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Plasma circulating microRNAs in patients with stable coronary artery disease - Impact of different cardiovascular risk profiles and glomerular filtration rates

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1st Editorial decision
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Ref.: Ms. No. JCTRes-D-21-00018
Plasma circulating microRNAs in patients with stable coronary artery disease – impact of different cardiovascular risk profiles and glomerular filtration rates
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Dear Mr Lapšovs,

Reviewers have now commented on your paper. You will see that they are advising that you revise your manuscript. If you are prepared to undertake the work required, I would be pleased to reconsider my decision.

For your guidance, reviewers' comments are appended below.

If you decide to revise the work, please submit a list of changes or a rebuttal against each point which is being raised when you submit the revised manuscript.Also, please ensure that the track changes function is switched on when implementing the revisions. This enables the reviewers to rapidly verify all changes made.

Your revision is due by Mar 30, 2021.

To submit a revision, go to https://www.editorialmanager.com/jctres/ and log in as an Author. You will see a menu item call Submission Needing Revision. You will find your submission record there.

Yours sincerely

Michal Heger
Editor-in-Chief
Journal of Clinical and Translational Research

Reviewers' comments:

Reviewer #1: The authors submit an orignial research paper entitled "Plasma circulating microRNAs in patients with stable coronary artery disease - impact of different cardiovascular risk profiles and glomerular filtration rates". They looked at the levels of plasma circulating microRNA-126, -145, and -155 in patients with stable coronary artery disease, different cardiovascular risk profiles and different glomerular filtration rates. Plasma circulating miRNA-126 levels were increased in patients with severe atherosclerosis as determined by the SYNTAX score. Elevated miRNA-155 expression was observed in patients with stage 1 GFR but was lower in patients with stage 2 and 3 GFR. Plasma circulating miRNA- 155 had positive correlations with higher levels of BMI, HOMA index, C-peptide and triglycerides.
The study is interesting although it is dealing ith a small cohort (40 patients), and the patients in the various CKD stages are sometimes too few to reach unambiguous conclusions. Results are sometimes confirmatory of other previous publications that should be cited. For example, Fourdinier et al (Sci Rep 2019) looked at plasma levels of mIR-126 in a cohort of more than 600 patients at various CKD stages with cardiovascular outcomes. Result son miR-145 and miR-155 palsma levels in CVD diseases are summarized in Maitrias et al ATVB, 2017. They should be mentioned in discussion.
As the glomerular filtration rate (GFR) was calculated by the MDRD and CKD-EPI formulas, it should be stated as estimated GFR, not GFR.
Concerning RNA extraction, what sort of Plasma (or serum) was used?
Justify use of cel-miRNA-39 as a spike-in control (see ROberts et al, PLOSONE 2014). What controls were performed to show that RNA was not degraded?
Concerning the 2-ΔΔCT method, what was compared to what, exactly?
Please give the exact number of patients for each CKD stage.
In graph 2, error bars should be provided

Authors' response

Questions from Reviewer (I answer them here and also all answers are included in the latest manuscript version):

- The study is interesting although it is dealing with a small cohort (40 patients), and the patients in the various CKD stages are sometimes too few to reach unambiguous conclusions. Results are sometimes confirmatory of other previous publications that should be cited. For example, Fourdinier et al (Sci Rep 2019) looked at plasma levels of mIR-126 in a cohort of more than 600 patients at various CKD stages with cardiovascular outcomes. Result son miR-145 and miR-155 plasma levels in CVD

diseases are summarized in Maitrias et al ATVB, 2017. They should be mentioned in discussion. - **Included in the discussion part.**

- As the glomerular filtration rate (GFR) was calculated by the MDRD and CKD-EPI formulas, it should be stated as estimated GFR, not GFR. - **Changed**.
- Concerning RNA extraction, what sort of Plasma (or serum) was used? - **We used venous blood circulating miRNA.**
- Justify use of cel-miRNA-39 as a spike-in control (see Roberts et al, PLOSONE 2014). What controls were performed to show that RNA was not degraded? Concerning the 2-ΔΔCT method, what was compared to what, exactly? **Fasting venous blood samples in EDTA-containing tubes were taken before PCI. To isolate plasma, we used rapid centrifugation for 20 min at 4°C. The supernatant was stored at –80°C in RNAse/DNAse-free tubes. The MiRNeasy Serum/Plasma kit protocol (Qiagen, Valencia, CA) was used to obtain total RNA containing miRNAs. Before the RNA purification process, cel-miRNA-39 was added as a spike-in control to avoid differences in template quality and confirm the efficiency of the reverse transcription reaction and normalize the relative Ct values of miRNA-126, miRNA-145 and miRNA-155 in subsequent data analyses. Cel-MiRNA-39 was used as a spike-in control since it has no mammalian homologue [14]. A TaqMan microRNA Reverse Transcription kit (Applied Biosystems, CA, USA) was used to perform reverse transcription. TaqMan miRNA assay kits (Applied Biosystems) were used for miRNA amplification, and real-time polymerase chain reaction (RT-PCR) was performed to detect miRNA-126, miRNA-145, and miRNA-155 expression. For further data analysis, the relative expression levels of miRNAs were calculated using the comparative delta *Ct* (threshold cycle number) method (2-ΔCT) implemented in the RT-PCR System software. The relative *Ct* for cel-miRNA-39 was used as a control in the normalization of miRNA-126, miRNA-145, and miRNA-155 *Ct*.**
- Please give the exact number of patients for each CKD stage. - **Included in Table 1.**
- In graph 2, error bars should be provided. - **Included in the graph.**

2nd Editorial decision
27-Mar-2021

Ref.: Ms. No. JCTRes-D-21-00018R1
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Dear authors,

I am pleased to inform you that your manuscript has been accepted for publication in the Journal of Clinical and Translational Research.

You will receive the proofs of your article shortly, which we kindly ask you to thoroughly review for any errors.

Thank you for submitting your work to JCTR.

Kindest regards,

Michal Heger
Editor-in-Chief
Journal of Clinical and Translational Research

Comments from the editors and reviewers:

Reviewer #1: changes are satisfactory