and TT genotypes and the frequency of the rs8099917 TT, GT and GG genotypes were not emphasized.

They were mentioned however as noted above since this article was written with a clinical perspective and considering the word count limits it was not further emphasized (Page: 9, Line: 15-17).

RESULTS

- Lack of subheadings within this section

Subheadings were added.

- Results of liver biopsy and assessment of the stage of fibrosis were described in this section without being mentioned in section of materials and methods.

It was mentioned in the material and methods (Page: 7, Line: 4).

- Some data were mentioned not in its proper place; e.g.: “Treatment course had to be withdrawn in one subject due to a suicidal attempt.”

After adding the subheadings the sentences were transferred to their appropriate places.

- SVRA & SVRN are better to be replaced by R & NR denoting responders and non responders.

It was changed accordingly.

- The authors only evaluated subjects with rs12979860 "CC" vs. non-CC genotypes as well as rs8099917 "TT" genotype, vs. non-TT genotypes. Thus missing other alleles; CT and TT genotypes for rs12979860 and GT and GG genotypes for rs8099917.

This comment has been answered in one of the previous responses.

- Table one needs editing revision for the last 2 rows; Genotype 3 and Genotype Both1&3.

Some changes were made but if more changes are needed please point them out more clearly.

- Table two/ Three: P value is missing and has to be mentioned

P values were mentioned in Table 2 for the significant differences.

DISCUSSION

- An overall theoretical analysis of the study core results is poorly covered. A great part of the discussion was dealing with the response rate and genotype which is irrelevant to the study main topic (Mainly the second paragraph).

One of the main targets of this study was to evaluate the response rate in our patients necessitating some discussion about this matter and comparing the results with the findings of other local or international studies. Some paragraphs were also added to talk about the main goal (*IFNL* polymorphisms and treatment response) of our study (Page: 12-13).

- Paragraph 2:" In 2010, Alavian": it is" In 2010, Alavian et al" This has to be corrected Also, citation is missing regarding "In 2011 another Iranian article reported a 77.8% success rate in 216 treatment naïve patients with 83.8% in genotype 3 and 72.6% in genotype 1.". Thus the related reference has to be mentioned here and within the reference section.

Both were corrected accordingly (Page: 11, Line: 27).

- Paragraph 3: It was mentioned that identification of INFL (formerly thought to be IL28B). This statement is better to be interleukin (IL) 28B/interferon (IFN) lambda 3 (λ3) genes. Both are synonymous and in use.

It has been explained in the introduction part that these genotypes are also known as IL28B polymorphism (Page: 6, Line: 18).

- Paragraph 7: "In 2009 Gheorghe reported..." has to be replaced by "In 2009 Gheorghe et al reported..."

It was corrected (Page: 12, Line: 14).

REFERENCES

- PMID is well maintained for all included references except ref.No: 16

PMID is missing because the journal is not indexed in PubMed. The reference you mentioned is the official journal of the Iranian Association of Gastroenterology and Hepatology and is published in Persian.

CONFLICT OF INTEREST STATEMENT

- This study was financially supported by Pooyesh Darou which is the local manufacturer of pegylated interferon alpha-2a in Iran (Pegaferon®) that could be considered as a potential conflict of interest.

Although I think this part was addressed to the editors but some notes worth mentioning; three brands of pegylated interferon are available in Iran from which Pegaferon is produced in Iran and the other two are Roche's Pegasys and PEG-Intron manufactured by Schering company and are imported to Iran from abroad. Among these, Pegaferon and PEG-Intron are covered by the medical Insurances thus seriously limiting the use of Pegasys in our country. Despite insurance coverage, PEG-Intron costs three times higher than Pegaferon, leaving Pegaferon (pretty much without any competition) as the most affordable choice in our country. Although this study was financially supported by this manufacturer, the funder had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

3. References and typesetting were corrected

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

Sincerely yours,

Dr. Maryam Keshvari

Blood Transfusion Research Center, High Institute for Research and Education in Transfusion Medicine, Tehran, Iran

Tell: (+98) 021 66592126

Fax: (+98) 21 66900386

Email: M.keshvari@ibto.ir