Serum Osteopontin and Cytokeratin-18 in Chronic Hepatitis C Patients

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Abstract

Chronic hepatitis C is a global health problem; most patients are at risk for developing fibrosis and cirrhosis. Histological examination of liver biopsies is currently the gold standard for the detection of early liver damage, but there is a strong need for better noninvasive methods. The aim of this study was to evaluate the association between serum osteopontin (OPN) level, serum cytokeratin 18 M30 (CK-18 M30) neoepitope level and the histological severity of hepatic fibrosis in hepatitis C virus (HCV) induced patients.

Subjects and methods: This study included 89 subjects, 70 with chronic hepatitis C virus infection, they were classified into 2 groups according to METAVIR fibrosis stage as follows: group I (stage 2 or less was considered as mild liver fibrosis) included 50 patient, group II (stage 3 or more was considered as extensive fibrosis) included 20 patients and 19 healthy matched age and gender as a control group. All subjects were submitted to the following: Through history taking, complete clinical examination, and serum concentrations of osteopontin and cytokeratin 18 M30 neoepitope were measured by enzyme linked immunosorbent assay (ELISA). The results revealed that there were high significant differences of OPN and CK-18 M30 between patients and control (P<0.001). There was a high significant difference of OPN (P<0.001) and a significant difference of CK-18 M30 (P=0.02) when compared between mild fibrosis and extensive fibrosis groups. There was a high significant correlation of serum OPN concentrations with severity of liver fibrosis degree (r=0.75, P<0.001), while the serum CK-18 M30 concentrations showed a significant correlation (r=0.33, P=0.005). In ROC curve serum OPN at the cut-off point of 3.1 ng/ml could discriminate mild from extensive fibrosis with sensitivity of 95%, serum CK-18 M30 at the cut-off point of 293 ng/ml could discriminate mild from extensive fibrosis with sensitivity of 70%. Finally from obtained study, results showed that serum OPN levels was better than CK-18 M30 in identification the degree of hepatic fibrosis and could be used as a biomarker to assess the stage of fibrosis in HCV patients which would help to reduce the number of liver biopsies.

Keywords: Chronic hepatitis C; Osteopontin; Virus infection; Cytokeratin

Introduction

Detection and quantification of hepatic fibrosis represents a longstanding challenge in hepatology [1]. With ongoing liver damage, fibrosis may progress to cirrhosis, predisposing to liver failure and primary liver cancer [2].

Assessment of liver fibrosis has become increasingly important in order to make therapeutic decisions, determine prognosis and to follow-up disease progression. Osteopontin (OPN) is a phosphorylated acidic glycoprotein with a diverse range of biologic activities including cell adhesion, proliferation and migration [3]. OPN is a strong chemoattractive and pro-inflammatory molecule, which is involved in a number of pathologic events, e.g. angiogenesis, apoptosis, inflammation, wound healing and tumor metastasis [4]. OPN also induces extracellular matrix accumulation by binding to type I collagen, fibronectin and osteocalcin, contributing to tissue fibrotic process [5].

Cytokeratin-18 (CK-18) is a major intermediate filament protein in liver cells and it is important for hepatocyte integrity [6]. CK-18 is a substrate for caspases that are activated during apoptosis. Fragmented CK-18 accumulates in apoptosing cells and then is released into the blood. Because many liver injuries increase hepatocyte apoptosis, an ELISA for detecting caspase-cleaved CK-18 was developed with M30, an antibody that recognizes a specific caspase cleaved CK-18 epitope [7].

Aim of the Study

It is to assess serum concentrations of osteopontin and cytokeratin 18 M30 neoepitope in relation to liver fibrosis in hepatitis C virus (HCV) patients.

Subjects and Methods

This study was carried on Clinical Pathology Department, Faculty of Medicine, Menoufia University Hospitals in collaboration with hepatology medicine, National Liver Institute in the period between December 2013 to December 2014. Written consents were obtained from all subjects studied and the study protocol was approved by the ethics committee of the Faculty of Medicine, Menoufiya University.

This study included 89 subjects, 70 with chronic hepatitis C virus infection who were defined by presence of HCV-RNA by RT-PCR, they were not previously treated with any form of interferon alfa selected from the outpatient clinics of the National Liver Institute, Menoufia University and the patients were classified into 2 groups according to METAVIR fibrosis stage as follows: group I (Stage 2 or less was considered as mild liver fibrosis) included 50 patient (28) males and (22) females And group II ( stage 3 or more was considered as extensive fibrosis) included 20 patients (12) males and (8) females and the third group was of 19 healthy volunteers (10) males and (9) females as a
control group who they are gender and age matched and they were with no previous history of liver diseases and were negative for HBV and HCV infections.

All individuals included in the study were subjected to the following: through history taking, complete clinical examination and serum concentrations of osteopontin and serum cytokeratin 18 M30 neoepitope were measured by ELISA according to the protocol provided by the manufacturer (MyBioSource, San Diego, USA). The optical density was measured at 450 nm using a microplate reader. Sample collection: 4 ml of blood was withdrawn from the subjects under complete aseptic conditions. The samples were collected in plain vacutainer tube and left to clot for 10-20 min at room temperature. Samples were separated by fully centrifugation at 3000 rpm for 20 min. Clear sera were separated and kept frozen at -80 until the time of the assay.

Analytical Statistics

Student test (t) was used to compare between two groups of normally distributed quantitative data, Mann-Whitney test for comparison between two groups not normally distributed, Spearman Correlation coefficient (r), diagnostic sensitivity, specificity, positive predictive value, negative predictive value, diagnostic accuracy and Receiver Operator Characteristic Curve (ROC curve) a graphic representation of the relationship between sensitivity and specificity at different cutoff points for a diagnostic test [8].

Results and Discussion

Fibrosis and cirrhosis of the liver are major causes of morbidity and mortality worldwide and are associated with increasing economic and social impact [9]. Chronic infection with HCV is a leading cause of liver related morbidity and mortality worldwide and predisposes to liver fibrosis and end-stage liver complications [10] (Tables 1-4 and Figures 1-4).

In the present study, it was found that median OPN levels were higher in patients than healthy controls (2.21 ng/ml vs. 0.98 ng/ml respectively; P<0.001). The mean plasma OPN concentration of individuals with extensive fibrosis (fibrosis degree ≥ 3) was highly significant than that with mild fibrosis (fibrosis degree ≤ 2) (4.76 ± 0.87 vs. 2.05 ± 0.58 ng/ml, respectively; P<0.001). The serum OPN concentrations were highly significantly correlated with severity of liver fibrosis degree (r=0.75, P<0.001). The ability of serum OPN as a biomarker to discriminate the severity of fibrosis was assessed by ROC curve. Serum OPN at the cut-off point of 3.1 ng/ml could discriminate mild (stage 1, 2) from extensive fibrosis (stage 3, 4) with an area under ROC curve (AUC) of 0.98, sensitivity of 95%, specificity of 92%. These findings were agreed with Huang et al., who mentioned that those were high significant differences were noted in the mean plasma OPN levels between subjects with extensive fibrosis and those with mild fibrosis (P<0.001) [3]. The correlation between the plasma OPN levels and the severity of liver fibrosis degree was noted (r=0.945, P<0.001).

<table>
<thead>
<tr>
<th>OPN (ng/ml)</th>
<th>Mean ± SD</th>
<th>Median</th>
<th>Range</th>
<th>Test of significance</th>
<th>P-value</th>
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<tbody>
<tr>
<td>OPN (ng/ml)</td>
<td>N=70</td>
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<tr>
<td>Mean ± SD</td>
<td>2.82 ± 1.40</td>
<td>2.21</td>
<td>0.98–5.75</td>
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<td>Median</td>
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<td>t-test 15.23</td>
<td>&lt;0.001</td>
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<tr>
<td>Range</td>
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<td>281.5</td>
<td>221.5–513</td>
<td>t-test 4.98</td>
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Table 1: Comparison between all groups as regard OPN and CK-18 M30.

<table>
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<tr>
<td>Mean ± SD</td>
<td>0.88 ± 0.20</td>
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Table 2: Correlation between CK-18, OPN and fibrosis stage.

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<tr>
<td>OPN (ng/ml)</td>
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<tr>
<td>CK-18 M30 (ng/ml)</td>
<td>0.005</td>
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AUC: Area under Curve; PPV: Positive Predictive Value; NPV: Negative Predictive Value

Table 3: Validity of OPN and CK-18 M30 for differentiation between patients with liver fibrosis and the control group.

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Table 4: Validity of OPN and CK-18 M30 for differentiation between mild and extensive fibrosis.

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Table 4: Validity of OPN and CK-18 M30 for differentiation between mild and extensive fibrosis.
Patouraux et al. [11], also reported that the serum OPN level correlated strongly with fibrosis ($r=0.61$, $P<0.001$). The area under the ROC curve in patients with chronic hepatitis C to estimate advanced (F $\geq 3$) fibrosis with the serum OPN level was 0.81. Matsue et al. [12], mentioned that serum OPN levels were remarkably increased from F0 through F4 in a progressive manner and the differences were highly significant ($P<0.001$) between each group. The data were highly correlated with the degree of hepatic fibrosis. In ROC curve for the discrimination of F1/F2 from F3/F4, the AUC of serum OPN was 0.99. Furthermore, circulating OPN level is characterized as an excellent predictor of cirrhosis in patients with hepatitis B infection [13]. Overall, it was observed that, OPN level in liver, adipose tissue and serum is correlated with fibrosis in patients with alcoholic liver disease. The gained results were explained by that OPN is a key component of the extracellular matrix (ECM) that is associated with the fibrotic process during tissue remodeling [14]. OPN is dramatically unregulated both at mRNA and protein levels during the pathogenesis of hepatic fibrosis and in other inflammatory processes [15]. Activated hepatic stellate cells are mainly responsible for the increased expression and synthesis of OPN during fibrogenesis and the increased OPN induces collagen type I production [16].

In the present study, it was found that serum CK-18 M30 levels were higher in patients compared to healthy controls (313.69 ± 68.39 ng/ml vs. 342.89 ± 73.52, respectively; $P<0.001$). The serum CK-18 M30 levels are higher in extensive fibrosis group compared to mild fibrosis group (342.89 ± 73.52 ng/ml vs. 302.01 ± 63.27 ng/ml, respectively; $P<0.001$). The serum CK-18 M30 concentrations significantly correlated with severity of liver fibrosis degree ($r=0.33$, $P=0.005$). Serum CK-18 at the cut-off point of 293 ng/ml could discriminate mild (stages 1 and 2) from extensive fibrosis (stages 3 and 4) with an area under ROC curve (AUC) of 0.70, sensitivity of 70%, specificity of 60%.

These results were comparable with Jazwinski et al., they reported that median CK-18 M30 levels were higher in CHC patients compared...
to controls (P<0.001). They also reported that fibrosis stage was associated with increasing serum CK-18 M30 levels (P=0.015) [16]. Bantel et al., studied the apoptotic marker CK-18 M30 in the serum, low levels of CK-18 M30 were detectable in the control group, and in contrast patients with different grades of disease activity revealed considerably elevated levels of caspase generated fragment CK-18 which agreed with this study. But no correlation was found between caspase activity and stage of fibrosis in contrast to this study which showed significant correlation between caspase activity and grade of liver fibrosis [6].

Cavalgia et al. [17], in their study showed a correlation between CK18 M30 and liver fibrosis (r=0.329; P=0.0126). Abdel et al. [18], showed that serum levels of CK-18 M30 are higher in liver disease than the healthy controls (P<0.01) and the highest serum CK-M30 levels were observed in patients with severe liver fibrosis. It was found also a statistically significant correlation between the serum levels of CK-18 M30 and fibrosis (P<0.01) which agreed with this study.

These results also agreed with Kowerda et al. [19], study which showed that median serum levels of CK18 M30 was higher in patients than control (P<0.001) and was associated with liver fibrosis(P<0.0001). Papatheodoris et al. [20], studied CK 18 M30 as a marker for disease severity in CHC infection. The serum levels of CK 18 M30 in patients are higher than the control group and highly significant correlation between serum levels of CK18 M30 and fibrosis grading (P<0.001).

It has a good diagnostic accuracy for differentiating patients with advanced fibrosis than patients with minimal fibrosis AUC (0.74). This study showed that CK-18 levels are higher in patients with CHC infection and serum levels of CK18 M30 were positively correlated with the fibrosis stage in patients with chronic HCV which in turn support the correlation between caspase activation and apoptotic cell death in the development of liver fibrosis in patients with chronic hepatitis C. Apoptosis is programmed cell death initiated by many stimuli leading to resorption and shrinkage of cells [20]. Apoptosis has long been recognized as a key feature in chronic liver diseases [21]. CK-18 is an intermediary filament protein, expressed in hepatocytes, which is proteolytically cleaved during liver damage. The resultant CK-18 fragments are released by hepatocytes and can be detected in the serum [22]. Patoureaux et al. [11], evaluated OPN and total cytokeratin 18 levels in alcoholic liver disease, and mentioned that OPN and total cytokeratin 18 areas under the ROC curve that estimated significant fibrosis was 0.89 and 0.83, respectively. Finally it was concluded that serum OPN levels was better than CK-18 in identification the degree of hepatic fibrosis and could be used as a biomarker to assess the stage of fibrosis in HCV patients which would help to reduce the number of liver biopsies.

References

18. Abdel HH, Zayed N, Abdel HH, Fouad A, Akl M, et al. (2013) Evaluation of the diagnostic value of serum and tissue apoptotic cytokeratin-18 in patients with chronic hepatitis C. Apoptosis is programmed cell death initiated by many stimuli leading to resorption and shrinkage of cells [20]. Apoptosis has long been recognized as a key feature in chronic liver diseases [21]. CK-18 is an intermediary filament protein, expressed in hepatocytes, which is proteolytically cleaved during liver damage. The resultant CK-18 fragments are released by hepatocytes and can be detected in the serum [22]. Patoureaux et al. [11], evaluated OPN and total cytokeratin 18 levels in alcoholic liver disease, and mentioned that OPN and total cytokeratin 18 areas under the ROC curve that estimated significant fibrosis was 0.89 and 0.83, respectively. Finally it was concluded that serum OPN levels was better than CK-18 in identification the degree of hepatic fibrosis and could be used as a biomarker to assess the stage of fibrosis in HCV patients which would help to reduce the number of liver biopsies.