Peer-review report & answers

Authors thank the reviewers for positive comments.

Reviewer #1:

Comment:

1- Title: Diffusion-weighted Magnetic Resonance Imaging(MRI) and micro-RNA in diagnosis and staging of hepatic Fibrosis in chronic Hepatitis C…….must add…….. Diffusion-weighted Magnetic Resonance Imaging (MRI) and micro-RNA in diagnosis and staging of hepatic fibrosis in chronic hepatitis C virus infection

Answer 1:

***Authors thank the reviewers for positive comments. The revised title was reviewed and added (virus infection).**

2- INTRODUCTION = Early detection of hepatic fibrosis has important for HCV…….??? =, with variable but imperfect in dependent diagnostic accuracy in staging……..???

Answer 2:

- Early detection of hepatic fibrosis has important for HCV…….??? Was corrected to:

***Early detection of hepatic fibrosis has important therapeutic and prognostic implications for HCV-infected patients, since antiviral treatment can reduce hepatic decompensation and increase patients ' survival.**

- with variable but imperfect in dependent diagnostic accuracy in staging……..??? Was corrected to:

***with variable diagnostic accuracy in staging of liver fibrosis.**

3-RESULTS =Table (5) shows the cut-off values of ADC, miR used to differentiate early from late fibrosis with area under the, specificity, sensitivity and accuracy……??? Was corrected to

Answer 3:

***Table (5) shows the cut-off values of ADC and miR used to differentiate early from late fibrosis with Areas under the ROC Curves (AUROCs), specificity, sensitivity and accuracy**

4-DISCUSSION =These changes associated with restricted diffusion and lower ADC value….??? = However multicenter studies are recommended upon a large number of patients to increased validity of this study….???

- These changes associated with restricted diffusion and lower ADC value….??? Was corrected to:

Answer 4:

***These changes is responsible for restricted diffusion with low ADC value in patients with hepatic fibrosis.**

- However multicenter studies are recommended upon a large number of patients to increased validity of this study….??? Was corrected to:

Answer 5:

***However multicenter studies are recommended upon a large number of patients to increased validity of this study Therefore, further studies are needed at a larger scale to confirm the results of this work.**

Reviewer #2:

The study by Besheer et al. describes the use of a combinatorial approach of using DWI and miRNA analyses to improve the diagnosis and staging of hepatitis C to reduce the reliance on biopsy. The strengths of this study are the appropriate age and sex matching of controls and providing sensitivity, specificity and accuracy analyses for each metric. Overall, this study is relevant and important to those in the field. That being said, there are aspects the authors should address to improve the quality of their submission:

1) Why are the authors reporting values as medians versus mean values? It would be more appropriate to report mean values with standard error of the mean.

Answer 1:

But when the data are non-parametric, the presentation of data was by median range and our data was nonparametric.

2) In table 1 are the authors certain about the p value Reported? It would appear that albumin should not be significant just quickly looking at the values and error reported.

Answer 2:

- **In vital signs , lab parameters such as HB and albumin always the standard deviation is very small**
- **Minimum difference yields significance difference on comparison**

3) In table 1 are the authors certain about the p value reported?

In the results text the authors state that age was not significantly different but the p value reported is 0.001. I believe the P value on this chart was for differences in early and late fibrosis using a T test rather than using ANOVA for all groups. The authors need to specify but should include p values for the variable itself with all groups and for differences between groups. Also there was no title for Table 1 provided.

Answer 3:

- **That is correct (P value on this chart was for differences in early and late fibrosis using a T test)**
- **Student t test was done between each two groups**

Table 1 Comparison of demographic and laboratory data in F0, Early fibrosis and Late fibrosis groups

Parameters	**Control (n=82)**	**Early Fibrosis (n= 112)**	**Late Fibrosis (n = 96)**	**P value**
Age	38.3±10.2	34.1±8.9	@#41.4±7.8	0.001
Gender [M:F]	47:35	69:43	60:36	0.8
ALT	36.29±17.24	*52±36.07	@#57.17±36.88	0.001
AST	35±15.71	*49±25.12	@#58±35.12	0.001
Albumin	4.1±0.45	4.2±0.44	@#3.8±0.69	0.001
Bilirubin	0.88±0.48	0.81±0.26	#1.02±0.46	0.001
PCR	95746±10111	*391000±213876	@#254500±129314	0.001
AFP	7.5±1.57	*5.007±2.95	@#10.47±6.78	0.001

Data expressed as mean and standard deviation, F one way annova test was used for Comparison between the three groups.

Student was used to compare between each two groups.

* Significance between control and early.
# Significance between control and late.
@ Significance between late and early

4) Figure 2 and 3 are not referenced in the results section or text of the study. This should be corrected.

Answer 4:

- **It was corrected and cited in our manuscript**

5) When was the blood collected for the serum miR assay? Was this prior or after biopsy as this could influence results.

Answer 5:

- **Blood collected for the serum miR assay just prior biopsy**

6) There are wording and grammatical errors with some impacting the readability of the manuscript. For example, in the introduction, "…with variable but imperfect in dependent diagnostic accuracy in staging" and "Ultrasound elastography used for grading of hepatic fibrosis but it is operator dependent." The authors need to correct these throughout.

Answer 6:

- **The revised article is reviewed by language editor and corrected.**

7) The abbreviation use is not consistent. For example, ADC is used prior to being defined in the text. Micro-RNAs are

sometimes listed as miR and at other times miRNA. The authors should correct throughout.

Answer 7:

it was corrected throughout the text.

Reviewer #3:

In current study,Tarek Besheer et al. try to established an new methods via assess diffusion-weighted MR imaging (DWI) and micro-RNAs (miR) in diagnosis and staging of hepatic fibrosis in patients with chronic hepatitis C, that's a new idea for diagnosis of cirrhosis and the study have good design and also well construction .But ,some issues need consideration:

1. accessibility, only few medical institute could test micro-RNA in routine practice and both micro-RNA and MR are not the criteria of clinical judgment for diagnosis of cirrhosis according general guideline

Answer 1:

- It is a research work
- Combination between imaging and laboratory results in detection and staging of hepatic fibrosis resulting from chronic HCV infection is a valuable method because it is easy and noninvasive
- however if more studies done on large number of patients, the results will be more accurate and could test micro-RNA in routine practice

2. Necessity, there are numbers of new Non-invasive diagnosis index for estimate cirrhosis in recently, so, I don't think this study have potential useful for future.

Answer 2:

- Combination between imaging and laboratory results in detection and staging of hepatic fibrosis resulting from chronic HCV infection is a valuable method because it is easy and noninvasive.
- The available methods to detect and stage fibrosis are either invasive like biopsy or noninvasive like laboratory and imaging but not accurate. The combination of serum markers of hepatic fibrosis (miR 21, 200b and 29b) together with MRI with Diffusion-Weighted Imaging was challenging to introduce new dependable and noninvasive method helping in early detection and staging of hepatic fibrosis and cirrhosis.
- The merits of diffusion MR imaging is sensitive method for assessment and grading of hepatic fibrosis with short examination time and it is not operator dependent and is found in some imaging center. The ultrasound is operator dependent with variable results from machine to other.

3. for a research work, it's have value for published

Answer 3

- **Authors thank the reviewer for positive comments**

Reviewer #4:

- The manuscript is interesting and novel. I just suggest to authors to have their manuscript revised by a native speaker.

Answer:

- **Authors thank the reviewers for positive comments. The revised article is reviewed by language editor and corrected.**

43491 Cross-check report:

Comment:

Diffusion-weighted magnetic resonance imaging and micro-RNA in the diagnosis and staging of hepatic fibrosis in chronic hepatitis C virus infection

Besheer et al: Hepatic fibrosis diagnosis with micro-RNA and MRI

Tarek Besheer, Hatem Elalfy, Mohamed Abd El-Maksoud, Ahmed Abd El-Razek, Saher Taman, Khaled Zalata, Wagdy Elkashef, Hossam Zaghloul, Heba Elshahawy, Doaa Raafat, Wafaa Elemshaty, Eman Elsayed, Abdel-Hady El-Gilany, Mahmoud El-Bendary.

Response: This is our abstract accepted in UEG2018 in Vienna as described in the cover letter and of course

those are the authors of the abstract and the submitted paper upon your request.

Comment: Diffusion-weighted MR imaging and micro-RNA in the diagnosis and staging of hepatic fibrosis in chronic hepatitis C virus infection

Response:This has been changed as follow:

Diffusion-weighted magnetic resonance imaging and micro-RNA in the diagnosis and staging of hepatic fibrosis in chronic hepatitis C virus infection

Comment:

The biopsy was performed 2 months prior to the MR imaging, to avoid artifacts related to early post biopsy changes. The right lobe of the liver was sampled by using a 16-gauge needle. Analgesic was given to 23 patients after biopsy. No major complications after biopsy have been observed. The specimens of 1.2 cm in length or longer were formalin fixed, paraffin-embedded and stained with hematoxylin–eosin, reticulin, and Masson trichrome stain. Pathological analysis was carried out by pathologist who was blinded to MR imaging findings and clinical stage of the CHC. The liver biopsy specimen had to contain at least ten portal tracts to be included in the analysis. The histological stages of hepatic fibrosis were classified according to the criteria of METAVIR score into F0, no fibrosis; F1, portal fibrosis without septa; F2, portal fibrosis with few septa; F3, numerous septa without cirrhosis; and F4, cirrhosis[32].

Response:

The biopsy was conducted at least 60 days before imaging to avoid misinterpretation attributed to early post-biopsy changes. The length of the specimen was not less than 1.2 cm (contain at least ten portal tracts) which was fixed in

formalin, put in paraffin and stained with special stain according to the international criteria for pathological analysis. According to METAVIR score, hepatic fibrosis staging were classified as

Comment:
Table (1) shows the demographic data of patients and controls
Response
Table (1) reveals the characteristics of patients and controls.

Comment

The main findings in this study were as follows: the ADC value progressively and significantly decreases from controls to patients with early fibrosis to those with late fibrosis; the combination of ADC and miR-200b is the best predictor for differentiating patients from controls with high accuracy (96.9%); and the combination of ADC and miR-200b is the best predictor for differentiating early fibrosis from late fibrosis with good accuracy (80.2%).

Response
These sentences have been deleted as it is repetition of the results.
Comment:
restricted diffusion with low ADC values in patients with hepatic fibrosis.
Response
restricted diffusion with reduction in the ADC values in patients with hepatic fibrosis
Comment:
studies by Boulanger et al. and Soylu et al. were not able to find a correlation between the stages of fibrosis and the values.
Response
On the other hand, other studies did not find an association between grading of fibrosis and the ADC values.

Comment:
tissue in the liver in addition to deposition of collagen fibres, fatty infiltration, hepatitis, cell necrosis/apoptosis, inflammatory cell infiltration, which are responsible for.
Response:
The results in our study may be explained by the increase of hepatic connective tissue with accumulation of collagen, fatty tissue, and inflammatory cells which may lead to restricted diffusion with low ADC values in patients with late fibrosis.
Comment:
Moreover, TGF-β stimulates processing of the primary miR-21 precursor into mature miR-21.
Resonse;
Moreover, induction of maturation of primary miR-21 precursor into mature miR-21 occurs via TGF-β.
Comment;
MiR-29b is capable of suppressing HSC activation, production of type I collagen [44] and expression of extracellular matrix genes in HSCs through the transforming growth factor β (TGF-β)/SMAD-CTGF signalling network [19, 45, 46]. Overexpression of miR-29 attenuates collagen and fibrotic matrix accumulation in HSCs through directly targeting genes of interest.
Resonse:

MiR-29b interfere with the process of fibrogenesis via inhibition of HSC activation, production of type I collagen [44] and expression of extracellular matrix genes in HSCs through the transforming growth factor β (TGF-β)/SMAD-CTGF signalling network [19, 45, 46]. Overexpression of miR-29 weaken collagen and matrix deposition in HSCs through interfering with genes of fibrogenesis[46].

Comment:
A few limitations are present in the current study. First, the small number of patients.
Response:
As regard limitations of the current work. First, the small sample size which limits the statistical power.
Comment:

Second, this study used diffusion-weighted MR imaging. Further studies using advanced diffusion modules such as diffusion kurtosis imaging.

Response:

Second, the use of diffusion-weighted MR imaging. Further studies using recent diffusion modules such as diffusion kurtosis imaging and diffusion tensor imaging at 3-tesla will improve the results.

Comment:

an alternative surrogate non-invasive diagnostic tool.

Response:

This study concluded that combining ADC and miRs offers a new non-invasive method for diagnosis and staging of hepatic fibrosis in patients with chronic hepatitis C.