ANSWERING REVIEWERS

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Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 14478-review.doc).

Title: Assessment of the correlation between serum prolidase and alpha-fetoprotein levels in patients with hepatocellular carcinoma

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The manuscript has been improved according to the suggestions of reviewers:

1 Format has been updated

2 Revision has been made according to the suggestions of the reviewer

Reviewed by 02937214 minor revizyon

Minor points: 1. The ***Reviewed by*** authors should be careful about drawing conclusions of observed results. In Figure 1, the authors just can only get that there is a significant correlation (r=0,616; P<0.001) between prolidase and AFP values in patients with HCC, but can't obtain the conclusion that prolidase and AFP values are related to tumor size, number and BCLC staging classification and macrovascular invasion. Moreover, it's better to show r=0,616; P<0.001 in Figure 1. 2. Please analyze the relationship among serum prolidase levels, AFP levels and lymph node metastasis in table 2. 3. AFP is the most

commonly used serological marker worldwide for diagnosing hepatocellular carcinoma, but the accuracy and specificity of AFP are not high. The authors should detect serum prolidase and L3AFP level in patients with HCC regarding tumor size, number and BCLC staging. 4. The tumor node metastasis (TNM) system classifies can be used for staging of patients with HCC, why does the author prefer Barcelona-Clinic Liver Cancer (BCLC) criteria.

1. Thanks for your comments.We have reanalyzed the relationship between serum prolidase and alpha fetoprotein levels in patients with HCC regarding tumor size, number, macrovascular invasion and BCLC staging according to reviewer's recommendation. The outcomes of the analysis have been showed at the **Figure1a and b.**
2. We have used BCLC staging system to classify the patients with HCC (A: Early B: Intermediate C: Advanced D: End-stage). The stage C of BCLC contains macrovascular invasion or extrahepatic spreads, therefore we emphasized macrovascular invasion instead of lymph node metastasis. However, lymph node metastasis is a main criterion of TNM staging system, it takes a place in extrahepatic spread of HCC in BCLC staging (1,2).
3. However, its diagnostic value is more and more questioned, due to poor sensitivity and specificity, in clinical practice AFP is the most commonly used tumor marker in HCC worldwide (3). Therefore, we prefered AFP to compare with prolidase level instead of L3 AFP. We agree with the reviewer about using L3 AFP. Unfortunately, we are not able to analyze L3 AFP in two weeks that is the given period for us to make the changes in the manuscript.
4. There are several staging systems for HCC such as the Cancer of the Liver Italian Program (CLIP), tumour-node-metastasis (TNM) system, French classification, Okuda classification, Chinese University Prognostic Index (CUPI), Japan Integrated Staging (JIS) and the Barcelona-Clinic Liver Cancer (BCLC) staging system. American Joint Committee on Cancer (AJCC) uses tumour-node-metastasis system as staging system for many malignancy diseases to predict prognosis. Nevertheless, in HCC, AJCC/TNM system fails to stratify patients adequately with respect to prognosis because TNM system evaluates only tumour extension. The BCLC staging system has come to be widely accepted in clinical practice and is also being used for many clinical trials of new drugs to treat HCC. Therefore, it has become the de facto staging system that is used. The Barcelona-Clínic Liver Cancer (BCLC) classification divides HCC patients in 5 stages (0, A, B, C and D) according to preestablished prognostic variables, and allocates therapies according to treatment-related status. Thus, it provides information on

both prognostic prediction and treatment allocation. Prognosis prediction is defined by variables related to tumor status (size, number, vascular invasion, N1, M1), liver function (Child–Pugh's) and health status (ECOG). Treatment allocation incorporates treatment dependant variables, which have been shown to influence therapeutic outcome, such as bilirubin, portal hypertension or presence of symptoms-ECOG. This classification uses variables related to tumor stage, liver functional status, physical status, and cancer-related symptoms, and links the stages described with a treatment algorithm. The BCLC classification aims to incorporate prognosis estimation and potential treatment advancements in a single unified proposal. It has been suggested that it is best suited for treatment guidance, and particularly to select early-stage patients that could benefit from curative therapies. It may be applied to the majority of HCC patients, although individual cases may warrant special consideration, particularly candidates for liver transplantation with impaired liver function (1,2,4,5,6,7,8,9).

For these reasons, we have prefered BCLC staging system for this study.

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Reviewed by 2447122 major revizyon

In the present study, Ilikhan et al. compared serum prolidase activity and AFP values in HCC and cirrhotic patients and, healthy volunteers. They found a significant increase in prolidase activity in HCC patients with respect to cirrhotic patients and controls. Also, prolidase levels showed a significant correlation with tumor size and number. Besides, authors showed that in patients with HCC, there was a significant correlation between the prolidase activity and AFP values in terms of tumor size, number and BCLC staging classification. Major comments: 1. The authors conclude that both serum prolidase activity and AFP levels could be useful in early diagnosing of HCC. But, how the measurement of prolidase activity would be an advantage compared to uniquely determine the levels of AFP? 2. As the authors mention, metastatic tumor cells secrete high levels of proteases that enable them to degrade basement membranes and the extracellular matrix, and thus, invade other tissues. However, the presence of macrovascular invasion was not significantly correlated with serum prolidase, probably because of the reduced number of samples. Therefore, this study would be enriched if more patients with vascular invasion are included. It would be very interesting if the correlation between serum prolidase activity and the presence of distant metastasis or other invasive characteristics (incomplete encapsulation, multiple tumor number) in HCC patients were evaluated. Also, authors should analyze patient's survival and recurrence.

1. First of all we thank you for your comments. We have checked the present study to reveal the probable advantages of prolidase levels compared to the AFP levels in patients with HCC. It is known that AFP levels are normal (less than 10 ng/ml) in up to 40% of patients with HCC,

particularly during the early stage of the disease (1,2), and elevated AFP levels are seen in patients with cirrhosis or exacerbation of chronic hepatitis (3,4). Thus we have attempted to view the value of prolidase measurement among the patients with HCC whose AFP levels were low (less than 80 ng/ml) and both cirrhotic patients and controls. We have seen that HCC patients with low AFP levels have significantly higher serum prolidase levels compared to both cirrhotic patients and controls. We added these outcomes in the results as ''Additionally, prolidase values were significantly higher in HCC patients (n=9) with low AFP levels (less than 80 ng/ml) compared to both cirrhotic patients and controls ($p<0.001$,$p<0.001$, respectively). Unfortunately, the small sample size including low AFP levels has not enough patients to let us make powerful analysis about the value of prolidase. ''

2. A major limitation to our study is the relatively small sample size. And a second limitation of this study may be related to not having the outcomes of long-term follow-up of the patients with HCC. Future investigations may elucidate differences in prolidase patterns in long-term follow-up within the groups of the patients with chronic liver diseases. Unfortunately, we have no time for including the new cases. The big size study can be realized in the future. This manuscript is a preliminary descriptive study about the importance of prolidase for screening of HCC. We emphasized the limitations of our study in conclusions as ''Our study has several limitations, such as the low number of patients and not having long term outcomes of HCC patients regarding prolidase levels. Nevertheless, future comprehensive studies covering larger populations are needed to elucidate the value of prolidase activity during follow-up of the patients with chronic liver diseases. ''

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Reviewed by 2537576 rejected

The author presented a study of the prognostic ability of serum prolidase in hepatocellular carcinoma. In this study, serum prolidase levels are significantly correlated with the tumor size, number and stage of HCC. Meanwhile, the level of prolidase is positive related with AFP, and accompanied with similar results of AFP values in HCC patients. However, it still has some points should be made clear. 1. AFP is considered as a specific biomarker in the progress of HCC, it is associated with the progress of disease and also the patients' clinic outcomes. Prolidase exhibited higher sensitivity and specificity than AFP in predicting the tumor size and number, which are adverse prognostic factors for HCC survival, but it is interesting to know the prognostic value of prolidase in patients' survival, which is a more important and considerable end point. 2. The serum prolidase levels are positive related with AFP levels. In addition, both of them could predict the progress of HCC and are significantly associated with tumor size, number and stage of HCC. What is the significant difference between them in the prognostic prediction?

1. Thank you for the comments of the reviewer. Our main purpose about this manuscript is the correlation between prolidase and tumor load and spreading. We observed a correlation about them. Maybe the prolidase activity can reflect the tumor behavior except the survival. The survival study is out of our purpose about this manuscript. Actually, we did not looking for a prognostic marker in this study. We were just interested in prolidase activity regarding tumor features. But, it can be planned as a future study.
2. It can be considered as an advantage of serum prolidase activity among the patients with HCC whose AFP levels were low, that is statistically higher from both cirrhotic patients and controls.

3 References and typesetting were corrected

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology.*

Sincerely yours,

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