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PEER-REVIEW REPORT

Name of journal: *World Journal of Translational Medicine*

Manuscript NO: 76831

Title: Cell-free mitochondrial DNA quantification in ischemic stroke patients for non-invasive and real-time monitoring of disease severity and outcome

Provenance and peer review: Unsolicited Manuscript; Externally peer reviewed

Peer-review model: Single blind

Reviewer's code: 05114388
Position: Peer Reviewer
Academic degree: MD

Professional title: Doctor

Reviewer's Country/Territory: China

Author's Country/Territory: India

Manuscript submission date: 2022-04-02

Reviewer chosen by: AI Technique

Reviewer accepted review: 2022-04-07 13:53

Reviewer performed review: 2022-04-16 03:37

Review time: 8 Days and 13 Hours

Scientific quality	[ ] Grade A: Excellent [ ] Grade B: Very good [Y] Grade C: Good [ ] Grade D: Fair [ ] Grade E: Do not publish
Language quality	[Y] Grade A: Priority publishing [ ] Grade B: Minor language polishing [ ] Grade C: A great deal of language polishing [ ] Grade D: Rejection
Conclusion	[ ] Accept (High priority) [ ] Accept (General priority) [ ] Minor revision [Y] Major revision [ ] Rejection
Re-review	[Y] Yes [ ] No

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Peer-reviewer statements	Peer-Review: [Y] Anonymous [ ] Onymous Conflicts-of-Interest: [ ] Yes [Y] No

SPECIFIC COMMENTS TO AUTHORS

The authors provides a primitive platform for non-invasive and cost-effective diagnosis and prognosis of patients with AIS using circulating cell-free mitochondrial DNA (cf-mtDNA) quantification and validation. I think that this paper may be precious providing useful data to the literature and adding new evidence, but I have some concerns: Major concerns: 1. The inclusion and exclusion criteria for subjects in the manuscript are not clearly expressed, which affects the overall quality of the manuscript, please elaborate. 2. The severity of ischemic stroke needs to be evaluated from multiple aspects. Our commonly used evaluation methods are NIHSS score and mRS score. In addition, we often discuss strokes in the anterior and posterior circulations separately. In this manuscript, I do not see a description related to it. If the authors have conducted research in this area, please specify.

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Title: Cell-free mitochondrial DNA quantification in ischemic stroke patients for non-invasive and real-time monitoring of disease severity and outcome

Provenance and peer review: Unsolicited Manuscript; Externally peer reviewed

Peer-review model: Single blind

Reviewer's code: 05368193
Position: Peer Reviewer
Academic degree: MD

Professional title: Doctor

Reviewer's Country/Territory: China

Author's Country/Territory: India

Manuscript submission date: 2022-04-02

Reviewer chosen by: AI Technique

Reviewer accepted review: 2022-05-11 02:58

Reviewer performed review: 2022-05-14 14:28

Review time: 3 Days and 11 Hours

Scientific quality	[ ] Grade A: Excellent [ ] Grade B: Very good [ ] Grade C: Good [Y] Grade D: Fair [ ] Grade E: Do not publish
Language quality	[Y] Grade A: Priority publishing [ ] Grade B: Minor language polishing [ ] Grade C: A great deal of language polishing [ ] Grade D: Rejection
Conclusion	[ ] Accept (High priority) [ ] Accept (General priority) [ ] Minor revision [Y] Major revision [ ] Rejection
Re-review	[ ] Yes [Y] No

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Peer-reviewer statements	Peer-Review: [Y] Anonymous [ ] Onymous Conflicts-of-Interest: [ ] Yes [Y] No

SPECIFIC COMMENTS TO AUTHORS

In this prospective article, the authors attempted to investigate the role of cf-mtDNA in determine severity and outcome in ischemic stroke patients, but there are many problems that need to be solved. (1) Intravenous administration of tissue plasminogen activator (TPA) is the gold standard treatment for acute ischemic stroke within window period, but not all patients benefit from this treatment, and a small number of patients even get worse because of the use of TPA. I want to know the effect of treatment with TPA on these patients. It is important because different treatment results may have a great impact on detection indicators, such as cf-mtDNA concentrations and relative ND1 expression levels in your study. (2) ROC analysis can't describe the correlation between two variables or two groups, but can often be used to evaluate the diagnostic and discriminating efficiency for diagnostic test. Sentences such as "ROC analysis for cf-mtDNA concentration between control and disease at onset showed significant association with almost linear response","The ROC analysis for cf-mtDNA concentration between disease at onset and 24hrs of treatment showed significant association with 65.84% sensitivity and 55.12% specificity", and so on, are not accurately expressed in my opinion. (3) "patients after 24hrs of treatment didn't show significant difference with patients with onset. Similarly, no significant difference was observed between 24hrs and 72hrs of treatment". Since the intergroup comparison has shown no significant difference, ROC analysis seems to be of little significance. (4) Section "Intergroup analysis and diagnostic significance of cf-mtDNA concentration" is confusing and inconsistent with the Figure, which needs to be clearly explained. (5) Sentences "Moreover, the values of relative expression of ND1 were comparable in patients at 24hrs (0.4474±0.4784 vs

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0.9790±0.2605) and 72hrs (0.4474±0.4784 vs 1.105±0.03871) of treatment with control individuals ($p>0.05$)" should not be placed in this section, because the comparative analysis of relative expression of ND1 at 24hrs and 72hrs with control individuals has been described in the previous paragraph. (6) Sentences"While, no diagnostic significance of ND1 relative expression values was observed between patients at disease onset and 72hrs of treatment and represented only 51.14% sensitivity and 50.28% specificity" is not consistent with the previous expression, and the description of the related figure also should be changed. (7) The r value is -0.82 instead of -0.62 in the correlation analysis of circulating cf-mtDNA concentrations between 24hrs and 72hrs of treatment. (8) Why are there two values 0.867 and 0.863 between onset and 24hrs of treatment in the correlation matrix analysis of relative expression levels of ND1? (9) You don't correlate the patient's disease status including severity and outcome with the quantification of cf-mtDNA and don't apply some research tools for clinical neuroscience such as NIHSS, so you can't convince me to believe the role of cf-mtDNA in determine the severity and outcome in ischemic stroke patients.

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RE-REVIEW REPORT OF REVISED MANUSCRIPT

Name of journal: *World Journal of Translational Medicine*

Manuscript NO: 76831

Title: Cell-free mitochondrial DNA quantification in ischemic stroke patients for non-invasive and real-time monitoring of disease severity and outcome

Provenance and peer review: Unsolicited Manuscript; Externally peer reviewed

Peer-review model: Single blind

Reviewer's code: 05368193
Position: Peer Reviewer
Academic degree: MD

Professional title: Doctor

Reviewer's Country/Territory: China

Author's Country/Territory: India

Manuscript submission date: 2022-04-02

Reviewer chosen by: Li-Li Wang

Reviewer accepted review: 2022-06-27 16:42

Reviewer performed review: 2022-06-28 16:46

Review time: 1 Day

Scientific quality	[ ] Grade A: Excellent [ ] Grade B: Very good [Y] Grade C: Good [ ] Grade D: Fair [ ] Grade E: Do not publish
Language quality	[ ] Grade A: Priority publishing [Y] Grade B: Minor language polishing [ ] Grade C: A great deal of language polishing [ ] Grade D: Rejection
Conclusion	[ ] Accept (High priority) [ ] Accept (General priority) [Y] Minor revision [ ] Major revision [ ] Rejection
Peer-reviewer	Peer-Review: [Y] Anonymous [ ] Onymous

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statements	Conflicts-of-Interest: [ ] Yes [Y] No

SPECIFIC COMMENTS TO AUTHORS

The authors have done a lot of laboratory work for this study and have revised the article carefully, which is commendable. Section "Correlation of NIHSS score at baseline with cf-mtDNA concentration and relative ND1 expression" is added according to the suggestion of peer-reviewers, however, authors don't correlate the outcome of patients to cf-mtDNA quantification for some reasons, so I suggest that authors should make appropriate changes to the title and other parts of the article. And there are some minor errors that need to be corrected: (1) It should be "as a non-invasive" instead of " as s non-invasive" in the aim section, (2) Is there any mistake in the description of temperature in sentence "The RT-qPCR conditions were 94oC for 3 minutes, 94oC for 30 s, 54oC for 30 s and 72oC for 30 s for 40 cycles along with melt-curve analysis"? (3) The cf-mtDNA was not quantified at 48hrs, so the word "48hrs" in the section "2.3.3 correlation analysis" should be deleted, (4) "Sensitivity" should be added after "82.66%" in the last sentence in the section "4.3 Discriminative analysis and diagnostic significance of relative ND1 expression levels", (5) The numbers in the table of Figure 5 should be aligned, (6) Article format, spacing, punctuation marks, spelling errors, abbreviations, P value, AUC value, MD value, CI value should be reviewed wholly.