Response Sheet

First of all, I'd like to thank you for your precious time during reviewing this manuscript, and hope the following lines cover all requested issues.

Name of journal: World Journal of Gastroenterology
ESPS manuscript NO: 31642
Title: Validation of a panel serum microRNA as biomarkers for early diagnosis of hepatocellular carcinoma post hepatitis C infection in Egyptian Patients.

## FOR COMMENTS OF THE REVIEWER I:-

In this manuscript, the authors investigated 7 miRNAs to verify if they could be used as prospective biomarkers for HCC related HCV. Sensitivity and Specificity of each miRNA has been demonstrated. This work is of potential interests and significance. The following issues need to be addressed:

1. Why did the author choose 7 miRNAs mentioned in the paper, please give your explanation.
   Answer; as mentioned in the background part (lines 130-131) "miRNAs profiling studies in HCV-induced HCCs compared with controls have found that a number of miRNAs are significantly changed in HCV-induced HCCs [17-19], compared with normal controls."

2. I wonder if the level of serum miRNA will be discordance in different stage of HCC, could the author state the serum miRNA in different stages of HCC?
   Answer; yes and the following figure illustrate that
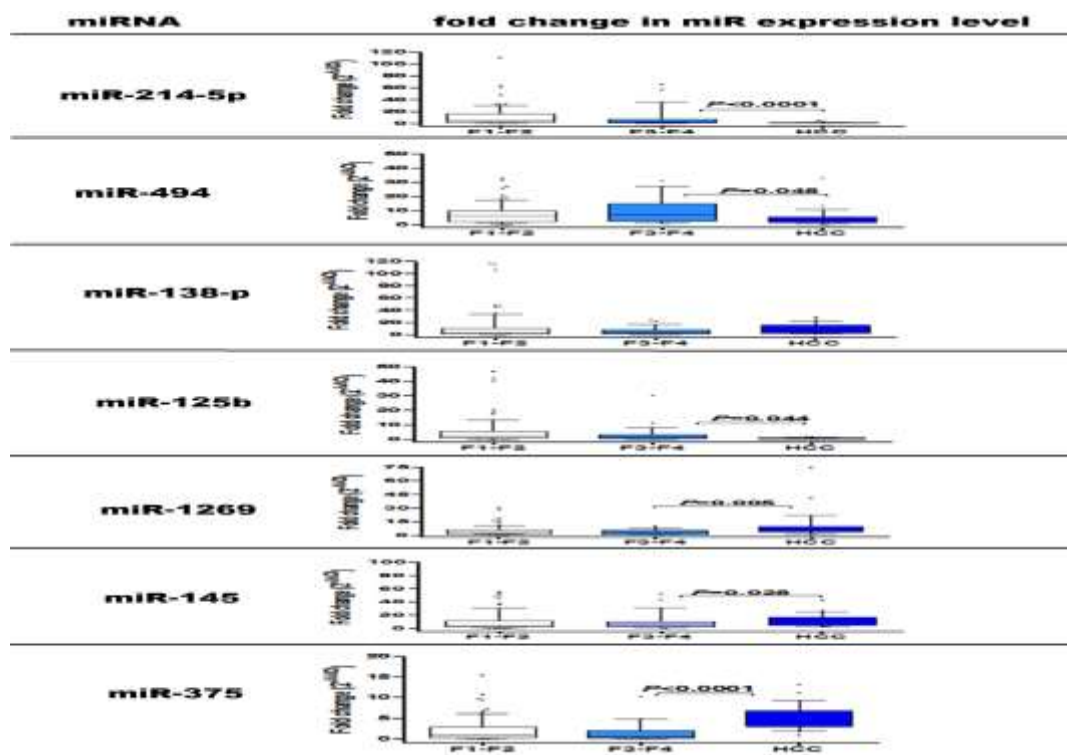
3. <mark>I would suggest giving the reader a standard table, such as omitting redundant vertical moulding…</mark>

Answer; omitting such a data like this may make the reader lack a necessary details for each scoring grade.

4. In Fig 2, the separately graphs can be combined as one picture. There had the same phenomena in Fig 3.

Answer; Both figure 2 and figure 3 images are combined as one picture.

5. Please the author checked the manuscript carefully; eg, "Non-malignant chronic HCV (n = 125)" in Table 1??

Answer; Corrected to 250 (it was a typing error).

6. "Circulating miRNA panel (HCC against early fibrosis)" appeared twice in Fig.4.

Answer; Corrected to early and late fibrosis (it was a typing error).

FOR COMMENTS OF REVIEWERS II:-

Reviewer's code: 00069015
Reviewer's country: China

This manuscript aimed to look for a non-invasive method for early diagnosis of HCV-related HCC. The authors demonstrated that a panel of four serum miRNA (miR-214-5p, miR-375, miR-1269, and miR-125b) that had a significant correlation with HCC and that serum miR-145 monitoring might be a possible diagnosis tool for progression of fibrosis to HCC. The finding is very interesting and valuable. However,

There are several questions which should be concerned by authors.

1. The abstract and introduction parts are a little bit long, the authors should make it briefer.
   Answer; some unnecessary paragraphs have been shortened, we try not to elongate nor make it too short.

2. In the table 1, there might be a mistake about the number of Non-malignant chronic HCV.
   Answer; Corrected from 125 to 250.

3. In the table 1, the meaning of P value is not clear enough, the authors should explain it more specifically.
   - Answer; Illustrated by signs; where Groups with different signs show significant difference (P<0.05), while those with same signs show no significant difference (P>0.05).

4. There are several grammar mistakes in this manuscript.
   Answer; Many grammar mistakes corrected upon revision and the manuscript reviewed by another native language scientific group.

FOR COMMENTS OF REVIEWERS III:-

Reviewer's code: 03536815
Reviewer's country: Italy

I read with great interest the manuscript titled "Validation of a panel serum microRNA as biomarkers for early diagnosis of hepatocellular carcinoma post hepatitis C infection in Egyptian Patients. By MN Elemeery et al. It deals with the advancement of more dependable markers for diagnosis of HCC at early stage and better methodologies for HCC screening and early detection. A collection of little non-coding circulating RNAs associated with HCC in HCV patients have been studied. Authors investigated microRNAs, as prospective biomarkers for HCC in patients with HCV related hepatitis, and found miR-214-5p and miR-1269 as a biomarker for liver fibrosis progression to HCC.

I think the manuscript need extensive major revisions before to considerate it for publication.

1. In the Introduction, Please specify the acronyms FLD and CLD.
   Answer; in line 106; FLD specified (Fatty-Liver disease)
   In line 108; CLD specified (chronic-liver disease).

2. Authors stated: The urinary miRNAs marks found in this study might be of incredible value and applied for diagnosis of HCC early, before the onset of progression in patients infected with HCV. The manuscript does not involve urinary biomarker. Please specify that it is a citation.
   Answer; Corrected to; *"It was reported that the urinary miRNAs might be of incredible value and applied for diagnosis of HCC early, before the onset of progression in patients infected with HCV* [20]*"* (with mention to citation within the text)

3. I suggest to add a brief mention on urinary biomarkers of HCC. This concise review could be helpful. Trovato FM, Tognarelli JM, Crossey MM, Catalano D, Taylor-Robinson SD, TrovatoGM. Challenges of liver cancer: Future emerging tools in imaging and urinary biomarkers. World J Hepatol. 2015 Nov 18;7(26):2664-75.
   Answer; Although it is an important issue to mention but our study does not include any urinary test. So, it seems to be unnecessary elongation in background section.

In the Patients and methods section
1. Please mention the 84 controls.
   Answer; Mentioned in line 152; *"and 84 control (non-infected) patients whom were treated and resorted in clinical hepatology department"*

2. The miRNA selection section is inconsistent, please improve it.
   Answer; Line 166-171 was corrected to be easier to understand.

3. In the statistical analysis do you use Mean or Median?
   Answer; we usually use mean during this study except for fold change (ex., in Differential expression of serum miRNA levels in HCC patients).

In Clinical and pathological Aspects for HCC patients

1. Please check the number and reformulate this sentence, it is not clear: Concerning CT imaging, 112 patient showed one focal lesions in liver, 128 patients of them showed focal size more than 5 cm, with 48 patients with portal vein thrombosis as shown in table (2).

   Answer; Numbers corrected to *"112 patient showed one focal lesions in liver, <u>64 patients</u> of them showed focal size more than 5 cm,"*

- Previously mentioned (128) was total numbers of patients showed focal size more than 5 either to be single or multiple. (It was mentioned by mistake in text, so we correct it as reviewer note).

In the section Serum miRNA profile in HCC patients

2. please specify this sentence, it is not clear: In comparison to HCV patients, miR-1269, miR-214-5p, miR-375 and miR-145 significantly increased in expression fold, whereas miR-125b, miR-138-5p, and miR-494 significantly decreased in HCV groups

   Answer; line 213-221, it corrected to; *"Differential serum miRNA levels were measured in HCC patients compared to healthy control and HCV-infected patients (Mann-Whitney U-test). The result showed that miR-1269 and miR-494 were positively regulated in HCC patients with median fold change (8.71 and 5.45 respectively) comparing to healthy non-infected control, at p<0.0001. While, miR-125b, miR-138b, miR-214-5p, miR-375 and miR-145 were negatively regulated in HCC patients with median fold change (0.51, 0.46, 0.23, 0.25 and 0.6 respectively) in comparison to healthy non-infected control as shown in figure 1."*

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In the Regression analysis for studied miRNA section:

3. Please specify the word "circularibg" used in the AUROC figure 4.
   Answer; It was a typing mistake; replaced by circulating

4. In table 4 please highlight in bold the statistically significant results.
   Answer; Done

The Discussion is confusing.

1. I suggest to mention that miR-1269 and miR-494 are related also to other types of cancer: for example: Bu P, Wang L, Chen KY, Rakhilin N, Sun J, Closa A, Tung KL, King S, Kristine Varanko A, Xu Y, Huan Chen J, Zessin AS, Shealy J, Cummings B, Hsu D, Lipkin SM, Moreno V, Gümü? ZH, Shen X. miR-1269 promotes metastasis and forms a positive feedback loop with TGF-β. Nat Commun. 2015 Apr 15;6:6879.
   Answer; added in line 347-349.

2. In the Discussion please specify the acronyms PV.
   Answer; In line 332; PV specified as portal vein

I think that this manuscript needs extensive improvements.

The reading is difficult. Grammatical corrections and punctuation are needed in the entire manuscript, in order to make the discussion fluent and understandable. There are also some distraction mistakes.   I suggest to require the help of an English native speaker.

Answer; Many grammar mistake has been corrected, and the manuscript passed to another native speaking scientific group to check it.