Hepatitis Monthly
Volume 24 · Issue 1 · e139309

OPEN PEER REVIEW

Interleukin-6 Gene (-174 G>C) Polymorphisms in Patients with Chronic Hepatitis C Virus; A Case-Control Study

Mitra Ahadi, Mahdieh Khoshakhlagh, Negin Masoudifar, Sina Gerayli, Samaneh Sepahi, Sina Rostami, Zahra Meshkat, Alireza Pasdar

SUBMITTED	REVISED	ACCEPTED	DOI
9 Aug 2023	17 Jan 2024	18 Feb 2024	10.5812/hepatmon-139309

About this report

This open peer review (OPR) report shows the public comments made on this article during peer review, by the reviewers, the associate editor and the editor-in-chief, for every revision round, together with the authors' replies. Comments appear only with the consent of the people who wrote them. Reviewers who chose to sign their report are named; the others are shown as "Referee". Comments are edited for language only, and confidential remarks to the editors are not included.

More about open peer review: brieflands.com/knowledgebase/opr

Contents

1 **Original submission** Referee 1, Referee 2, Associate Editor 2
2 **Revision 1** Referee 2, Associate Editor 4
Authors' reply to reviewers 5
Reviewer and editor comments 10

1 Original submission

3 comments · 15 Nov 2023 – 21 Nov 2023

Referee 1 Reviewer

MINOR REVISION 15 Nov 2023

Please see the comment in the file.

Referee 2 Reviewer

MAJOR REVISION 21 Nov 2023

Dear AE,
The manuscript titled "Association of Interleukin-6 Gene (-174 G>C) Polymorphisms with Chronic Hepatitis C Virus Infection; A case-control study" investigates the potential association between IL-6 gene polymorphisms and chronic hepatitis C virus (HCV) infection. The study employs a case-control design to analyze the genetic variations in the IL-6 gene (-174 G>C) among HCV-infected patients and healthy controls. While the study addresses an important topic, several issues need to be considered and addressed before it can be considered for publication. Below are the major points and recommendations:

The abstract provides a concise summary of the study; however, it lacks key details, such as the methodology and main results. Expanding the abstract to include these elements would be beneficial.
Introduction:

The introduction provides relevant background information about HCV infection and the role of IL-6 in the immune response. However, it could benefit from more context and a clearer statement of the research hypothesis or research question.
Methods:

The methods section is generally well-structured and provides sufficient information to understand the study design and procedures. However, there are some areas that need improvement:
Clarify the criteria for patient and control group selection. For example, specify how HCV infection was confirmed, and provide details about the exclusion criteria.
Describe the demographic characteristics of the control group more comprehensively.
Include information on the primer sequences used for genotyping.
Mention the source of the commercial ELISA kit used for HCV antibody testing.
The statistical analysis section lacks detail. Provide more information about the statistical tests used and how potential confounders were controlled for.
Results:

The presentation of results is clear and concise, but some additional details are needed:
Include the actual number of individuals in each genotype category.
Clarify the adjusted analysis, including which confounders were adjusted for.
Discussion:

The discussion section interprets the results well but lacks a thorough comparison and discussion of the findings in the context of existing literature. Provide a more comprehensive review of relevant studies and their results.

Associate Editor MAJOR REVISION 21 Nov 2023

Thanks to the authors and appreciation to the researchers. The manuscript presents valuable research. But some revisions are needed as requested by the reviewers. Also, minor changes should be made in the abstract:
ABSTRACT:
The study design should be mentioned in the first line of the methods section of the ABSTRACT.

2

Revision 1

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

Hepatitis Monthly

Dear Prof. Seyed-Moayed Alavian

Editor-in-Chief

The authors gratefully acknowledge you for handling the manuscript and thank the reviewers for their comments on earlier version of this paper. I have modified the manuscript according to their valuable comments and your helpful instructions. The changes are highlighted in yellow in the text. The detailed corrections are listed below point by point:

Reviewer 1:

1. I think the title should be revised as the data could not support the relationship between Interleukin-6 Gene (-174 G>C) Polymorphisms and HCV chronic infection, therefore, authors cannot claim the "Association of Interleukin-6 Gene (-174 G>C) Polymorphisms with Chronic Hepatitis C Virus Infection"

Answer: Thanks for your consideration. The title was corrected according to reviewer comment. "**Interleukin-6 Gene (-174 G>C) Polymorphisms in patient with Chronic Hepatitis C Virus; A case-control study**"

2. Use the complete form of odds ratio (OR) before its first use in "The OR for the CC genotype was 3.84 (95% CI 0.71-21.09), which was not significant"

Answer: Thanks for your consideration. We correct according to reviewer comment: in line 18 odds ratio for the first time

3. "Based on our findings, the polymorphism of the -174 G>C position of the IL-6 gene may confer some degree of risk in hepatitis C virus-infected patients" the data for this conclusion was not presented clearly in text

Answer: Thanks for your consideration. We clarify risk associated with HCV: it means several factors associated with -174 G>C position of the IL-6 gene and hepatitis C virus.

4. It would be better that other IL-6 polymorphisms also discussed in brief in the manuscript

Answer: Thanks for your consideration. We discussed other IL-6 gene in different part of introduction such as line 51-54: Different single nucleotide polymorphism (SNP) at positions −597 G/A (rs1800797), −572 G/C (rs1800796), and −174 G/C (rs1800795) of the IL6 promoter have been reported to influence IL6 transcription. The most frequently studied polymorphism is −174 G/C.

Line 59-63: But one investigation have shown the association between -174G/C polymorphism and HCV infection. However, −572C/G, and −597G/A, have been reported to be related to a wide

range of diseases, including cancer, autoimmune diseases and hepatitis. Few studies have been conducted as to the relationship between HCV and IL-6 promoter polymorphisms.

Reviewer 2:

Please see the comment in the file

Q: Flaviviridae was italic.

Answer: Thanks for your consideration. We correct referring to reviewer comment with write in italic *Flaviviridae*

Q: The aim of our investigation: not our

Answer: Thanks for your consideration. We correct according to reviewer comment in line 63: The aim of this investigation was to find out whether one of the main *IL-6* promoter polymorphic positions (-174 G>C) is associated with HCV infection in our population.

Method

Q: So after HBsAg+ how many participant remain in the study???

Answer: According to reviewer comment, the sentence “Afterward, a total of 146 individuals were recruited to partake in the investigation, and subsequently allocated into two groups, consisting of 67 patients diagnosed with HCV and 79 individuals as a control.” was added to method section.

Q: where is the reference of primer? Did you design the primers?

Answer: The forward and reverse primer sequences were selected based on the two references bellow: and added to method section.

1. A Settin, S Nassar, A Abdel-Latif, R Elbaz, S El-Mongy, AA Hassan, and AM Hassan. Association of Cytokine Gene Polymorphism with Susceptibility and Clinical Types of Leprosy. Int J Health Sci (Qassim). 2007 Jan; 1(1): 25–33.
2. Saeed Daneshmandi, Abbas Ghasemi, Ali Akbar Pourfathollah. IL-6 -174G/C Promoter Polymorphism in Acute Appendicitis. LABMEDICINE. Volume 40 Number 10. October 2009.

Discussion

Q: Where is the number? Please write the p value

Answer: thanks for your consideration. The number of P value was 0.024.

Reviewer 3:

The abstract provides a concise summary of the study; however, it lacks key details, such as the methodology and main results. Expanding the abstract to include these elements would be beneficial.

Answer: thanks for your consideration, line 11-13: Expand the result: The C allele was more frequent in HCV-infected patients compared to controls (p<0.05; *odds ratio* (OR) = 0.21, 95% CI: 1.85-3.16) however after adjustment for confounders this association did not remain significant. In addition, in a recessive analysis model of this position (CC vs. GG+GC), CC was more common in HCV-infected patients compared to healthy individuals which were not significant (p=0.21; OR= 2.91, 95% CI: 0.55-15.53). GG polymorphism was the most prevalent genotype in both case and control groups (P=0.44), however CC was the least common (P=0.12).

Introduction:

The introduction provides relevant background information about HCV infection and the role of IL-6 in the immune response. However, it could benefit from more context and a clearer statement of the research hypothesis or research question.

Answer: Thanks for your consideration. We correct the introduction

Methods:

The methods section is generally well-structured and provides sufficient information to understand the study design and procedures. However, there are some areas that need improvement:
Clarify the criteria for patient and control group selection. For example, specify how HCV infection was confirmed, and provide details about the exclusion criteria.

Answer: In this study; the inclusion criteria was individuals HCV patients whose test for HCV infection with enzyme- linked immunosorbent assay kits (Delaware Biotech, Inc.,Wilmington, DE) was positive. HCV viral load test was carried out by real time polymerase chain reaction (PCR) and people with $HBsAg^{+}$ were excluded from the study. This sentence was added to method section too.

Describe the demographic characteristics of the control group more comprehensively.

Include information on the primer sequences used for genotyping.

Answer: HCV genotyping was performed according to the method described by Afshari et al. and Ohno et al. All individuals involved in the current investigation underwent testing for HIV infection, with the outcomes indicating negativities for both the subjects and the individuals serving as representative samples of the general population. In the control group, there were no drug addicts and no tattooed persons, while in the patient group, 30 people were addicts and 21 people respectively. Percentage of transfusion was 41.71 in patients and 10.12 in control groups. This sentence was added to result section too.

Mention the source of the commercial ELISA kit used for HCV antibody testing.

Answer: The source of the commercial ELISA kit (Delaware Biotech, Inc.,Wilmington, DE, USA) was added to the main text.

The statistical analysis section lacks detail. Provide more information about the statistical tests used and how potential confounders were controlled for.

Answer: According to reviewer comment, more details was added to statistical analysis section.

Results:

The presentation of results is clear and concise, but some additional details are needed:
Include the actual number of individuals in each genotype category.

Answer: According to reviewer comment, more details was added to result section. “As shown in Table 2, the frequencies of the GG, CC/GC and CC, GG/GC genotypes among cases were 46.5%, 53.5% and 7% ,93%, respectively. But, in control these genotypes frequencies were 64.6%, 35.4% and 2.5, 97.5% respectively. There was also no significant difference in genotypes frequencies between case and control after adjusted to confounders. The G allele frequency was 81.01% in control and 69.72% in case group, and C allele frequency was in control was 18.99% and in case was 30.28%. The allele frequencies distribution was significant difference between two groups only before adjustment (P=0.024)”.

Clarify the adjusted analysis, including which confounders were adjusted for.

Answer: The confounders were transfusion, tattoo and addiction. This sentences added to result section too.

Discussion:

The discussion section interprets the results well but lacks a thorough comparison and discussion of the findings in the context of existing literature. Provide a more comprehensive review of relevant studies and their results.

Answer: Line 184-200 comparison and discussion of the findings in the context of existing literature. In the study of Ali Deeb et al, whether IL-6 (-174G/C), (-597G/A), and (-572G/C) promoter polymorphisms are associated with serum IL-6 levels in HCV patients. A significant increase was -174GG in group of healthy in comparison to patients group (Deeb, Nasr, Badra, & El Sayed, 2019). In 2010, Falleti et al. conducted a study on patients with HCV and persistently normal transaminases (PNALT) to examine disease progression and documented a link between the high-producer genotype (GG) in IL6 – 174 polymorphism and a more unfavorable progression of HCV infection (Falleti et al., 2010). In 2015, a study investigated an influence of the IL-6 (−174) C allele on the clearance of HCV, without detecting any genetic association between the IL-6 (-174G/C) SNP and susceptibility to HCV infection (Fabrício-Silva et al., 2015).

Minton and colleagues (Minton et al., 2005), along with Park in 2003 (Park et al., 2003) and Ribeiro in 2007 (Ribeiro, Visentainer, & Moliterno, 2007), as well as Migita (Migita et al., 2005), and Chang (Chang et al., 2015) in a meta-analysis, did not find support for the involvement of IL-6 (-174G/C) SNP in HCV clearance. Chang et al.'s meta-analysis specifically showed that the lack of association between (-147G/C) IL-6 polymorphisms and the outcome of chronic HCV infection might be attributed to the near absence of the C allele of -174GC polymorphism in East Asian and South American populations.

2 Revision 1

2 comments · 17 Feb 2024 – 17 Feb 2024

Referee 2 Reviewer

ACCEPT 17 Feb 2024

Dear AE,
I read the manuscript, and the authors corrected the requested items. The article could be accepted at this stage.
Sincerely;

Associate Editor

ACCEPT 17 Feb 2024

Dear EIC.
Previous errors have been corrected, and there are no further recommendations.