OPEN PEER REVIEW

Open Peer Review (OPR)

As a new feature for progressing towards transparency, we decided to open a new window for all of our editors as well as authors titled "**Open Peer Review**". We hope by this new facility, our reviewers will be more motivated and authors will be more satisfied with the review process. We believe that publishing our peer review reports could make a transparent and clear environment for all our efforts within a journal, but not all reviewers tend to publish their comments.

What is "Open Peer Review" process?

An "Open Peer Review" process is making the details of all review process (including reviewers, associate editors, and EICs comments) as "Public" as it is agreed by EIC, Authors, and reviewers.

Advantages of "Open Peer Review" process

- More transparency, constructiveness and tactful comments of the peer review process: leads to an increase in the quality of reviews
- More motivations for all involved roles in the review process
- Authors' satisfactions from the review process: Increases honesty between authors and reviewers
- Education of both authors and new students
- Prevents reviewers from following their individual agendas and leading to the detection of reviewers' conflicts of interests

You can find out more at:
https://sites.kowsarpub.com/kowsar/knowledgebase/category/tree.html#opr.html

Hepatitis Monthly
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Peer Review Report for "Evaluation of microRNA Gene Polymorphisms in Liver Transplant Patients with Hepatocellular Carcinoma"

Author(s): Sahar Dehbidi, Zhaleh Farokhizadeh, Mohammad Hossein Karimi, Afsoon Afshari, Mehrdad Behmanesh, Mohammad Hossein Sanati, Bita Geramizadeh, Ramin Yaghobi

Review Timeline:

Submit Date: 15 Mar 2020
Revised Date: 15 Jul 2020
Accepted Date: 19 Jul 2020

Revision (0)

Here, you can see the **Reviewers**, **Associate Editors** and **EICs'** comments from the beginning to the end of the revision process.

REFEREE: Reviewer | **Revision** (0) 11 Apr 2020

Dear AE,
There are some comments which should be addressed by the author::
- The name of the manuscript is too general to transmit the main idea to readers
- In keywords, the "polymorphism" word should also be included
- Some typing errors detectable in the text that should be omitted: such as "DNPTM kit (Cinna Gene"), A figure displaying the digestion pattern (even a schematic one) is better to include in the manuscript.
- The number of references is too high and needs to be refined
Thanks.

REFEREE: Reviewer | **Revision** (0) 22 Apr 2020

Dear AE,
The present study has studied gene polymorphisms of four microRNA in patients who underwent liver transplantation and compare them with those of the control group. The authors also studied the effects of these polymorphisms on the outcome of liver

transplantation. So the title of the manuscript is overstated and inappropriate. On the other hand, in the background section, mainly the effects of microRNAs on the progression of HCC have reported and there isn't any evidence about the possible role of these genes in liver tissue rejection. It is clear that several variables have effects on liver transplantation outcomes such as surgeon skill or patients disease severity or …. Also, more pieces of evidence are needed to describe the rationale of the selection of these microRNAs.
Thanks.

Jamal Sarvari: Reviewer | **Revision (0)** 22 Apr 2020

Dear AE,
There are some points regarding the manuscript entitle: The pattern of microRNA gene polymorphisms in liver transplanted patients included:
1- Abbreviation and words should be the same in all parts of the manuscript.
For example mir in the sentences:
Discussion
1- The miRNA genes, located in cancer-associated genomic regions can function either as tumor suppressors or oncogenes can lead to malignant transformation (20, 32-34). MiRNAs have a role in introducing and developing
And in miR-146a G>C, miR-499A>G, miR 149C>T and 196a-2 C>T gen
2- The location of some sentences should be changed in the manuscript; for example:
A) This sentence is related to the statistical analysis section but it is bringing in the result section: Armitage's trend test was also used to check the association of genotypes with acute rejection whenever the Hardy-Weinberg equilibrium did not apply.
B) This sentence is related to the study population of the material and method but it is bringing in the DNA extraction part: Tissue samples from HCC patients were selected from the sample bank of Shiraz Transplant Research Center and blood samples of the control group were collected from Shiraz Blood Transfusion Center.
C) And this sentence is related to the result section but it is bringing in the materials and method section: Demographic data of the studied patients are shown in Table 1.
D) Also this sentence that brings in the discussion part recommended being transfer to the introduction part:
Mir 146a G>C rs2910164 polymorphism is located in the stem region opposite to the mature miR-146a sequence. The optimal free energy was decreased for C alleles compared with the G allele, suggesting a less stable secondary structure for the C allele. Pre-microRNAs genetic variation of mir-146 changes the secondary structure and alters the expression of mature miRNAs (29).
3- The Statistical analysis section is too long therefore it should have been shorter.
4- The sequences of primes appear in bout table and text section that should be removed from the text
5- In Table1 with legend: "Demographic characteristics of HCC and liver rejected and non-rejected transplant patients" does not show any demographic characteristic (at least age and gender) and showed only the number of patients groups. The demographic characteristic should be included in the table
6- The 3 paragraphs at the beginning of the discussion should be merging and appear in one small paragraph.

Thanks.

Heidar Sharafi: Associate Editor | **Revision** (0) 26 Apr 2020

Dear EIC,
This manuscript was evaluated by the peer-review team. While I am happy with the novelty of this study, there are technical issues with the study design and results, and presentation of the manuscript. I invite the authors to respond to the reviewers' comments carefully to give them a chance for another round of rigorous peer-review. In addition, I have a few major and minor comments as well:
Major Comments:
1. It is unclear what was the sample obtained from the HCC patients. It was included that authors obtained informed consent from participants, so they could take a blood sample instead of using preserved liver tissue if they intended to evaluate participant's genetics. Moreover, It is not clear if the specimen was the liver of the recipient of the liver of the donor. In my point of view, both can be important in the rejection phenomenon however the genetics of donated liver looks a bit more influencing in terms of rejection. These concerns should be clearly included and discussed as a study limitation considering that only one was evaluated either donated liver or recipient liver.
2. Although I am sure that was very difficult to find 60 HCC patients who underwent liver transplantation, I observe the current sample size as a study limitation in terms of statistical power. Was there any sample size calculation while they were designing the study? Is 60 enough to have the optimum statistical power? The authors talked about the power of tests in "Statistical analysis" however I couldn't find any result for it. Finally, I believe the authors should include it as a study limitation, in case they didn't estimate the sample size.
3. I found another study by the author assessed the association of the same MIRs and HCC and they found few of them to be linked with HCC. I have the main concern, why the authors selected these SNPs for evaluation of liver-transplant rejection. This should be included briefly in the introduction and discussion as well as a response to this concern. I believe, it is a very important issue if the authors have a pre-study hypothesis for inclusion of each SNP considering that liver transplant rejection is an immunologic process apart of hepatocarcinogenesis.
4. I am confused with the statistical analysis for comparisons of genotypes and alleles distribution between the two groups. in each analysis (allele or genotype), one domain (one allele or one genotype) should be included as the reference for calculation of the P-value or OR obtained from the other domains. Without the reference (OR=1) other ORs are meaningless. For genotype analysis, regularly one of the homozygous genotypes which have less frequency in the risk group than the control group is selected as the reference genotype and the other two genotypes can be compared with the reference to obtain an OR and P-value for each. Moreover, I calculated the OR and P for MIR-146G>C using SPSS and resulted in a P-value of 0.071 (Fisher's exact) which is confirmed by the stated 95%CI of OR as included 1 (0.87-6.89). I observed the latter issue for the other P values reported less than 0.05. I expect that when $P<0.05$ the 95%CI of OR should not include 1 (such as 1.2-5.6 or 0.14-0.82).
Minor Comments:

1. The title should be revised to present the study conducted by the authors.
2. I suggest authors to include both rs# (such as rs2910164) and SNP designation (such as miR-146a G>C) once in the first use in each abstract and manuscript body preferably as rs# (SNP designation). In the next uses, they can only include the rs#.
3. The study aim(s) should be clearly included in both the abstract and manuscript body.
4. "Relative risk" should be deleted from statistical analysis.
5. The subgroup analysis by the underlying disease should be deleted from a manuscript in addition to Tables 5 and 6. These are not adding more data.
6. Table numbers should be amended in the manuscript body.
7. Information in Table 2 are duplicated. Primer sequences, fragment sizes, enzymes can remain in the Table and Thermal and PCR mix conditions can be moved to the manuscript body.
8. Table 3 can be deleted.
9. Where are AluI and BclI restriction sites?
10. Data of Table 1, can be deleted from the manuscript body or included briefly with referring to the Table while data of age and gender can be added to this Table.
Regards

Seyed Moayed Alavian: EIC | **Revision** (0) 27 Apr 2020

Dear Author,
The reviewers' comments are mentioned. Please correct the details as mentioned above carefully according to reviewers' request.
Thanks.

OPEN PEER REVIEW

Revision (1)

Reply to Reviewers

Ideally, the reviewing process can significantly improve the submitted manuscripts by allowing the authors to take into account the advice of reviewers. Author(s) must reply to all reviewers' comments in a separate Word file, point by point. A "**Reply to Reviewers**" document is submitted along with revised manuscript during submission of revised files, summarizing the changes that the authors made in response to the reviewers' comments. The responses to reviewers' comments specifies how the authors addressed each comment the reviewers made.

You can read the authors' responses to the reviewers' comments in the next page.

Hi Dear Prof. Seyed-Moayed Alavian

The Editor in Chief of Hepatitis Monthly

We tried to response to all reviewer comments of manuscript with No. 102690 and incorporate correction highlighted with yellow colour throw all parts of manuscript and sent the revised manuscript and response to reviewers in separate files.

Reviewer 1:

1- Abbreviation and words turned the same in all part of the manuscript.
2- The location of mentioned and recommended sentences changed in the text of manuscript.
3- The Statistical analysis section was summarized.
4- Duplicate presentation of molecular methods in both text and Table 2 was removed.
5- Table1 was completed with more demographic characteristics.
6- Paragraphs in the beginning of discussion were merged.

Reviewer 2:

1- The title of manuscript was revised.
2- Keyword "polymorphism" was included.
3- Mentioned typing errors were corrected in the text.
4- Digestion pattern (fragment size) of each SNP was included in Table 2.
5- The number of references was refined.

Reviewer 3:

1- The title of manuscript was revised.
2- Documents about the possible role of microRNAs in liver tissue rejection were not exist till now and based on earlier reports announced the importance of microRNAs genetic polymorphisms in HCC outcomes that included more in the background section, we hypothesize and evaluate this importance for the first time in liver recipients with HCC experiencing graft rejection.

Associate Editor's Comments:

Major Comments:

1- The FFPE liver tissue samples of transplant recipients with HCC were selected from Namazi hospital sample bank and evaluated retrospectively.
2- Limitations about sample size and type, and also time of sample collection were included in the end of the discussion.

3- This study was hypothesized and designed in continuing of our and other earlier studies emphasized on the importance of microRNAs genetic polymorphisms in HCC outcomes. Based on earlier facts and reports added to the text of introduction and discussion, studied microRNAs SNPs are the most determinative SNPs in microRNA regulation and function we think need to evaluate in livre tissue rejection.

4- The statistical analysis was re-checked and revisions were included in the text and tables.

Minor Comments:

1- The title of manuscript was revised.

2- The writing format of SNPs was uniformed throw all parts of the text.

3- The study aims were uniform and clear in both abstract and manuscript body.

4- The Relative risk" deleted from statistical analysis.

5- Tables 5 and 6 were deleted.

6- The table numbers corrected in the manuscript body.

7- Duplicate presentation of molecular methods in both text and Table 2 was removed.

8- Table 3 was deleted.

9- The missing restriction sites were added.

10- Table 1 was completed with more demographic characteristics.

Sincerely

Dr.Yaghobi

OPEN PEER REVIEW

Revision (1)

Here, you can see the **Reviewers**, **Associate Editors** and **EICs'** comments from the beginning to the end of the revision process.

Heidar Sharafi: Associate Editor | **Revision** (1) 5 Jun 2020

Dear EIC,
The authors addressed most of the raised comments and concerns however, there are remained points:
1. Regarding using FFPE tissues, I am not convinced yet. While they called the patients for written informed consent, they could sample a blood specimen. Authors can let us know if they have any justification for it.
2. Regarding limitation with sample size, I could not find any statement in the discussion.
3. Regarding the statistical analysis, I would add my previous concern to be considered "I am confused with the statistical analysis for comparisons of genotypes and alleles distribution between the two groups. in each analysis (allele or genotype), one domain (one allele or one genotype) should be included as the reference for calculation of the P-value or OR obtained from the other domains. Without the reference (OR=1) other ORs are meaningless. For genotype analysis, regularly one of the homozygous genotypes which have less frequency in the risk group than the control group is selected as the reference genotype and the other two genotypes can be compared with the reference to obtain an OR and P-value for each."
Regards

Seyed Moayed Alavian: EIC | **Revision** (1) 6 Jun 2020

Dear Author,
Your article is reviewed by the reviewers carefully. According to their comments, this document needs minor revision. Please follow their comments as requested.
Thanks.

OPEN PEER REVIEW

Revision (2)

Reply to Reviewers

Ideally, the reviewing process can significantly improve the submitted manuscripts by allowing the authors to take into account the advice of reviewers. Author(s) must reply to all reviewers' comments in a separate Word file, point by point. A "**Reply to Reviewers**" document is submitted along with revised manuscript during submission of revised files, summarizing the changes that the authors made in response to the reviewers' comments. The responses to reviewers' comments specifies how the authors addressed each comment the reviewers made.

You can read the authors' responses to the reviewers' comments in the next page.

Hi Dear Prof. Seyed-Moayed Alavian

The Editor in Chief of Hepatitis Monthly

Thanks for your valuable recommendations about manuscript with No. 102690. I incorporate correction highlighted with yellow colour throw manuscript.

1- This study was retrospective and we interested to collect both blood and tissue samples from all participants. However, after calling patients to written and complete informed consent, we could collect blood samples from only some limited patients and we revise the project to focus on only FFPE samples, perforce.
2- A paragraph describes limitations of study was added in the end of discussion.
3- This notification was concerned and the reference genotypes was marked and determined for each studied miRNA genes (Tables 3, 4).

Sincerely

Dr.Yaghobi

OPEN PEER REVIEW

Revision (2)

Here, you can see the **Reviewers**, **Associate Editors** and **EICs'** comments from the beginning to the end of the revision process.

Heidar Sharafi: Associate Editor | Revision (2) 9 Jul 2020

Dear EIC,
I reviewed the revised manuscript. The remained concerns addressed by the authors however, I am not yet happy with Tables 3 and 4.
1. Each domain and data should be inserted in a single cell such as GG in one cell, CG in one cell, 74 (61.7) in one cell, 33 (27.5) in one cell and etc. This will clarify the information in the table to let the production team construct it in final version. They should use cells, rows, and columns to construct their tables.
2. Some 95% CI of ORs are (upper limit - lower limit) such as (1.54-0.34). These should be corrected to (lower limit - upper limit).
3. One allele should be designated as a reference as well.
4. The reference allele or genotype does not need P-value.
5. Some data are presented as N (%) and some as (%) N. One format is needed.
Regards

Seyed Moayed Alavian: EIC | Revision (2) 10 Jul 2020

Dear Author,
Your article is reviewed by the reviewers carefully. According to their comments, this document needs minor revision. Please follow their comments as requested.
Thanks.

OPEN PEER REVIEW

Revision (3)

Reply to Reviewers

Ideally, the reviewing process can significantly improve the submitted manuscripts by allowing the authors to take into account the advice of reviewers. Author(s) must reply to all reviewers' comments in a separate Word file, point by point. A "**Reply to Reviewers**" document is submitted along with revised manuscript during submission of revised files, summarizing the changes that the authors made in response to the reviewers' comments. The responses to reviewers' comments specifies how the authors addressed each comment the reviewers made.

You can read the authors' responses to the reviewers' comments in the next page.

Hi Dear Prof. Seyed-Moayed Alavian

The Editor in Chief of Hepatitis Monthly

Thanks for your recommendations about tables 3 and 4 of manuscript with No. 102690. I incorporate correction highlighted with yellow colour.

Sincerely

Dr.Yaghobi

OPEN PEER REVIEW

Revision (3)

Here, you can see the **Reviewers**, **Associate Editors** and **EICs'** comments from the beginning to the end of the revision process.

Seyed Moayed Alavian: EIC | **Revision** (3) 19 Jul 2020

Dear Author,
The document is reviewed carefully by the reviewers. According to their comments and your revisions as requested, this manuscript is accepted.
Thanks.