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HMGB1 and SEPP1 as predictors of hepatocellular carcinoma in patients with viral C hepatitis: Effect of DAAs

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ABSTRACT

Background: Hepatitis C viral infection (HCV) and hepatocellular carcinoma (HCC) are potential health problems. New directly acting antivirals (DAAs) changed HCV treatment strategies. Selenoprotein P1 (SEPP1) is a hepatokine implicated in HCC pathogenesis. High mobility group box1 (HMGB1), a nuclear DNA-binding protein, involved in immune and inflammatory responses in HCV and HCC. Therefore, the aim of current study was to investigate HMGB1 and SEPP1 levels in HCV and HCV + HCC patients and exploring DAAs effect on them.
Methods: 15 healthy volunteers, 25 HCV and 25 HCV + HCC patients were included. Liver function tests, alpha fetoprotein (AFP), SEPP1 and HMGB1 serum levels were evaluated. For HCV group blood samples before and after treatment with sofosbuvir/daclatasvir combination were collected.
Results: HMGB1 was significantly higher in HCV + HCC group than in control and HCV groups ($p < .05$). SEPP1 decreased significantly in HCV and HCV + HCC groups compared to control group ($p < .001$). SEPP1 significantly elevated after treatment with DAAs ($p = .001$). HMGB1 and SEPP1 were negatively correlated with each other in HCV group ($p = .047$). Logistic regression analysis showed that HMGB1 and SEPP1 could be used as predictors for HCC in HCV infected patients ($p = .02$,$p = .002$) respectively. Receiver operating characteristic curve (ROC) revealed HMGB1 had 32% sensitivity and 100% specificity in differentiating HCV from HCV + HCC patients, both SEPP1 and HMGB1 had high sensitivity (92%,60%) and 93% specificity in differentiating healthy from HCV + HCC group.
Conclusion: HMGB1 and SEPP1 are involved in pathogenesis of HCV and HCV induced HCC. Both of them could serve as predictive biomarkers for HCC in HCV patients.

1. Introduction

Hepatitis C virus (HCV) is a potential disease worldwide, about 3% of the world's inhabitants are HCV infected and > 170 million are chronically infected and having risk of liver cirrhosis and/or hepatocellular carcinoma (HCC) development [1].On the other hand HCC is the fifth common type of cancer, the third major reason of cancer-related death, also it is responsible for about one million deaths annually [2]. Its incidence is increasing dramatically due to several risk factors as HCV, hepatitis B viral infection (HBV), nonalcoholic fatty liver disease, primary biliary cirrhosis, food contamination with carcinogens such as aflatoxin, cirrhosis and smoking [3].

Nowadays unlike HBV, HCV is curable. Ten to fifteen million patients are infected with HCV genotype 4 (HCVG4); large percentage is found in Egypt (14.7%) where HCVG4 represents > 90% of the infected population [4].

Ideal treatment for patients with HCVG4 is changing rapidly; in the past pegylated interferon/ribavirin combination showed good response rates and extensive side effects [5]. Modern progress in treatment development emerged a number of directly acting antiviral agents (DAAs) that achieve better sustained virologic response (SVR) with minimal side effects. One of these DAAs is sofosbuvir, an HCV NS5B polymerase inhibitor, has newly been permitted for HCV cure in Egypt [6].

Hepatokines are group of liver derived proteins that have a role in metabolism of lipid and glucose in liver, skeletal muscles and adipose tissue. One of them is selenoprotein P1 (SEPP1) which acts as selenium transporter [7]. Studies revealed that serum SEPP1 levels slightly increased in HCV and significantly elevated in type2 diabetic patients

Abbreviations: HCV, hepatitis C virus; HCC, hepatocellular carcinoma; HMGB1, high mobility group box1; SEPP1, selenoprotein P1; DAAs, direct acting antiviral agents; AFP, alpha fetoprotein; AST, aspartate transaminase; ALT, alanine transaminase

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with HCV [1]. Also SEPP1 has a major role as an antioxidant as it is involved in peroxynitrate degradation, that plays a role in inflammatory toxicity [8]. Hepatic disease can affect selenoproteins hepatic synthesis as SEPP1 and selenium (Se) containing proteins as albumin [9]. Also small intestine mucosal abnormalities in cirrhotic patients lead to Se malabsorption and this could contribute to decreased levels of serum SEPP1 [10]. Decreased expression and decreased level of SEPP1 were reported in different types of cancer as renal cell carcinoma, colorectal adenoma, prostate cancer, and gastric adenocarcinoma [3]. Additionally, quantitative analysis of serum SEPP1 in other studies revealed marked decrease in SEPP1 levels in newly diagnosed HCC patients with or without HCV compared to chronic liver disease and healthy groups [11].

High mobility group box1 (HMGB1) is a non-histone, nuclear DNA-binding protein. Its role relies on its position in the cell. In the nucleus, it is involved in a number of important DNA events [12]. Under certain conditions as starvation and exposure to reactive oxygen species (ROS), HMGB1 translocates to the cytoplasm and transits to the extracellular environment [13]. HMGB1 as an extracellular signaling molecule has inflammatory and carcinogenetic roles, it is actively released from natural killer cells, monocytes and active macrophages and passively released from necrotic cells in cancer tissues resulting in local inflammation. HMGB1 serum levels increased significantly in chronic hepatitis leading to progression to liver fibrosis [14]. Previous studies revealed that toll like receptors (TLRs), specifically TLR4 signaling, contribute in pathogenic fibrosis in hepatic diseases of several causes. Being endogenous TLR4 ligand, HMGB1 could be secreted during hepatic injury. It contributes in liver fibrosis via TLR4 signaling [15]. In addition, TLR4 signaling is implicated in progression of HCC in HBV patients leading to increased proliferation and prevention of apoptosis [16]. Previous studies demonstrated that serum HMGB1 levels increased significantly in HCC compared to chronic hepatitis and cirrhotic groups. Such findings propose that HMGB1 might have a crucial role in HCC progression and many issues may lead to increase in HMGB1 levels in HCC patients due to production and secretion of HMGB1 by HCC cells [17]. However the exact role of HMGB1 in HCV or in HCV + HCC needs further elucidation.

Therefore, the present study aimed at investigating serum levels of SEPP1 and HMGB1 in HCV and HCV + HCC patients and evaluating their possible utilization as non-invasive predictors for HCC in HCV patients, as well as examining the effect of DAAs therapy on their serum levels in HCV patients.

2. Subjects and methods

This study was carried out in Cairo, Egypt. Sixty five subjects ($n = 65$) were included in this study and divided into three groups. The first group was the control group, it comprises 15 healthy volunteers ($n = 15$), the second group comprises 25 naïve patients infected with chronic HCVG4 ($n = 25$), it received DAAs therapy for 12 weeks ($n = 25$), and the third group comprises 25 HCV patients with HCC ($n = 25$). For the second group, blood samples were collected before and after anti-viral treatment combination. The healthy participants had no preceding hepatic diseases or tumor history. All patients were histopathologically diagnosed and underwent serum viral test. All participants were age- and sex-matched. They were recruited from Medical Ain Shams Research Institute (MASRI viral hepatitis research and treatment unit) faculty of Medicine Ain Shams University and Manshyet EL Bakry general hospital, Cairo, Egypt. For inclusion, patients infected with HCVG4, their age was 40–60 years. For exclusion the patients should not have history of co-infections, alcohol abuse, thyroid dysfunction, type 2 diabetes, HBV infection, autoimmune diseases and HCC due to etiologies other than HCV.

Before treatment, full medical history was obtained from all participants including history of previous HCV treatment and any features of decompensated cirrhosis and a thorough clinical examination. Baseline laboratory studies were done including liver function tests as serum aspartate transaminase (AST), alanine transaminase (ALT), albumin, total bilirubin also white blood cells (WBCs), international normalized ratio (INR), platelet count, alpha fetoprotein (AFP), SEPP1 and HMGB1 were measured.

HCV infected patients were scheduled to receive combined therapy of sofosbuvir 400 mg and daclatasvir 60 mg daily for 12 weeks, and after the completion of treatment blood samples were withdrawn again, while HCC patients did not receive this combination therapy.

Blood samples obtained from all patients were collected in vacutainer serum collecting tubes then centrifuged for serum separation. The separated serum then divided into aliquots and stored at −80 °C till measurements. A quantitative measurement serum viral load of HCV was carried out by real-time PCR and it then repeated at the end of treatment.

This study was approved by the local research ethics committee of faculty of Pharmacy Ain-Shams University, and a written informed consent was obtained from all participants in this study. The study was conducted in accordance with the provisions of the declaration of Helsinki and Good Clinical Practice guidelines.

3. Laboratory measurements

Serum aminotransferases (AST and ALT), total bilirubin and albumin were assayed colorimetrically using kits of (Biodiagnostics, Egypt) by using appropriate photometer (Shimadzu UV-1650 PC, Japan). Serum SEPP1 was determined using commercially available ELISA kits from (Elabscience, China, Catalog No. E-EL-H2177) according to sandwich-ELISA method. The micro ELISA plate was precoated with specific antibodies to human SEPP1. Standards and samples were added to proper wells for binding to the antibodies. Biotinylated detection antibody specific for human SEPP1 and horseradish peroxide HRP conjugate were then added successively to each well in the microplate. After incubation, unbound components were washed away. When the substrate solution was added, only wells with human SEPP1, biotinylated detection antibody and avidin-HRP complex appeared blue in color. Then stop solution was added and the color became yellow. The optical density (OD) then measured spectrophotometrically at wavelength of 450 nm ± 2 nm. The OD value was directly proportional to human SEPP1 concentration.

Serum HMGB1 was measured by using human HMGB1 ELISA kit (Elabscience, China, Catalog No. *E*-EL-H1554) according to sandwich-ELISA method. Level of AFP was estimated using ELISA kit (Chemux BioScience, INC., USA, Catalog No. 10101). SEPP1 and AFP kit principle were similar to that of HMGB1 kit.

ELISA had been conducted in accordance to the manufacturer's directions using Chromate microplate reader.

3.1. Statistical analysis

IBM SPSS statistics (V. 22.0, IBM Corp., USA, 2013) was used for data analysis. Data were expressed as mean ± SEM for quantitative parametric measures, in addition to median percentiles for quantitative non-parametric measures and both number and percentage for categorized data. Student's *t*-test was used for comparison of two independent groups for parametric data and Wilcoxon Rank Sum for non-parametric data. However, for comparison between > 2 groups for parametric data we used analysis of variance (ANOVA) and Kruskall Wallis for non-parametric data. For statistical comparisons within HCV group, Chi-square test and Mann–Whitney *U* test were used as appropriate. Covariates with a $p < .05$ at univariate analysis were included in a multivariate logistic regression model, and results were reported as odds ratio (OR) and their 95% confidence intervals (CIs). A receiver operating characteristics (ROC) analysis was used to evaluate overall predictive value and cut-off level of the test and to detect the area under the curve which reflects the sensitivity, specificity, positive predictive

Table 1
Demographic data and laboratory characteristics of the studied groups.

Parameter	Control (n = 15)	HCV (n = 25)	HCV + HCC (n = 25)	*P*1	*P*2	*P*3
Age (years)	43.47 ± 0.92	46.6 ± 2.3	54.52 ± 1.57	–	–	–
Gender M/F	5/10	11/14	19/6	–	–	–
Fibrosis stage F0/F1/F2/F3/F4	–	9/12/2/2/0	0/0/0/9/16	–	–	–
AST (IU/L)	34.4 ± 1.23	46 ± 2.06	68.4 ± 3.92	0.013	0.000	0.000
ALT (IU/L)	18 ± 1.32	42.8 ± 3.87	51.84 ± 2.53	0.000	0.000	0.032
Total bilirubin (μmol/L)	66.3 ± 5.3	61.88 ± 3.5	155.58 ± 21.2	0.852	0.000	0.000
Albumin (g/dL)	6.53 ± 0.3	4.06 ± 0.07	2.93 ± 0.15	0.000	0.000	0.000
AFP (ng/ml)	3.09 ± 0.17	10.5 ± 0.55	70.06 ± 2.67	0.01	0.000	0.000
HMGB1 (pg/ml)	820.68 ± 31.25	899.69 ± 19.05	1014.97 ± 51.38	0.184	0.002	0.027
SEPP1 (ng/ml)	11.44 ± 0.29	9.01 ± 0.31	8.17 ± 0.33	0.000	0.000	0.053

Results are represented as mean ± SEM. *P*1 control group compared to HCV group, *P*2 control group compared to HCV + HCC group, *P*3 HCV group compared to HCV + HCC group. AST: aspartate transaminase, ALT: alanine transaminase, AFP: alpha fetoprotein, HMGB1: high mobility group box 1, SEPP1: selenoprotein P1, HCV: hepatitis C virus, HCC: hepatocellular carcinoma.

value (PPV), Negative predictive value (NPV), and the accuracy of SEPP1 (ng/ml) and HMGB1 (pg/ml) as diagnostic biomarkers in the development of HCC in HCV patients. For all tests, $p < .05$ was considered statistically significant.

4. Results

The clinical and laboratory variables of the studied subjects are shown in Table 1. Regarding liver function tests albumin levels showed a significant decrease in both HCV and HCV + HCC groups compared to control group ($p < .001$). Both AST and ALT levels were significantly higher in HCV group compared to control group ($p < .05$, $p < .001$) and also showed significant increase in HCV + HCC group compared to control group ($p < .001$) and to HCV group ($p < .001$, $p < .05$). Total bilirubin showed slight increase in HCV group compared to control group, however a high significant increase in HCV + HCC group was found compared to control group and HCV group ($p < .001$).

Notably, HMGB1 showed slight increase from 820.68 ± 31 pg/ml in control group to 899.69 ± 19 pg/ml in HCV group while significant increase was found from 820.68 ± 31 pg/ml in control group to 1014.97 ± 51 pg/ml in HCV + HCC group ($p < .01$), and from 899.69 ± 19 pg/ml in HCV group to 1014.97 ± 51 pg/ml in HCV + HCC group ($p < .05$).(Table 1).

On the other hand SEPP1 showed a significant decrease from 11.44 ± 0.29 ng/ml in control group to 9.01 ± 0.31 ng/ml in HCV group and from 11.44 ± 0.29 ng/ml in control group to 8.17 ± 0.33 ng/ml in HCV + HCC group ($p < .001$).(Table 1).

The effect of treatment with DAAs on levels of AST, ALT, total bilirubin, albumin, AFP, HMGB1 and SEPP1 was found as follows:

AST, ALT and total bilirubin showed significant decrease in HCV group after treatment ($p < .001$), while albumin level increased significantly after treatment ($p < .05$).(Table 2).

Also both platelet count and INR revealed significant decrease in HCV group after treatment ($p < .05$), but WBC's showed non-significant decrease. AFP showed significant decrease after treatment ($p < .001$).(Fig. 1A).

While HMGB1 showed slight decrease from 899.69 ± 19 pg/ml in HCV group before treatment to 830.48 ± 28 pg/ml after treatment (Fig. 1B) and significant increase was found with SEPP1 from 9.01 ± 0.31 ng/ml before treatment to 10.17 ± 0.29 ng/ml after treatment ($p < .01$).(Fig. 1C).

Pearson correlation revealed that HMGB1 was significantly positively correlated with ALT, total bilirubin, AFP and negatively correlated with platelet count and albumin. (Table 3). Also it revealed that SEPP1 level was positively correlated with albumin, platelet count and negatively correlated with AST, ALT, total bilirubin and AFP (Table 3). Interestingly HMGB1 and SEPP1 were negatively correlated with each

Table 2
Laboratory results in HCV group patients before and after treatment with DAAs.

Parameter	HCV before treatment (n = 25)	HCV after treatment (*n* = 25)	*P*-value
Viral load	2,305,443 ± 1,613,997	–	–
AST (IU/L)	46 ± 2.06	32.12 ± 1.63	0.000
ALT (IU/L)	42.8 ± 3.87	23.96 ± 0.98	0.000
Total bilirubin (μmol/L)	61.88 ± 3.5	53.92 ± 3.5	0.015
Albumin (g/dL)	4.06 ± 0.07	4.60 ± 0.22	0.036
Platelet count (10^3/μL)	257.48 ± 12.92	235.08 ± 10.19	0.035
WBC (10^3/μL)	7.6 ± 0.43	7.29 ± 0.36	0.573
INR	1.1 ± 0.02	1.06 ± 0.02	0.038

Results are represented as mean ± SEM. AST: Aspartate transaminase, ALT: Alanine transaminase, DAAs: Direct acting antivirals, WBC: white blood cells, INR: international normalized ratio.

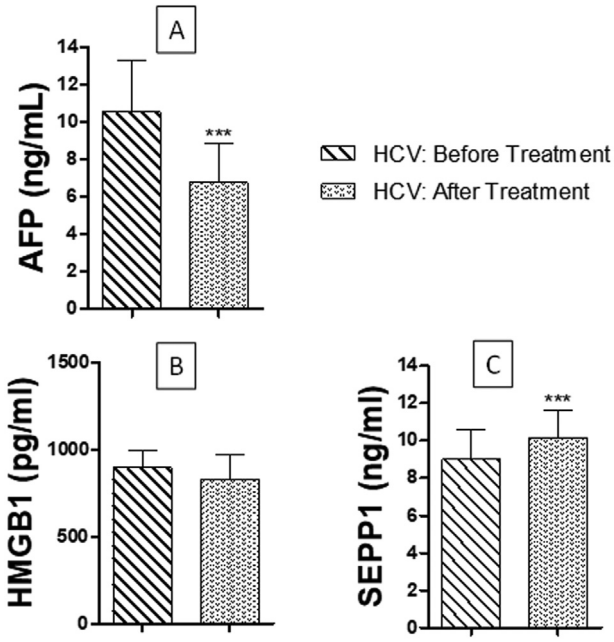

Fig. 1. Serum levels of AFP (A), HMGB1 (B) and SEPP1 (C) in HCV patients before and after treatment with DAAs. *** $P < .001$.
AFP: alpha fetoprotein; HMGB1: high mobility group box 1; SEPP1:selenoprotein P 1; HCV: hepatitis C virus.

Table 3
Correlation between High mobility group box1 (HMGB1), Selenoprotein P1 (SEPP1) and other studied parameters in all the studied groups.

	HMGB1		SEPP1	
Variable	r	*P*	r	*P*
AST	0.241	0.053	−0.611	0.000***
ALT	0.254	0.041*	−0.504	0.000***
Total bilirubin	0.352	0.004**	−0.332	0.007**
Albumin	−0.38	0.002**	0.497	0.000***
AFP	0.349	0.004**	−0.611	0.000***
Platelet count	−0.492	0.013*	0.511	0.009**
WBC	−0.167	0.426	0.113	0.591
INR	0.255	0.219	0.011	0.959
SEPP1	−0.163	0.194	–	–

AST: Aspartate transaminase; ALT: Alanine transaminase; AFP: Alpha fetoprotein; WBC: White blood cells; INR: International normalized ratio; HMGB1: High mobility group box1; SEPP1:Selenoprotein P1.* significant; ** and *** both are highly significant.

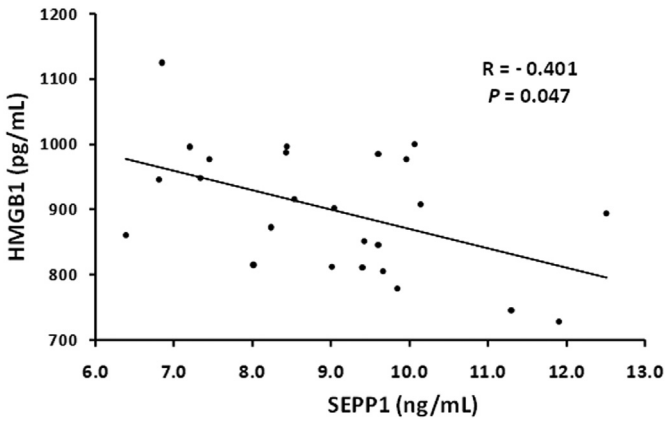

Fig. 2. Correlation between HMGB1 and SEPP1 in HCV group.
HMGB1: high mobility group box 1; SEPP1: selenoprotein P 1.

other in HCV group (p = .047) (Fig. 2).

Logistic regression analysis revealed that serum SEPP1 could be used as predictor marker of HCV group from control group (OR = 0.268; 95%CI:0.118–0.607; p = .002) and for HCV + HCC group from the control group (OR = 0.135; 95%CI:0.039–0.471; p = .002).

Also it showed that serum HMGB1 could be used as predictor marker for HCV + HCC group from the control group (OR = 1.006; 95%CI:1.001–1.011; p = .02) and for HCV + HCC group from HCV group (OR = 1.004; 95%CI:1.000–1.007; p = .05).

Regarding the differentiation between healthy subjects and HCV + HCC patients the ROC analysis detected 92% sensitivity and 93.33% specificity with a best cut off value of 10.11 ng/ml for SEPP1 and 60% sensitivity and 93.33% specificity with a best cut off value of 941 pg/ml for HMGB1. (Fig. 3).

5. Discussion

Millions of HCV infected population all over the world develop a number of chronic liver diseases as hepatic fibrosis and cirrhosis in about 20% of patients and within 5 years about 10–20% of patients with cirrhosis might develop HCC [2]. HCC is the most common cause of death in HCV infected patients, and epidemiological findings propose that the death rate is growing [18]. A chief result of chronic HCV infection is production of free radicals causing oxidative DNA damage and this induces mutations, epigenetic change and genomic instability via proinflammatory cytokine activities leading to oncogenes activation and tumor suppressor genes inactivation leading to inflammation associated carcinogenesis [19]. Therefore, the current study aimed to investigate serum levels of SEPP1 and HMGB1 in HCV and HCV + HCC patients and to evaluate their possible utilization as non-invasive predictors for HCC in HCV patients, as well as to examine the effect of DAAs therapy on their serum levels in HCV patients.

Introduction of highly potent DAAs improved the management, clinical outcomes and overall prognosis of HCV patients. DAAs were demonstrated to be highly efficacious in HCV eradication even in cirrhotic patients [20]. DAAs induced downregulation of circulating proinflammatory cytokines, chemokines, and growth factors causing improvement in liver function [21].

This study investigated HMGB1 level in HCV patients and healthy people. Significant increased HMGB1 was detected in chronic hepatitis infected patients compared to healthy people. HMGB1 is released into the extracellular milieu during infection with HBV or HCV. HMGB1 could be important in initiation and mediation of hepatitis [22]. HMGB1 might act as chemotactic factor in smooth muscle cells, endothelial cells and fibrocytes which participate in fibrogenic reactions and injury [15].

In agreement with those studies this study revealed an association between HCV infection and increased HMGB1 level. Additionally, significant high levels were observed in HCV patients who developed HCC compared to healthy group.

HMGB1 was found to play essential role in HCC development and in metastasis and bad outcomes. HMGB1 induces several intracellular signaling pathways when binds to its receptor; the receptor for advanced glycation end products (RAGE). This explains why immature and malignant cells express high HMGB1 and RAGE levels [17]. Furthermore, HMGB1 upregulates mitochondrial biogenesis in HCC cells promoting tumor survival and proliferation through stimulation of TLRs signaling pathways [23].

Also this study detected significant positive correlation between AFP and HMGB1 levels which discussed by earlier study of Cheng et al. [17] that was carried on HCC patients with HBV in majority and only few HCC patients with HCV. In contrast, the current study was carried on HCC patients with HCV only and not HBV. In addition this study found that HMGB1 had significant positive correlation with ALT and total bilirubin and significant negative correlation with albumin and platelet count.

Concerning the effect of DAAs on serum levels of HMGB1 this study found only a slight decrease after treatment. Although a change in the inflammatory pattern is coinciding with HCV clearance, several baseline factors like advanced liver disease, fatty liver and/or markers of liver steatosis were reported to contribute to persistent inflammation after completion of DAAs treatment [24].

SEPP1 is a liver-derived secretory protein and the major selenoprotein in plasma acting as selenium transporter. SEPP1 as an antioxidant protects LDL and plasma proteins against oxidation [25]. Liver injury could impair selenoproteins hepatic synthesis as SEPP1 and proteins containing Se as albumin, leading to decreased Se levels [9].

Although a previous study showed slight increase in serum SEPP1 in HCV patients [1] this study found significant decrease in serum SEPP1 in HCV group when compared to control group. Another study revealed down regulation of SEPP1 expression in patients with HBV leading to decrease in the defense ability against lipid peroxidation [26].

Further, an earlier study revealed that decrease in serum Se levels was significantly related with liver fibrosis severity in HCV patients. The decline of SEPP1 activity is also speculated to be in proportion to Se decrease because SEPP1 serves as its carrier protein [10].

It was reported that therapeutic doses of DAAs as sofosbuvir and daclatasvir are hepatoprotective due to their antioxidant effect as they significantly reduce oxidative stress parameters as malondialdehyde, superoxide dismutase and catalase activities in hepatic tissue and similarly cause a decrease in hepatic tissue content of tissue inhibitor metalloproteinase-1and tumor necrosis factor alpha [27].

Although there are few studies that examine DAAs effect on SEPP1

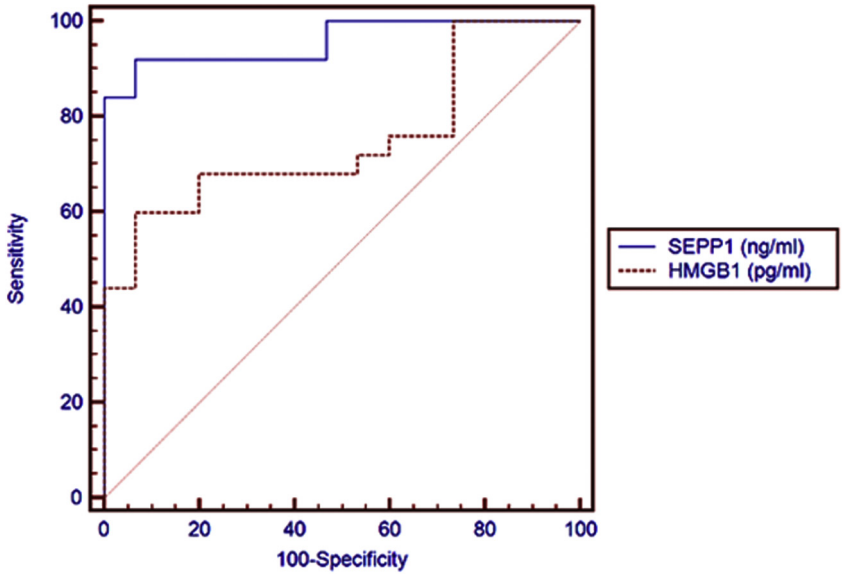

Fig. 3. ROC curve for SEPP1(ng/ml) and HMGB1 (pg/ml) as predictors to differentiate between control group and HCV + HCC group.
ROC: receiver operating characteristic; SEPP1:selenoprotein P1; HMGB1:high mobility group box1; HCV: hepatitis C virus; HCC:hepatocellular carcinoma.

levels and oxidation state in HCV in general, this study showed a highly significant increase in SEPP1 level in HCV group after treatment compared to HCV group before treatment with DAAs, and this discusses the antioxidant role of DAAs.

This study showed a significant negative correlation between HMGB1 and SEPP1 in HCV group which explains that both of them may have common molecular mechanism and this was explained by several studies that revealed direct relation between them and some growth factors. One of them found that HMGB1 might play role in upregulation of vascular endothelial growth factor-A in retinal ganglion cells after exposure to advanced glycation end products leading to bad prognosis of diabetic retinopathy [28]. Another study revealed that SEPP1 is negatively regulated by transforming growth factor ß1 that plays chief role in processes of inflammation as wound healing so that SEPP1 levels in patients of intensive care suffering from systemic inflammatory response syndrome or sepsis showed extremely decreased plasma SEPP1 levels [29].

Interestingly logistic regression analysis of this study showed that SEPP1 and HMGB1 may be used as predictive biomarkers for HCC. Additionaly, it revealed that HMGB1 could be used as predictive biomarker for HCC in HCV patients.

The ROC analysis performed in this study indicated a moderate sensitivity but high specificity for predictive validity of HMGB1 in differentiation between HCV and HCV + HCC patients. It revealed high sensitivity and specificity for predictive validity of both SEPP1 and HMGB1 in differentiating between healthy subjects and HCV + HCC patients knowing that SEPP1 has higher sensitivity than HMGB1. These findings should encourage clinicians to include both cytokines in their screening profiles for HCV and HCC patients.

6. Conclusion

This study indicates crucial role for HMGB1 and SEPP1 in HCV and HCV induced HCC and both of them may be targets of treatment to prevent progression of HCC. Also this study suggests that both serum HMGB1 and SEPP1 could serve as predictive biomarkers for HCC in HCV patients. However, the small number of patients is one of its limitations. So further studies on larger scale should be conducted to confirm our findings.

Disclosure of interest

The authors declare that they have no competing interest.

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All authors have approved the final article.

References

[1] S.A. Ali, W.M.H. Nassif, D.H.A. Abdelaziz, Alterations in serum levels of fetuin a and selenoprotein P in chronic hepatitis C patients with concomitant type 2 diabetes: a case-control study, Clin Res Hepatol Gastroenterol 40 (2016) 465–470, https://doi.org/10.1016/j.clinre.2015.12.003.

[2] Y. Han, J. Niu, D. Wang, Y. Li, Hepatitis C virus protein interaction network analysis based on hepatocellular carcinoma, PLoS One 11 (2016) 1–13, https://doi.org/10.1371/journal.pone.0153882.

[3] S. Guariniello, G. Di Bernardo, G. Colonna, M. Cammarota, G. Castello, S. Costantini, Evaluation of the selenotranscriptome expression in two hepatocellular carcinoma cell lines, Anal. Cell. Pathol. (2015), https://doi.org/10.1155/2015/419561.

[4] E.M. El-gindy, E.F.A. Mostafa, I.M. Montasser, F.M. Ismail, Comparative study between different modalities of treatment of HCV in new era of direct acting antiviral drugs (DAAs) in Aswan governorate, Egypt J Hosp Med 71 (2018) 3591–3600, https://doi.org/10.12816/0047680.

[5] M.A.A. Claassen, Knegt R.J. De, D. Turgut, Z.M.A. Groothuismink, H.L.A. Janssen, Negative regulation of hepatitis C virus specific immunity is highly heterogeneous and modulated by Pegylated interferon-alpha/ribavirin therapy, PLoS One 7 (2012), https://doi.org/10.1371/journal.pone.0049389.

[6] A. Elsharkawy, R. Fouad, W. El Akel, M. El Raziky, M. Hassany, G. Shiha, et al., Sofosbuvir-based treatment regimens: real life results of 14 409 chronic HCV genotype 4 patients in Egypt, Aliment. Pharmacol. Ther. 45 (2017) 681–687.

[7] T.W. Jung, H.J. Yoo, K.M. Choi, Implication of hepatokines in metabolic disorders and cardiovascular diseases, BBA Clin 5 (2016) 108–113, https://doi.org/10.1016/j.bbacli.2016.03.002.

[8] A.J. Pellatt, R.K. Wolff, E.M. John, G. Torres-Mejia, L.M. Hines, K.B. Baumgartner, et al., SEPP1 influences breast cancer risk among women with greater native american ancestry: the breast cancer health disparities study, PLoS One 8 (2013), https://doi.org/10.1371/journal.pone.0080554.

[9] R.M. Rua, M.L. Ojeda, F. Nogales, J.M. Rubio, M. Romero-Gómez, J. Funuyet, et al., Serum selenium levels and oxidative balance as differential markers in hepatic damage caused by alcohol, Life Sci. 94 (2014) 158–163, https://doi.org/10.1016/j.lfs.2013.10.008.

[10] T. Himoto, H. Yoneyama, K. Kurokohchi, M. Inukai, H. Masugata, F. Goda, et al., Selenium deficiency is associated with insulin resistance in patients with hepatitis C virus-related chronic liver disease, Nutr. Res. 31 (2011) 829–835, https://doi.org/10.1016/j.nutres.2011.09.021.

[11] M. Tarek, M.L. Louka, E. Khairy, R. Ali-Labib, D.Z. Zaky, I.F. Montasser, Role of

microRNA-7 and selenoprotein P in hepatocellular carcinoma, Tumor Biol. 39 (2017), https://doi.org/10.1177/1010428317698372.
[12] R. Kang, R. Chen, Q. Zhang, W. Hou, S. Wu, L. Cao, et al., HMGB1 in health and disease, Mol. Asp. Med. 40 (1998) 1–116, https://doi.org/10.1016/j.mam.2014.05.001.
[13] D. Wu, H. Liang, H. Wang, C. Duan, H. Yazdani, J. Zhou, et al., Hepatitis B virus-X protein regulates high mobility group box 1 to promote the formation of hepatocellular carcinoma, Oncol. Lett. 16 (2018) 4418–4426, https://doi.org/10.3892/ol.2018.9178.
[14] A. Kargi, Ö. Demirpençe, Ş. Gündüz, S. Göktaş, A.S. Alikanolu, M. Yildirim, Serum levels of HMGB1 have a diagnostic role in metastatic renal cell cancer, Cancer Biomarkers 17 (2016) 17–20, https://doi.org/10.3233/CBM-160611.
[15] Z. Zhang, C. Lin, L. Peng, Y. Ouyang, Y. Cao, J. Wang, et al., High mobility group box 1 activates toll like receptor 4 signaling in hepatic stellate cells, Life Sci. 91 (2012) 207–212, https://doi.org/10.1016/j.lfs.2012.07.009.
[16] Y. Wang, J. Cai, X. Zeng, Y. Chen, W. Yan, Y. Ouyang, et al., Downregulation of toll-like receptor 4 induces suppressive effects on hepatitis B virus-related hepatocellular carcinoma via ERK1/2 signaling, BMC Cancer 15 (2015) 1–9, https://doi.org/10.1186/s12885-015-1866-9.
[17] B.Q. Cheng, C.Q. Jia, C.T. Liu, X.F. Lu, N. Zhong, Z.L. Zhang, et al., Serum high mobility group box chromosomal protein 1 is associated with clinicopathologic features in patients with hepatocellular carcinoma, Dig. Liver Dis. 40 (2008) 446–452, https://doi.org/10.1016/j.dld.2007.11.024.
[18] M. Sakae, S. Kubo, S. Takemura, C. Sakata, T. Uenishi, S. Kodai, et al., Effect of interferon therapy on first and second recurrence after resection of hepatitis C virus-related hepatocellular carcinoma, Hepatol. Res. (2012) 564–573, https://doi.org/10.1111/j.1872-034X.2011.00959.x.
[19] S.M. Shawki, S.S. Meshaal, A.S. El Dash, N.A. Zayed, M.O. Hanna, Increased DNA damage in hepatitis C virus-related hepatocellular carcinoma, DNA Cell Biol. 33 (2014) 884–890, https://doi.org/10.1089/dna.2014.2417.
[20] J.D. Yang, M.D. Leise, Damned if you do, damned if you don't: the evolving story of de novo and recurrent hepatocellular carcinoma amongst those treated with direct-acting antivirals for hepatitis C virus, Liver Int. 37 (2017) 809–811, https://doi.org/10.1111/liv.13422.
[21] G.N. Saraiva, N. Fonseca, T. Medeiros, P. Emílio, C. Leite, G.D.S. Lacerda, et al., Restoring inflammatory mediator balance after Sofosbuvir- induced viral clearance in patients with chronic hepatitis C, Mediat. Inflamm. 2018 (2018), https://doi.org/10.1155/2018/8578051.
[22] L. Zhang, J. Han, H. Wu, X. Liang, J. Zhang, J. Li, et al., The association of HMGB1 expression with Clinicopathological significance and prognosis in hepatocellular carcinoma: a meta-analysis and literature review, PLoS One 9 (2014) 1–9, https://doi.org/10.1371/journal.pone.0110626.
[23] S. Tohme, H.O. Yazdani, Y. Liu, P. Loughran, Windt D.J. Van Der, H. Huang, et al., Hypoxia mediates mitochondrial biogenesis in hepatocellular carcinoma to promote tumor growth via HMGB1 and TLR9 interaction, Hepatology 66 (2018) 182–197, https://doi.org/10.1002/hep.29184.
[24] C. Welsch, M. Efinger, M. von Wagner, E. Herrmann, S. Zeuzem, T.M. Welzel, C.M. Lange, Ongoing liver inflammation in patients with chronic hepatitis C and sustained virological response, PLoS One 12 (2) (2017) e0171755, , https://doi.org/10.1371/journal.pone.0171755.
[25] H. Steinbrenner, E. Bilgic, L. Alili, H. Sies, P. Brenneisen, Selenoprotein P protects endothelial cells from oxidative damage by stimulation of glutathione peroxidase expression and activity, Free Radic. Res. 40 (2006) 936–943, https://doi.org/10.1080/10715760600806248.
[26] Y.S. Yi, S.G. Park, S.M. Byeon, Y.G. Kwon, G. Jung, Hepatitis B virus X protein induces TNF-α expression via down-regulation of selenoprotein P in human hepatoma cell line, HepG2, Biochim. Biophys. Acta Mol. basis Dis. 1638 (2003) 249–256, https://doi.org/10.1016/S0925-4439(03)00090-5.
[27] M.A. Ibrahim, A. Abdel-aziz, A. El-sheikh, M. Kamel, A. Khalil, H. Abdelhaleem, Hepatic effect of sofosbuvir and daclatasvir in thioacetamide-induced liver injury in rats, Clin Exp Hepatol (2018) 175–181, https://doi.org/10.5114/ceh.2018.78121.
[28] J. Lee, C. Hsiao, I. Yang, M. Chou, C. Wu, Y. Wei, et al., High-mobility group box 1 protein is implicated in advanced glycation end products – induced vascular endothelial growth factor a production in the rat retinal ganglion cell line RGC-5, Mol. Vis. (2012) 838–850 http://www.molvis.org/molvis/v18/a89.
[29] V. Mostert, S. Wolff, I. Dreher, J. Köhrle, J. Abel, Identification of an element within the promoter of human selenoprotein P responsive to transforming growth factor-β, Eur. J. Biochem. 268 (2001) 6176–6181, https://doi.org/10.1046/j.0014-2956.2001.02565.