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Hepatitis C Virus as risk factor for development of hepatocellular carcinoma in Egypt: II-Enhancement role of matrix metalloproteinases-2 in dissemination of HCC

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ABSTRACT

Hepatitis C virus (HCV) infection is a major public health problem all over the world. Egypt has the highest prevalence of HCV worldwide (17-26%) with subsequent high morbidity from chronic liver disease, cirrhosis and hepatocellular carcinoma (HCC). Matrix metalloproteinase are proteolytic enzymes that play a role in the degradation of extracellular matrix (ECM) which is necessary for invasion and metastasis of tumor cells. The present work was designed to study the relationship between HCV infection and circulating MMP-2 level in chronic HCV patients (either without or with hepatic complication) and compared to that of non- HCV cirrhotic patients as well as healthy controls, in order to clarify the role of HCV in changing microenvironment and underlying mechanisms associated with dissemination of malignancy. The level of MMP-2 was estimated in sera collected at different stages of HCV infections as well as in ascetic fluids collected from those developing either HCC or cirrhosis. Statistical analysis of their results revealed that MMP-2 levels were significantly elevated in all patient groups as compared to healthy controls. The level of MMP-2 in HCV patients with HCC was significantly elevated when compared to other HCV patients. Meanwhile MMP-2 in ascetic fluids of cirrhotic patients were similar to that detected in their sera, while in HCC patients there were 2.4 times elevations in serum level of MMP-2 as compared to that in ascetic fluids. These results revealed that HCV infection is not only responsible for biochemical and hematological abnormalities recorded at chronic stages of infection but also creating a microenvironmental change by enhancing MMP-2 release, which effect on infected cell by obliging them to modify their phenotype in order to survive, thus increasing the invasion potential and facilitate tumor progression.

Keywords: Hepatitis C Virus, hepatocellular carcinoma, II-Enhancement, MMP-2 HCC

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INTRODUCTION

Hepatocellular carcinoma (HCC) of variable etiologies ranks 5th and 7th among most common cancers in men and women, respectively and 3rd among most frequent causes of cancer-related deaths worldwide (1). Chronic infection with Hepatitis C virus (HCV) is considered to be a prominent risk factor for the development of HCC, the molecular mechanisms underlying hepatocarcinogenesis is not fully elucidated (2).

HCV is an enveloped single-stranded RNA virus that belongs to the genus Hepacivirus of the family Flaviviridae (3). Viral infection is often asymptomatic, rendering very difficult its detection and treatment at an early stage; therefore, often referred to as a “silent disease”. In the majority of infected persons, infection does not resolve spontaneously; yet the virus mutates to escape host surveillance (4) and, infected individuals become chronic carriers. However, within this chronically infected population, the disease outcomes vary widely from minimal inflammation to severe liver tissue fibrosis propagating either to cirrhosis or HCC and ultimately death (5).

The viral molecule is thought to be unable to integrate into the host genome. HCV proteins interact with host-cell factors that are involved in cell cycle and transcriptional regulation, cell proliferation and apoptosis which contribute to the viral oncogenic processes (6).

The process of carcinogenesis is multistage which require alteration of the microenvironment for conversion of normal tissue to a tumor. Matrix metalloproteinases (MMPs) are principal mediators of alterations observed in the microenvironment during carcinogenesis and, not only have roles in invasion or late stages of cancer, but also in regulating initial steps of carcinogenesis in a favorable or unfavorable manner (7,8).

MMPs can act on a number of bioactive molecules and affect many behavioral patterns of the cell such as proliferation, differentiation, migration, angiogenesis and apoptosis (9). Elevated levels of MMPs in the tumor microenvironment can directly induce epithelial mesenchymal transition (EMT) in epithelial cells and produce more MMPs facilitating cell invasion and generate activated cells which drive cancer progression via further MMP production (10).

Similar to all proteins, MMP-2 mostly secreted as latent, inactive zymogen by various stromal and epithelial cell types, including mesenchymal cells, T cells, monocytes, macrophages, neutrophils, keratinocytes and also tumor cells (11). Activation of the pro-enzyme usually occurs in the pericellular or extracellular spaces. MMPs work together to create a cascade of activation, whereby, once one MMP is activated, it catalyses the conversion of other MMP zymogens to their active forms; so that many MMPs are “switched on” with the potential to degrade all classes of the extracellular matrix (12).

Overproduction of several types of MMPs including MMP-2 by tumor infiltrating cells may contribute to the marked increase in vascular permeability, opening of endothelial barriers and allowing passage of plasma proteins and inflammatory cells into otherwise privileged compartments(13). In addition to that, there was direct relation between expression of MMP-2 and degree of invasiveness, metastasis and presence of ascites in ovarian cancer patients concluding that MMP-2 and -9 are frequently over expressed in ovarian cancers cells disseminated in the peritoneal cavity (14).

Overexpression of MMP-2 mRNA expression was positively correlated to the degree of regional lymph node metastasis in HCC patients, increased tumor recurrence, advanced TNM stage and barcelona clinic liver cancer stage (15-17).

Li et al., (2012) (18) suggested that MMPs expression is consistently observed to be up-regulated by HCV. HCV NS4B protein activates STAT3 by enhancing its phosphorylation and translocation; thereby activating MMP-2 and Bcl-2 expression; a step that result in the regulation of cell transformation, apoptosis and possibly tumorigenesis as a consequence of HCV infection. Furthermore, HCV infection induces the generation of inflammatory cytokines and chemokine, potentially leading to the recruitment of inflammatory cells such as cytotoxic T lymphocytes, neutrophils, monocytes, dendritic cells, and natural killer cells to the liver, causing liver cell injury and chronic hepatitis (19).

So, it is foreseeable to study the levels of matrix metalloproteinase-2 (reflecting modifications in microenvironment) in patients with chronic hepatitis C virus and their correlation with progression towards cirrhosis and HCC in order to understand its potential role in HCC cancer initiation and development.

SUBJECTS AND METHODS

Subjects

The present study was conducted on a total of 58 subjects. Of them, 48 were patients recruited from those admitting and attending the in- and out-patient clinics of the Department of Internal Medicine, Medical Research Institute, Alexandria University. They included 4 main groups: The first group chronic HCV patients without manifestations of liver cirrhosis (12 patients), the 2nd was chronic HCV patients with radiological and serological evidence of liver cirrhosis (12 patients) and another group of 12 non-HCV patients with radiological and serological evidence of liver cirrhosis. The last group was chronic HCV patients with HCC confirmed by radiological and laboratory assessment (12 patients).

In addition, 10 healthy age- and sex-matched individuals were involved in the study as healthy controls.

The HCC group was diagnosed by abdominal ultrasonography, Triphasic CT abdomen, serum alfa fetoprotein and confirmed histopathologically. Cirrhotic group was diagnosed by abdominal ultrasonography, clinical examination, abdominal ultrasound, laboratory investigation and liver biopsy. All cases were newly diagnosed cases that had not received prior chemotherapy. The control group had no clinical or biochemical evidence of liver disease or known medical illness at recruitment and with normal abdominal ultrasonography. All controls had absent serological markers of HBV, HCV, Bilharziasis and autoimmune hepatitis. In addition, they had normal liver transaminase and with normal liver homoeostasis assessed by unremarkable radiographic findings on ultrasound study.

A detailed history, clinical assessment, biochemical liver profile, abdominal ultrasonography were done to all study groups in addition to serological testing and abdominal ultrasonography with Doppler examination to identify focal lesions and Triphasic CT scan for patients with hepatic focal lesions.

Ethics: Written informed consent was obtained from all participants prior to enrollment in the study, which conformed to the ethical guidelines of The 2004 Declaration of Helsinki. Ethical approval for the study was obtained from the Local Ethical Committee of the Medical Research Institute, Alexandria University.

Laboratory Clinical Investigations:

The laboratory investigations were included;

- Complete blood pictures, platelets count and prothrombin activity.
- Biochemical liver function tests (serum glutamic pyruvic transaminase (SGPT), bilirubin and albumin).

Assessment of MMP-2 level

Blood samples from peripheral veins (5 ml) were obtained from each patient, left to coagulate, and then centrifuged at 2000 rpm for 10 minutes. Serum was collected and then stored at -80°C until used. The patients were fasting at the time of sampling. MMP-2 levels were measured in sera collected from all subjects under study. Selected ascetic fluids was aspirated from selected groups of HCV patients with cirrhosis and HCC. Level of MMP-2 was estimated according to the method described by Smigielski et al., (2013) [20] employing commercially available Quantikine® Total MMP-2 sandwich ELISA kit (R&D Systems, China) and following the manufacturer`s recommendation.

Statistical analysis of the data: All analyses were performed using IBM SPSS software (SPSS for windows, version 21.0, Chicago, IL, USA). P-Value < 0.05 was considered significant, P-Value ~ 0.01 was considered highly significant and P-Value > 0.05 was considered insignificant.

RESULTS

Clinical Laboratory Data

Data of liver function and hematological picture was summarized in table (1).The platelet counts were reduced in all patient groups relative to healthy individuals (although within globally accepted reference range except only in HCV patients with end stage complications). These results were accompanied with parallel decrease in serum albumin level and prothrombin activity with elevated serum total bilirubin and SGPT.

Statistical analysis revealed that platelet count was significantly reduced in all patient groups as compared to normal controls (p=0.005, 0.0001, 0.005 and 0.0005 respectively). In addition, HCV patients with complications (cirrhosis and HCC) had significantly reduced platelet counts as compared to their partners without complications (p=0.0001 and 0.005 respectively). Furthermore, cirrhotic HCV patients had more significantly pronounced decrease in platelet counts when compared to those with non-HCV cirrhosis (p=0.005) but not when compared to HCC patients (p=0.1) table (1).

Statistical analysis of serum albumin levels showed a significant decrease in all patient groups when compared to healthy controls (p=0.0001). The same finding was recorded in HCV patients having hepatic complications (cirrhosis and HCC) when compared to HCV patients without complications (p=0.0001). Cirrhotic HCV patients had significantly reduced albumin levels when compared to non-HCV cirrhotic patients (p=0.005). Finally, no significant difference was recorded upon comparing albumin level in HCV cirrhotic and HCC patients (P=0.1) table (1).

Statistical analysis of serum total bilirubin revealed that there was a significant elevation in all patient groups as compared to their corresponding normal controls (p=0.0005, 0.0001, 0.0001 and 0.005 respectively). In addition, serum total bilirubin was found to be significantly elevated also in HCV patients with complications (cirrhosis and HCC) as compared to those without complications (p=0.005). On the other hand, no significant variation was recorded upon comparing serum total bilirubin in cirrhotic HCV patients as compared either to non HCV cirrhotic (p=0.25) or to HCC patients (p=0.31) table (1).

Statistical analysis of serum glutamic pyruvic transaminase (SGPT) revealed that significant elevation in SGPT levels in all patient groups as compared to healthy controls (p=0.0001, 0.0001, 0.005 and 0.0005 in groups II through V). In addition, HCV patients with HCC still had significantly elevated SGPT when compared to their partners without complications (p=0.025). On the other hand no significant variation in SGPT was recorded in cirrhotic HCV patients as compared either to HCV patients without complications (P= 0.025), HCC patients (P=0.1) or to non-HCV cirrhotic patients (P=0.25) table (1).

Statistical analysis of prothrombin activity these revealed that prothrombin activity was significantly affected in all patient groups as compared to normal subjects (p=0.0001). In addition, there was a significant decrease in prothrombin activity in HCV patients with complications (cirrhosis and HCC) as compared to those without complications (P=0.0005). Also, prothrombin concentration was significantly reduced in cirrhotic HCV patients relative to their non-HCV cirrhotic partners (p=0.025) but not to HCV patients with HCC (P=0.1) table (1).

Table (1): Statistical analysis of the results of laboratory data among HCV infected patient groups either without complications (group II), with liver cirrhosis (group III) or HCC (group V) as compared to non-HCV cirrhotic and healthy subjects.

Groups / Laboratory data	Healthy subjects (group I)	HCV patients without complications (group II)	HCV patients with liver cirrhosis (group III)	non-HCV cirrhotic patients (group IV)	HCV patients with HCC (group V)
platelet count x10^3/mm^3	296.8±84.7	188.9±49.65	87.8±44.5	173.3±82.9	109.1±59
Serum albumin level (gm/dl)	4.5±0.21	3.8±0.54	2.5±0.58	3.3±0.73	2.2±0.65
Total bilirubin level (mg/dl)	0.68±0.1	1.62±0.83	3.14±1.41	2.67±0.93	3.78±3.7
SGPT (U/L)	26.4±4.2	49.2±8.87	58.9±20.56	49.5±25.1	82.3±49.57
Prothrombin concentration (% activity)	108.7±9.33	76.17±14.83	54.3±9.8	68.21±19.9	55.62±8.8

Estimation of MMP-2 activity in serum and ascetic fluid

The MMP-2 level was measured in sera from all subjects under study as well as from selected ascetic fluid by ELISA using commercially available kits. Results were summarized in table 2 and expressed as ng/ml. MMP-2 in control subjects ranged from 130-275 ng/ml with mean±SD of 200±31.7; while in patients with chronic HCV, it ranged from 225-365 ng/ml with mean±SD of 295±40.6. In HCV patients with liver cirrhosis, MMP-2 ranged from 190-430 ng/ml with mean±SD of 289.1±60.3. In non-HCV cirrhotic patients, MMP-2 ranged from 130-410 ng/ml with mean±SD of 248.3±85.5. Finally, in HCV patients with HCC, it ranged from 285-460 ng/ml with mean±SD of 373.3±55.11 (table 2).

Statistical analysis of these results revealed that MMP-2 levels were significantly elevated in all patient groups as compared to healthy controls (p=0.0001, 0.0001, 0.05 and 0.0001 in groups II through V). The level of MMP-2 in HCV patients with HCC was significantly elevated when compared to other HCV patients either with or without complication (p=0.0001). Finally, no significant difference was recorded upon comparing MMP-2 levels in cirrhotic HCV patients relative to non-HCV cirrhotic (p=0.25) as well as in cirrhotic HCV patients relative to those without complications (p=0.25) (table 2).

Table (2): Statistical analysis of the results of MMP-2 level (ng/ml) among HCV infected patient groups either without complications (group II), with liver cirrhosis (group III) or HCC (group V) as compared to non-HCV cirrhotics and healthy subjects

Groups / MMP-2 (ng/ml)	Healthy subjects (group I)	HCV patients without complications (group II)	HCV patients with liver cirrhosis (group III)	non-HCV cirrhotic patients (group IV)	HCV patients with HCC (group V)
Range	130-275	225-365	190-430	130-410	285-460
Mean	200	295	289.1	248.3	373.3
SD	31.7	40.6	60.3	85.5	55.11
P_1		0.0001*	0.0001*	0.05*	0.0001*
P_2			0.25	0.1	0.0001*
P_3				0.25	0.0001*
ANOVA test **F=13.6, P=0.01**					

*Statistical significant at p ≤ 0.05; P1= p value between studied groups and control subjects; P2= p value between HCV patients and other studied groups; P3= p value between HCV patients with liver cirrhosis and other studied groups.

The concentrations of MMP-2 in ascetic fluids of cirrhotic patients with mean of 275 ng/ml; while in patients with HCC, its mean was 156.7ng/ml. In addition, serum MMP-2 levels in cirrhotic patients were 289.1ng/ml and in HCC patients were 373.3ng/ml.

The obtained data revealed that the concentrations of MMP-2 in ascetic fluids of cirrhotic patients were almost similar to that detected in their relevant sera while in patients with HCC, there was a 2.4 times elevation in serum levels of MMP-2 as compared to that in respective ascetic fluids. In addition, ascetic fluid MMP-2 levels in cirrhotic patients showed 1.8 times increase than their HCC partners; a figure that is contradictory to that encountered upon comparing serum levels of MMP-2 (1.3 times increase in HCC patients compared to cirrhotic patients) figure (1).

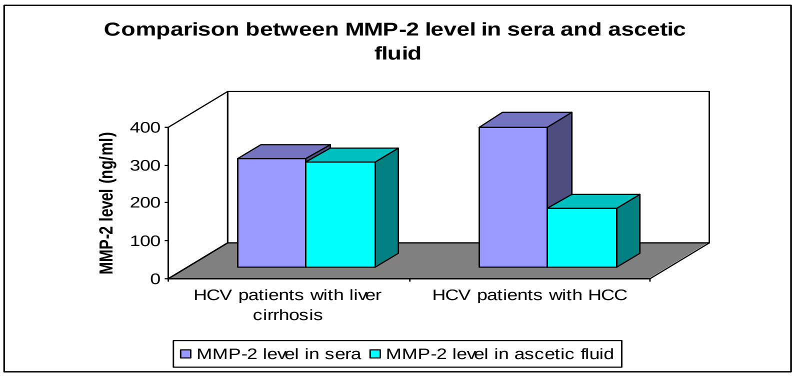
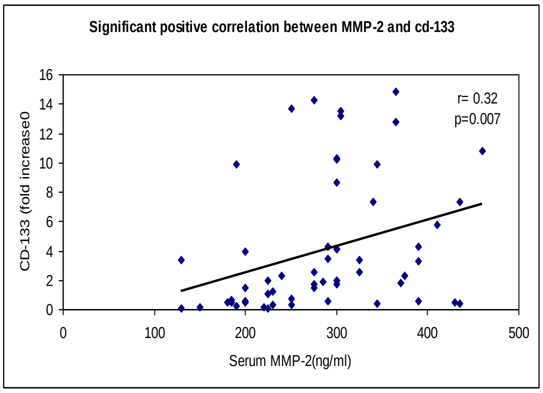
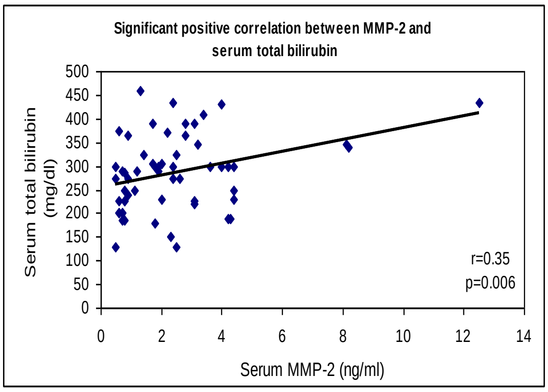


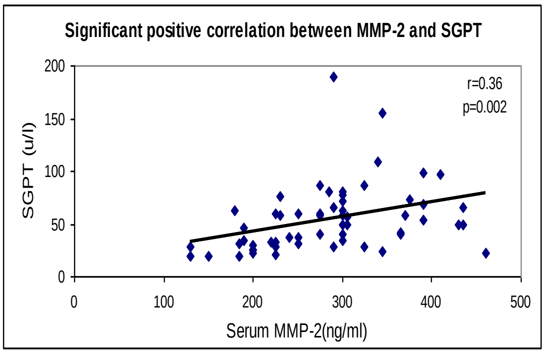
Figure (1): Comparison between MMP-2 levels in sera and ascetic fluid.

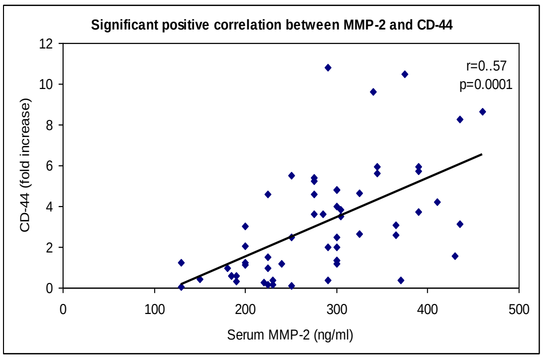
Correlation analysis

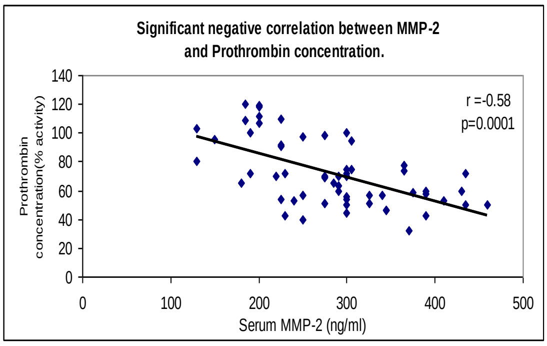
Pearson`s correlation was performed between all parameters among all subjects under study. There was significant positive correlation between MMP-2 and total bilirubin and SGPT. On the other hand significant negative correlation was found between MMP-2 and albumin, prothrombin activity and platelet count figure (2).

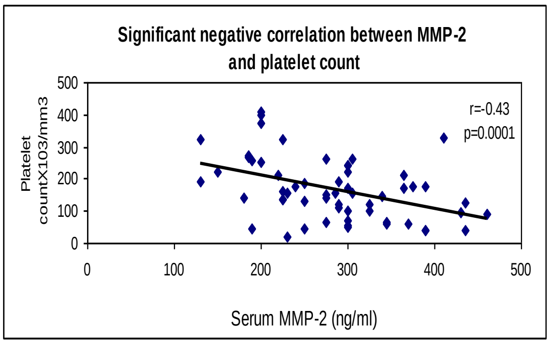












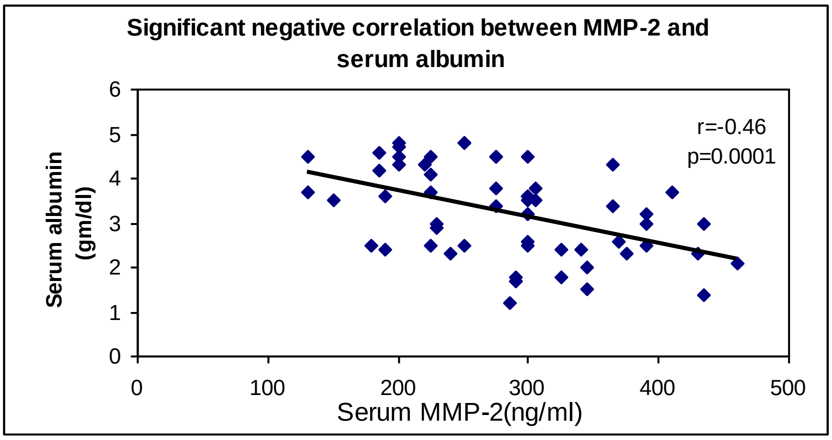


Figure 2: Significant correlations between serum MMP-2 (ng/ml) and other study parameters.

DISCUSSION

It is well established that the expression of various MMPs was detected in all types of primary tumors. Their circulating concentrations correlate with advanced stages of tumorgenesis, invasive and metastatic properties and, in general, with poor prognosis (21). This study was including the levels of MMP-2 in the sera and ascetic fluid in HCV infection.

The results revealed that maximal circulating MMP-2 levels was noticed in HCC patients that were even significantly higher than both chronic HCV patients and cirrhotic partners. No significant difference in MMP-2 level was recorded between chronic HCV patients without complications and cirrhotic patients. MMP-2 levels were correlated with biochemical parameters including: total bilirubin, SGPT, platelet counts, prothrombin activity and albumin which may reflect changes in extracellular matrix remodeling. Also the levels of MMP-2 in the sera and ascetic fluid of patients with HCV was evaluated.

Correlation analysis revealed that MMP-2 levels were negatively correlated with platelet counts, prothrombin activity and albumin while showed positive correlation with total bilirubin and SGPT. These data are going in agreement with Kuyvenhoven et al., (2003) (22) who found a significant correlation between MMP-2 and liver function (bilirubin, albumin, SGPT and prothrombin time) concluding that serum MMP-2 correlates with the severity of liver disease and may reflect changes in extracellular matrix remodeling.

Also our results showed a significant negative correlation between MMP-2 and platelet counts reflecting advancement of liver function impairment and extrahepatic metastasis. Owing to such a finding, Macías et al., (2011) (23) involved MMP-2 levels in combination with platelet counts to help in the diagnosis of liver fibrosis in HIV/HCV-coinfected patients.

Collectively, the results of the present work is a confirmation for preceding data denoting that the deterioration of liver homoeostasis due to chronic HCV infection is manifested by several biochemical and hematological characteristics. The elevation in liver aminotransferases and bilirubin were accompanied by impairment in profound liver functions; namely, serum albumin and prothrombin activity in addition to reduced platelet counts. Significant correlations were always recorded among these parameters as well as with advancement of the case towards liver cirrhosis and/or HCC.

Concerning the data of the circulating levels of MMP-2, our results are in accordance with Akca et al., (2013) (24) who detected high levels of circulating MMP-2 in patients diagnosed as HCV carriers either with or without positive plasma HCV RNA. They recommended the use of MMP-2 in monitoring the status of patients, determining the degree of damage to liver tissues, predicting the prognosis and over all management of HCV infection.

On the other hand, an earlier finding by El-Gindy et al., (2003) (25) indicated that serum levels of MMP-2 were almost similar in controls and in chronic HCV patients with or without fibrosis, although increased in cirrhotic patients, concluding that serum values of MMP-2 are able to detect cirrhosis with a high sensitivity. Also, Giannelli et al., (2002) (26) failed to report significant differences in MMP-2 concentration between healthy subjects and those with liver cirrhosis due to HCV. Instead, they reported remarkable tendency in localization of MMP-2 along the advancing edges of the tumor, suggesting possible involvement in the invasive and metastatic process. Of particular interest is their observation that MMP-2 serum levels were increased in patients with HCC compared to those with cirrhosis although the difference was statistically significant; data that goes in accordance with our results. The authors also found no difference in MMP-2 levels in sera and in crude extracts of primary nodules among HCC patients indicating that serum levels of MMP-2 might be taken as a good indicator for the metastatic potential of HCC bypassing the need for invasive interventions.

Our data are also in agreement with several observations by several authors who found increasing MMP-2 levels in chronic HCV patients where the most significant elevations were detected in those developing cirrhosis and HCC (27-29).

The finding that our cirrhotic patients (due to HCV or else where) did not show remarkable elevation in MMP-2 similar to their HCC partners is clearly compatible to that of Ishii et al., (2003) (30) and Ljumovic et al., (2004) (31) who showed that MMP-2 mRNA expression in patients with chronic HBV and HCV was relatively higher compared to non-viral chronic liver diseases. This observation is in contradiction to our finding where no difference in MMP-2 level was recorded among cirrhotic patients (with or without HCV); a controversy that can be attributed to initial exclusion of patients with HBV (known to contribute more significantly than HCV in MMP-2 overexpression) as well as to difference in method of assessment of MMP-2 under assay (mRNA expression versus circulating levels).

Our finding that MMP-2 levels were elevated also in non-HCV cirrhotics is in accordance with recent observation by Prystupal et al., (2015) (32) who found that MMP-2 activity in sera of patients with different stages of alcoholic liver cirrhosis was significantly increased compared to the control group. They showed also that among other MMPs, MMP-2 has been found to be the most sensitive as its activity was increased in all stages of liver cirrhosis. They further interpreted that the elevated serum MMP-2 level in cirrhotic patients may be explained by its over-production in the cirrhotic liver as a result of the propensity of hepatic stellate cells to compensate for the tissue damage and fibrosis. The authors also gave a notification of interest that the MMPs detectable in the liver and in the serum may derive from variable cells and tissues, of which the liver is not the most likely predominant one.

In the present study the level of MMP-2 in the ascetic fluid of selected patients with HCV-induced liver cirrhosis (3 patients) as compared to others with HCC (2 patients) was revealed that the concentrations of MMP-2 in ascetic fluids of cirrhotic patients were almost similar to that detected in their relevant sera while in patients with HCC, there was a 2.4 times elevation in serum levels of MMP-2 as compared to that in respective ascetic fluids. In addition, ascetic fluid MMP-2 levels in cirrhotic patients showed 1.8 times increase than their HCC partners; a figure that is contradictory to that encountered upon comparing serum levels of MMP-2 (1.3 times increase in HCC patients compared to cirrhotic patients). Indicating MMP-2 is more implicated in hematogenous dissemination and metastasis of malignant cells in HCC patients than in ascetic fluid. While increasing level of MMP-2 in ascetic fluid of cirrhotic patients meaning the infected cell begin to exit from liver or metastasis at this stage.

The diagnostic and predictive significance of MMPs in ascetic fluid has been evaluated by Noh et al., (2011) (33) who suggested that MMPs could be used as diagnostic markers in body fluids, and that MMP-2 in particular might be a prognostic marker in ascites of advanced gastric patients with disseminated metastasis. Also, Noh et al., (2012) (34) found that MMP-2 expression in body fluids can be used as an additive diagnostic marker in metastatic breast cancer patients.

Manenti, et al., (2003) (35) reported that MMP-2 and MMP-9 are expressed in ascites and plasma of ovarian cancer patients. Furthermore, experimental studies have shown that animals bearing ovarian carcinoma xenografts in the peritoneal cavity and treated with MMP inhibitors had lower amounts of ascites and survived longer indicating the potentially active involvement of MMP-2 in tumor progression and metastasis (36). In addition, Belotti, et al., (2003) (37) showed that MMPs contribute to the formation of ascites through the release of vascular endothelial growth factor (VEGF) and concluded, therefore, that targeting the respective MMP in tumor cells or host-derived cells could offer a way to control tumor progression and ascites in human ovarian carcinoma.

Collectively, the results of the present work is a confirmation for preceding data denoting that HCV infection increase the level of MMP-2 in chronic HCV patients without complication leading to change in extracellular matrix remodeling, and by advancement of liver function impairment due to viral infection or else is the reason for stabilize increase of MMP-2 during cirrhosis, and by its role in ascetic fluid formation it create environment suitable for migration of transformed cell from liver manifested by increase MMP-2 level in ascetic fluid from cirrhotic patients than those in HCC. On the other hand increase its level in sera of HCC patients than their ascetic fluid of the same patient indicating its role in hematogenous metastasis and tumor progression.

The current data together with other related investigations might promote potential assumptions that tumors not only arise as a result of successful escape from immune surveillance, but also as a result of breakdown of signaling network in normal cells due to persistent HCV infection that leads to their transformation into tumor cell. These events might lead to modification in surrounding microenvironment in order to survive and escape immune response, thus increasing the migration, invasion potential and facilitate tumor progression.

Reference

1. Liver Cancer Fact Sheet. Lyon: IARC; International Agency for Research on Cancer. Globocan 2012.
2. Bartosch B, Thimme R, Blum HE, Zoulim F. Hepatitis C virus-induced hepatocarcinogenesis. J. Hepatol 2009; 51: 810-20.
3. Moradpour DF, Penin, C. M. Rice. Replication of hepatitis C virus. Nat. Rev. Microbiol 2007; 5: 453-463.
4. Von HT, Yoon JC, Alter H, Rice CM, Rehermann B, Balfe P, et al. Hepatitis C virus continuously escapes from neutralizing antibody and T-cell responses during chronic infection in vivo. Gastroenterology 2007; 132: 667-78.
5. Levrero M. Viral hepatitis and liver cancer: The case of hepatitis C. Oncogene 2006; 25: 3834-47.
6. Banerjee A, Ray RB, Ray R. Oncogenic potential of hepatitis C virus proteins. Viruses 2010; 2: 2108-33.
7. Yadav L, Puri N, Rastogi V, Satpute P, Ahmad R, Kaur G. Matrix metalloproteinases and cancer -

roles in threat and therapy. Asian Pac J Cancer Prev 2014; 15 (3): 1085-91.

8. Gialeli C, Theocharis AD, Karamanos NK. Roles of matrix metalloproteinases in cancer progression and their pharmacological targeting. FEBS Journal 2011; 278: 16–27.
9. Kessenbrock K, Plaks V, Werb Z. Matrix metalloproteinases: Regulators of the tumor microenvironment. Cell 2010; 141: 52–67.
10. Radisky ES, Radisky DC. Matrix metalloproteinase induced epithelial-mesenchymal transition in breast cancer. Journal of Mammary Gland Biology and Neoplasia 2010; 15 (2): 201-12.
11. Visse R, Nagase H. Matrix metalloproteinases and tissue inhibitors of metalloproteinases: Structure, Function and Biochemistry. Circulation Research 2003; 92: 827-39.
12. Bonnans C, Chou J, Werb Z. Remodelling the extracellular matrix in development and disease. Nat. Rev. Mol. Cell Biol. 2014; 15: 786–801.
13. Krstic J, Santibanez JF. Transforming growth factor-beta and matrix metalloproteinases: Functional interactions in tumor stroma-infiltrating myeloid cells. The Scientific World Journal 2014; 10:115-29.
14. Abdelazim IA, Abu faza ML, Al kadi M. Immunoexpression of matrix metalloproteinase-2 (MMP-2) in epithelial ovarian cancers. Asian Pacific Journal of Reproduction 2013; 2(2); 136-41.
15. Zhao XI, Sun T, Che N, Sun D, Zhao N, Gu Q, et al. Promotion of hepatocellular carcinoma metastasis through matrix metalloproteinase activation by epithelial-mesenchymal transition regulator Twist1. J. Cell. Mol. Med. 2011; 15 (3): 691-700.
16. Xiang ZL, Zeng ZC, Fan J, Tang ZY, Zeng HY, Gao DM. Gene expression profiling of fixed tissues identified hypoxia-inducible factor-1alpha, VEGF, and matrix metalloproteinase-2 as biomarkers of lymph node metastasis in hepatocellular carcinoma. Clin Cancer Res 2011; 17(16): 5463-72.
17. Xiang ZL, Zeng ZC, Tang ZY, Fan J, Sun HC, Tan YS. Expression of cytokeratin 19 and matrix metalloproteinase 2 predicts lymph node metastasis in hepatocellular carcinoma. Mol Biol Rep 2011; 38: 3531-9.
18. Li Y, Zhang Q, Luo Z, Kang L, Qu J, Liu W, et al. Hepatitis C Virus activates Bcl-2 and MMP-2 expression through multiple cellular signaling pathways. J Virol 2012; 86: 12531-43.
19. Losikoff PT, Self AA, Gregory SH. Dendritic cells, regulatory T cells and the pathogenesis of chronic hepatitis C. Virulence 2012; 3: 610-20.
20. Smigielski J, Piskorz L, Talar-Wojnarowska R, Malecka-Panas E, Jablonski S, Brocki M . The estimation of metaloproteinases and their inhibitors blood levels in patients with pancreatic tumors. World J Surg Oncol 2013; 11: 137-44.
21. Wieland SF, Chisari FV. Stealth and Cunning: Hepatitis B and Hepatitis C Viruses. J Virol 2005; 79: 9369-80.
22. Kuyvenhoven JP, van Hoek B, Blom E, van Duijn W, Hanemaaijer R, Verheijen JH, et al. Assessment of the clinical significance of serum matrix metalloproteinases MMP-2 and MMP-9 in patients with various chronic liver diseases and hepatocellular carcinoma. Thromb Haemost. 2003; 89(4): 718-25.
23. Macías J, Mira J, Gilabert I, Neukam K, Roldán C, Viloria M, et al. Combined use of aspartate aminotransferase, platelet count and matrix metalloproteinase 2 measurements to predict liver fibrosis in HIV/hepatitis C virus-coinfected patients. HIV Med 2011; 12(1): 14-21.
24. Akca G, Tuncbilek S, SepiciDincel A. Association between matrix metalloproteinase (MMP)-2, MMP-9 and total antioxidant status of patients with asymptomatic hepatitis C virus infection. Letters in Applied Microbiology 2013; 57: 436-42.
25. El-Gindy I, El-Rahman AT, El-Alim MA, Zakj SS. Diagnostic potential of serum matrix metalloproteinase-2 and tissue inhibitor of metalloproteinase-1 as non invasive markers of hepatic fibrosis with HCV related chronic liver disease. Egypt J Immunol 2003; 10: 27-35.
26. Giannelli G, Bergamini C, Marinosci F, Fransvea E, Quaranta M, Lupo L, et al. Clinical role of MMP-2/TIMP-2 imbalance in hepatocellular carcinoma. Int J Cancer 2002; 97(4): 425-31.
27. Murawaki Y, Yamada S, Ikuta Y, Kawasaki H. Clinical usefulness of serum matrix metalloproteinase-2 concentration in patients with chronic viral liver disease. J Hepatol 1999; 30(6): 1090-8.
28. Boeker KH, Haberkorn CI, Michels D, Flemming P, Manns MP, Lichtinghagen R. Diagnostic potential of circulating TIMP-1 and MMP-2 as markers of liver fibrosis in patients with chronic hepatitis C. Clin Chim Acta 2002; 316: 71-81.
29. Sumer S, Demir NA, Kölgelier S, Inkaya AC, Arpaci A, Demir LS, et al. The clinical significance of serum apoptotic cytokeratin 18 neoepitope M30 (CK-18 M30) and matrix metalloproteinase 2 (MMP-2) levels in chronic hepatitis B atients with cirrhosis. Hepat Mon 2013; 13(6): 1-6.
30. Ishii Y, Nakasato Y, Kobayashi S, Yamazaki Y, Aoki T. A study on angiogenesis-related matrix metalloproteinase networks in primary hepatocellular carcinoma. J. Exp. Clin. Cancer Res 2003; 22: 461-71.
31. Ljumovic D, Diamantis I, Alegakis AK, Kouroumalis EA. Differential expression of matrix metalloproteinases in viral and non-viral chronic liver diseases. Clin. Chim. Acta 2004; 349: 203-11.

32. Prystupa A, Boguszewska-Czubara A, Bojarska-Junak A, Toruń-Jurkowska A, Roliński J, Załuska W. Activity of MMP-2, MMP-8 and MMP-9 in serum as a marker of progression of alcoholic liver disease in people from Lublin Region, eastern Poland. Annals of Agricultural and Environmental Medicine 2015; 22: 325-8.
33. Noh S, Jung M, Kim TS, Park CH, Lim SJ, Chung HC, et al. MMP-2 as a putative biomarker for carcinomatosis in gastric cancer. Hepato-Gastroenterology 2011; 58: 2015-9.
34. Noh S, Jung M, Lee HY, Wang B, Cho J, Kim TS, et al. Body fluid MMP-2 as a putative biomarker in metastatic breast cancer. Oncology Letters 2012; 3: 699-703.
35. Manenti L, Paganoni P, Floriani I, Torri W, Buda A, Taraboletti G, et al. Expression level of vascular endothelial growth factor, matrix metalloproteinase 2 & 9 and tissue inhibitor of metalloproteinases 1 & 2 in plasma of patients with ovarian carcinoma. Eur. J. Cancer 2003; 39: 1948-56.
36. Giavazzi R, Garofalo A, Ferri C, Lucchini V, Bone E A, Chiari S, et al. A synthetic inhibitor of matrix metalloproteinases, potentiates the antitumor activity of cisplatin in ovarian carcinoma xenografts. Clin. Cancer Res 1998; 4: 985-92.
37. Belotti D, Paganoni P, Manenti L, Garofalo A, Marchini S, Taraboletti G, et al. Matrix metalloproteinases (MMP9 and MMP2) induce the release of vascular endothelial growth factor (VEGF) by ovarian carcinoma cells: Implications for ascites formation. Cancer Research 2003; 63: 5224-9