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Research Article

Evaluation of Serum Matrix Metalloproteinase-7 (Mmp-7) In gyptian Patients with Hepatocellular Carcinoma

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Abstract

Background and Aim: HCV and hepatocellular carcinoma is increasing among the world. According to this regard, serum matrix metalloproteinase 7 in patients with hepatocellular carcinoma and chronic hepatitis C without hepatic focal lesions, was evaluated aiming to find any clinical usefulness for it as a new non- invasive diagnostic and/or prognostic bio marker.

Methods: This current study conducted on 90 participants; group 1 included 30 patients with HCV related chronic liver disease; group 2 included 30 HCV related hepatocellular carcinoma patients in early stages (BCLC 0 and BCLC A) and group 3 included 30 HCV related hepatocellular carcinoma patients in late stages (BCLC C and BCLC D). All medical and laboratory assessment including viral hepatitis markers, AFP and quantitative measurement of MMP-7 by enzyme-linked immunosorbent assay (ELISA) was done.

Results: This study revealed that HCC cases were statistically significantly higher ($p<0.05$) in elderly patients, as well in males than females. Both serum AFP and MMP-7 were highly statistically significantly increased ($p<0.000$) in HCC patients than CLD patients. Moreover, they were both statistically significantly higher ($p<0.05$) in late HCC group than early HCC. Performing the receiver operating characteristic curve (ROC) revealed that the best cut off for AFP and MMP-7 in prediction of HCC, was >60ng/mL and >5.97 ng/mL respectively. On the other hand the best cut off value was>152.2ng/mL and >7.86 ng/mL for AFP and MMP-7 respectively in prediction of late HCC. Finally, the combination of both AFP and MMP-7 for prediction of late HCC has increased sensitivity of MMP-7.

Conclusion: Serum MMP-7 may be a potential diagnostic and prognostic marker for HCC.

Keywords: Hepatocellular carcinoma; Serum MMP-7; Alpha-fetoprotein; Hepatitis C

Introduction

Hepatocellular Carcinoma (HCC) is the fifth most common cancer worldwide and the third leading cause of cancer-related mortality [1].

In Egypt, the annual proportion of HCC showed a significant rising trend from 4.0% in 1993 to 7.2% in 2002 [2]. Nowadays, Egypt has the highest prevalence of HCV in the world [3].

Alpha-fetoprotein (AFP) which is the golden marker for HCC is of low sensitivity; therefore, additional markers are suggested to be simultaneously evaluated in order to enhance the detection of HCC [4].

Matrix Metalloproteinases (MMPs) are zinc-containing endopeptidases and they are considered the main degrading enzymes of extracellular matrix proteins and basement membranes [5].

In various cancers, MMPs degrade the ECM (extracellular matrix) and thus involved in the invasion and metastasis of cancer cells. It is believed that MMP-9 degrades type IV collagen, which is a major constituent of the basement membrane and thus is the first step in cancer cell invasion and metastasis [6].

The MMPs reported to be over-expressed in many types of human malignant tumors including HCC and have been shown to participate in tumor invasion and the metastatic processes [7].

The purpose of this study was toevaluate serum matrix metalloproteinase 7 in patients with hepatocellular carcinoma and chronic hepatitis C without hepatic focal lesions, aiming to find its clinical usefulness as a new non- invasive novel diagnostic and prognostic marker for HCC.

Materials and Methods

This pilot cross sectional study was conducted between Tropical Medicine Department, Medical Biochemistry Department, and the outpatient HCC clinic, Faculty of Medicine, Ain Shams University Hospitals after approval from the Research and Ethics Committee of Ain Shams University was obtained in accordance with local research governance requirements. This study was performed in accordance with the 1964 Declaration of Helsinki and all subsequent revisions.

This study was conducted on 90 participants who were divided into 3 groups: Group A: included 30 patients with HCV related chronic liver disease. Group B: included 30 HCV related hepatocellular carcinoma patients in early stages (BCLC 0, A) Group C: included 30 HCV related hepatocellular carcinoma patients in late stages (BCLC C, D). Patients were classified into BCLC according to EASL guidelines.

Inclusion Criteria

The study included patients with chronic hepatitis C and hepatocellular carcinoma. Patients in all groups were subjected to full history taking, thorough clinical examination and laboratory investigations including (complete blood picture, ESR, liver function tests, kidney function, hepatitis viral markers and alfa fetoprotein, MMP-7 assay by ELISA kit), together with abdominal Ultrasonography (US) and triphasic spiral Computed Tomography (CT). HCC patients were diagnosed and classified according to AASLD Practice Guidelines [7].

Exclusion Criteria

Other etiologies of chronic liver disease (e.g. chronic hepatitis B CHB, autoimmune hepatitis…), intermediate stage HCC (BCLC B), previously treated HCC and refusal to participate in the current study.

Sample collection and processing

5 mL of venous blood was withdrawn from each participant under complete aseptic conditions in a Serum Separator Tube (SST); the sample was left at room temperature for 30-60 minutes for spontaneous clotting then serum was separated by centrifugation at 3000 rpm for 10 minutes. Serum samples were kept frozen at -80^{0}C until used for quantitative measurement of MMP-7.

Quantitative measurement of MMP-7 in serum

The quantitative determination of serum- MMP-7 was done by enzyme-linked immunoassay using the commercially available ELISA kit supplied by Quantikine (Minneapolis, MN, USA). ELISA was performed according to the manufacturer's instructions. Briefly, this assay employs the quantitative sandwich Enzyme Linked Immunoassay (ELISA) technique. A monoclonal antibody specific for MMP-7 has been pre-coated onto a microplate. Standards and samples were pipetted into the wells and so, the immobilized antibody binds the MMP-7. After washing away unbound substances, an enzyme-linked polyclonal antibody was added to the wells. Then, another wash was done to remove any unbound antibody–enzyme reagent. Next, a substrate solution was added to the wells and color developed in proportion to the amount of MMP-7 bound in the initial step. The color development was stopped and the intensityof the color was measured within 30 minutes; using a microplate reader set to 450 nm against zero standard optical density. Wavelength correction set to 540 nm or 570 nm was done. The MMP-7 concentration was calculated from the best drawn standard curve. The data were linearized by using log/log curve.

Statistical Analysis

All analysis was performed using the Statistical Package for Social Science (IBM SPSS) version 20. The data were expressed as quantitative data (mean, standard deviations and ranges) The comparison between two independent groups with quantitative data and parametric distribution were done by using Independent t-test. The data were expressed as qualitative data (number and percentages). Chi-square analysis was used in the comparison between two groups with qualitative data and Fisher exact test was used instead of the Chi-square test when the expected count in any cell found less than 5. Variables were cross-tabulated in all possible combinations against each other find any associations. The comparison between two groups with non-parametric data was done by using Mann-Whitney test. Receiver operating characteristic curve was used to assess the best cut off point of MMP-7 and AFP with its sensitivity, specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) in prediction of HCC and late HCC. The confidence interval was set to 95% and the margin of error accepted was set to 5%. So, the p-value was considered if > 0.05: Non significant (NS), while if <0.05: Significant (S) and if < 0.01: Highly Significant (HS).

Results

This study revealed that 73.3% of HCC cases were males with significant statistical p value <0.05. The age of patients ranged from 52-68 years, with a significant statistical (p<0.05) association of higher mean age in years (60.35±7.74) for HCC cases than CLD. 26.7 % of HCC patients were smokers in comparison to 6.7% in CLD group, history of dental procedures was higher in the HCC group (33.3%) than CLD group (6.7%) (Table 1).

		CLD	HCC	Mann-Whitney test	
		No. = 30	No. = 60	Z	p-value
AFP(ng/ mL)	Median (IQR)	8 (3.3 – 16)	37.6 (11.55 – 279.5)	4.468	0.000**
MMP-7(ng/mL)	Median (IQR)	3.13 (2.5 – 4.7)	4.95 (3.4 – 8.55)	3.676	0.000**
**p value= Highly statistically significant <0.001					

Table 2: Comparison between CLD and HCC Patients Regarding Alpha-Fetoprotein (AFP) and Matrix Metalloproteinase 7(MMP-7).

AFP level was statistically significantly higher ($p<0.05$) in late HCC group than early HCC with a median of (240.5 ng/mL) and (25.15ng/mL) respectively (Table 3).

		Early HCC	Late HCC	Mann-Whitney test	
		No. = 30	No. = 30	Z	p-value
AFP(ng/mL)	Median (IQR)	25.15 (7.6 – 73)	240.5 (16.2 – 523)	2.757	0.006*
MMP-7(ng/mL)	Median (IQR)	4.01 (2.94 – 7.5)	7.13 (3.88 – 10.1)	2.462	0.014*
*p-value: Statistically significant <0.05					

Table 3: Comparison between Early HCC Patients and Late HCC Patients Regarding Alpha Fetoprotein (AFP) and Matrix Metalloproteinase 7(MMP-7)

ROC curve analysis was performed to assess the diagnostic performance of AFP in the discrimination of HCC patients from patients with CLD which showed that the best cut off value was an AFP level >60 ng/mL with AUC=0.790, sensitivity was 48.3%, specificity was 100%, positive predictive value was 100% and negative predictive value was 49%. (Figure1).

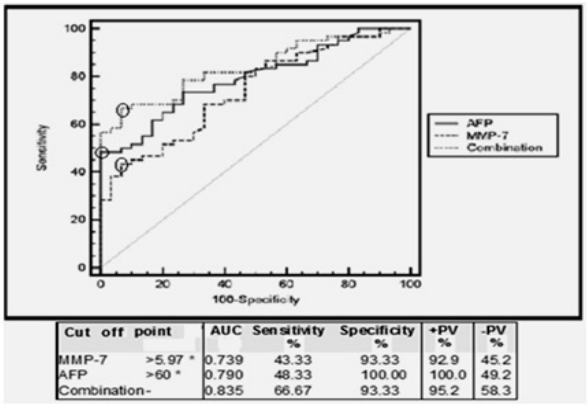

Figure 1: Receiver operating characteristic curve (ROC) for MMP7 and AFP best cutoff(s) in prediction of HCC group.

At that at a cut off value 200, sensitivity and specificity of AFP for detection of late HCC was 53.3% and 93.3% respectively. While at a cut off value 400, sensitivity and specificity of AFP for detection of HCC was 20% and 100% respectively (Table 4).

AFP	Early HCC		Late HCC		Chi-square test	
	No.	%	No.	%	X^2	p-value
< 200	28	93.3%	14	46.7%		
> 200	2	6.7%	16	53.3%	15.556	0.000
<400	28	93.3%	20	66.7%		
>400	2	6.7%	10	33.3%	6.667	0.010
Total	30	100.0%	30	100.0%		

In the current study,the ROC curve for AFP in prediction of late HCC showed the best cut off value was >152.2, with AUC=0.707, sensitivity 53,3%, specificity 93.3%, PPV 88.9% and NPV 66.7%. (Figure 2).

The ROC curve for the level of MMP-7 was significantly higher in HCC patients with a median of (4.95 ng/mL) in comparison with CLD patients with a median of (3.13 ng/mL) (Table 2). Moreover, the ROC curve for MMP7 in prediction of HCC group showed that the best cut off value was >5.97 ng/mL with area under the curve(AUC) 0.739, sensitivity 43.3%, specificity 93.3%, PPV 92.9% and NPV 45.2%. (Figure 1).

Also MMP-7 level was found to be higher in the late HCC group than the early HCC group with a median of (7.13 ng/mL) and (4.01 ng/mL) respectively (Table 3). Moreover, the ROC curve for MMP7 in prediction of late HCC group showed that the best cut off value was >7.86 ng/mL with area under the curve (AUC) = 0.685, sensitivity 46.6%, specificity 83.3%, PPV =73.7% and NPV =61%. (Figure 2).

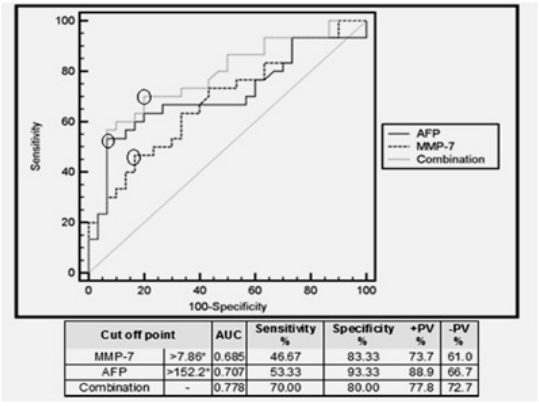

Figure 2: Receiver Operating Characteristic Curve (ROC) For MMP-7 and AFP In Prediction of Late HCC Group.

The combination of both AFP and MMP-7 for prediction of HCC has increased the sensitivity of MMP-7 from 43.33% to 66.67%. (Figure 1) In addition, the combination of both AFP and MMP-7 for prediction of late HCC has increased sensitivity of MMP-7 from 46.6 % to 70%. (Figure 2).

Discussion

HCC is the fifth most common cancer and is the third leading cause of cancer death worldwide [8]. Egypt has the highest prevalence of HCV in the world and the prevalence of HCC is increasing in the last years [3].

Unlike other solid malignancies, the coexistence of inflammation and cirrhosis makes a nearly diagnosis and prognostic assessment of HCC much more difficult [9]. In addition, the conventional tests of hepatic function do not distinguish HCC from cirrhosis, and thus they contribute little to the diagnosis of the tumor [10].

Although CT and MRI are reported to have a sensitivity of 60–94.4% and 58.5–93%, respectively, in diagnosis of tumors larger than 1 cm, their sensitivities for detecting tumors smaller than 1 cm are reduced by 33–45% and 33–67%, respectively [11]. Furthermore, small, arterially enhancing nodules are common in the cirrhotic liver, and majority of these nodules are benign [12].

Alpha Fetoprotein (AFP) which is the golden marker for HCC is of low sensitivity; therefore, additional markers are suggested to be simultaneously evaluated in order to enhance the detection of HCC [4]. Thus, a tremendous effort has been put forth and continues to be applied in the search for improved HCC biomarkers.

Matrix Metallo Proteinases (MMPs) are a large family of calcium-dependent zinc containing endopeptidases, which are responsible for the tissue remodeling and degradation of the Extracellular Matrix (ECM), including collagens, elastins, gelatin, matrix glycoproteins, and proteoglycan [13].

The Hepatic Stellate Cell (HSC) is the key cell type that contributes to liver fibrosis. Upon activation, the HSC undergoes proliferation, contraction and migration critical for the development of fibrosis. In addition, HSCs provide the major source of Extracellular Matrix (ECM) and Matrix Metalloproteinases (MMPs), the key regulators of matrix remodeling [14].

MMPs are implicated in a variety of physiological processes, including wound healing, uterine involution and organogenesis as well as in pathological conditions, such as inflammatory, vascular, autoimmune disorders, and carcinogenesis [15].

MMP-7 is able to cleave ECM and basement membrane [16], and mediate E-cadherin ectodomain shedding in injured lung epithelium leading to accelerated cell migration [17]. MMPs have been considered as potential diagnostic and prognostic biomarkers in many types and stages of cancer [18].

This study aimed to evaluate serum matrix metalloproteinase 7 in patients with hepatocellular carcinoma and chronic hepatitis C without hepatic focal lesions, aiming to find its clinical usefulness as a diagnostic and or prognostic marker.

This study revealed that 73.3% of HCC cases were males. This agrees with several studies [2] and an Egyptian study by Shaker et al., [3] performed on 1313 patients with HCC which revealed that male to female ratio was 3.7 :1.This may be the result of other associated factors such as the higher incidence of cirrhosis in males, and higher levels of smoking and alcohol intake in males [19].

In this study, the age of patients with HCC ranged from 52-68 years with a mean of 60.3±7.74. These findings were close to Hussein et al., [20], who reported that the age of patients with HCC was 40-77 years with a mean of 56 ± 8. Similarly, in another Egyptian study by Shaker et al., [3], they found that the most frequent age category affected by HCC was between 51 and 60 years (45.7%), followed by the category between 41 and 50 years (24.4%). This can be due to the fact that most patients with Hepatocellular Carcinoma (HCC) have liver cirrhosis, which develops following long periods of chronic liver disease [21].

The most common use of AFP is to screen for hepatocellular carcinoma in asymptomatic patients with HCV [22]. The present study revealed that AFP levels were significantly higher in HCC patients in comparison to CLD patients.

In the current study by performing, the Receiver Operating Characteristic curve (ROC) the best cutoff value for AFP in prediction of HCC, was >60 ng/mL with AUC=0.790, sensitivity= 48.3% ,specificity= 100%, positive predictive value= 100% and negative predictive value= 49%. On the other hand, Several previous studies reported different sensitivities and specificities for AFP in diagnosis of HCC. Sarwar et al., [23] have shown that AFP can best be used for screening of HCC at cut off value of 20.86 ng/mL with 72% sensitivity and 86% specificity. In addition, Durazo et al., [24] stated that level of AFP was significantly higher in patients with HCC than in those without HCC and that the best cutoff value was ≥25 ng/mL with sensitivity and specificity 69% and 87% respectively. Similarly Gupta et al., [22] by using the most commonly reported cutoff value of a positive test result for hepatocellular carcinoma (AFP level > 20 ng/mL), the ranges of test characteristics were as follows: sensitivity, 41% to 65% and specificity, 80% to 94%. Daniele et al., [25] also noted a 60% sensitivity of AFP with 20 ng/mL as cutoff value with positive predictive value (PPV) of only 50%, can be used for diagnosis of HCC.

The current study revealed that AFP level was higher in late HCC group than early HCC with a median of (240.5 ng/mL) and (25.15 ng/mL) respectively p value= 0.006. This corroborates with

a previous study by Tangkijvanich et al. [26] that found that HCC patients with high AFP tended to have greater tumor size, bi-lobar involvement, massive or diffuse types, and portal vein thrombosis. In addition to this it agrees with Peng et al. [27] who showed that HCCs with high AFP had larger sizes (>5 cm) and high-grade tumors as compared to those with lower AFP. Moreover, HCCs with high AFP had more frequent vascular invasion than those with lower AFP. Finally, Murugavel et al. [28] also, suggested that the AFP values are more likely to be elevated with the advanced stage of cancers and are of prognostic value to check the efficacy of treatment of HCC.

In the present study by using two of the previously proposed cut off values for prediction of late HCC 200 ng/mL and 400 ng/mL respectively the following was revealed ; at cut off value of 200 ng/mL, sensitivity and specificity of AFP for detection of HCC were 30% and 100% respectively, while at proposed a cut off value of 400 ng/mL, sensitivity and specificity of AFP for detection of HCC were 20% and 100% respectively which was close to a previous study that found that an AFP value ≥200 and 400 ng/mL had a sensitivity of 36.3% and 20.2%, respectively, and specificity of 100% in both groups [29].

In the present study, the ROC for AFP in prediction of late HCC showed the best cut off value at >152.2, with AUC=0.707, sensitivity 53,3%, specificity 93.3%, PPV 88.9% and NPV 66.7%.

Distinction of HCC from cirrhosis has become challenging because regenerative nodules may mimic tumors in cirrhotic livers and because of the elevated serum levels of AFP in patients with cirrhosis [30]. AFP has been widely used as a diagnostic marker for HCC. However, there are some patients showing continuous high AFP values but with no evidence of HCC [31].

MMPs reported to be over-expressed in many types of human malignant tumors including HCC and have been shown to participate in tumor invasion and metastatic processes. Serum MMP-9 was significantly increased in HCC patients with poor performance status, and in the presence of metastasis. Thus, MMP-9 may play an important role in HCC invasiveness and metastasis. This was in accordance with several authors [32-35]. Moreover, Hayasaka et al. found that plasma MMP-9 levels were significantly elevated in the patients with HCC with macroscopic portal vein invasion as compared to patients with HCC without portal vein invasion [36]. These data suggested the possibility of plasma MMP- 9 levels being a novel marker for HCC, particularly for metastatic potential [36].

To our knowledge, this pilot study was the first to investigate MMP-7 as a new biomarker for liver in serum as a non-invasive sample because previously it was only studied in tissue sample.

In the present study, serum MMP-7 was significantly higher in HCC patients with a median of (4.95 ng/mL) in comparison with CLD patients with a median of (3.13 ng/mL), which agrees with a previous study that studied the tissue expression patterns of MMP-7 in tissue samples of normal liver, cirrhotic liver, macro regenerative nodule, dysplastic nodule, and hepatocellular carcinoma of various grades of differentiation [37]. The increased expression of MMP-7 in cirrhotic liver in comparison to non-cirrhotic liver suggests a more favorable environment for invasion and metastasis of hepatocellular carcinoma. As their activity play a pivotal role in tumor growth and the multistep processes of invasion and metastasis, including proteolytic degradation of ECM, alteration of the cell–cell and cell–ECM interactions, migration and angiogenesis [38].

In the current study, the ROC for MMP-7 in the prediction of HCC group showed that the best cut off value was >5.97 ng/mL with area under the curve (AUC) 0.739, sensitivity 43.3%, specificity 93.3%, PPV 92.9% and NPV 45.2%.

In the present study, by comparing the MMP-7 serum level in early and late HCC. The MMP-7 was found to be significantly higher in late HCC group than early HCC with a median of (7.13 ng/mL) and (4.01 ng/mL) respectively.

Gao et al., stated that well-differentiated hepatocellular carcinoma in comparison to less differentiated tumors were associated with higher levels of MMPs. They suggested that tissue expression of MMPs and their inhibitors could be useful markers to predict the progression and metastasis of hepatocellular carcinoma [37].

In a previous study conducted by Ishii et al., it was found that over expression of MMP-7 has been shown to be associated with metastatic progression of colorectal carcinoma and cholangiocarcinoma, as well as hepatocellular carcinoma [39]. In a recent study conducted by Wang et al., it was found that elevated serum MMP-7 was highly predictive of unresectable pancreatic ductal adenocarcinoma and nodal involvement despite favorable preoperative cross-sectional imaging [40].

Moreover, Gao et al., concluded that higher levels of MMP-7 expression were found as hepatocellular carcinoma progressed from low to high grade [37]. Other authors reported that a high expression rate of MMP-7 mRNA in HCC tissues had a significant influence on the incidence of intrahepatic metastasis and portal vein invasion [41]. Similarly, Ishii et al., [39] Found that MMP-7 showed significant expression in cases of portal vein invasion. MMP-7 also tended to be highly expressed in cases of capsular infiltration, also in cases where the tumor size exceeded 5cm; the expression of MMP-7 was highly significant, suggesting its possible involvement in the proliferation [39].

All the previous findings were in congruence with our study, as the ROC for MMP-7 in prediction of late HCC group showed that the best cut off value was >7.86 ng/mL with area under the curve (AUC)= 0.685, sensitivity 46.6%, specificity 83.3%, PPV =73.7% and NPV =61%.

The present study finally, showed that by combining both AFP to serum MMP-7 the sensitivity of MMP-7 for detection of HCC increased from 43.3% to 66.6% and sensitivity of MMP-7 for detection of late HCC increased from 46.6% to 70%.

Conclusion

Serum matrix metalloproteinase 7 could provide useful tool as a potential novel diagnostic and prognostic marker for HCC.

Acknowledgments

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Conflict of Interest: All Authors declare to have no conflict of interest.

Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent: Informed consent was obtained from all individual participants included in the study.

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