Liver, Pancreas and Biliary Tract

Serum osteopontin levels as a predictor of portal inflammation in patients with nonalcoholic fatty liver disease

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A R T I C L E   I N F O

Article history:
Received 24 March 2012
Accepted 20 August 2012
Available online 18 September 2012

Keywords:
Biomarker
Inflammation
Nonalcoholic fatty liver disease
Osteopontin
Portal inflammation

A B S T R A C T

Background: Osteopontin is a secreted phosphorylated glycoprotein that is expressed by a variety of cell types and that mediates numerous and diverse biological functions. Osteopontin knockout mice are protected from obesity-induced hepatic steatosis. In the present study, we sought to investigate whether serum osteopontin concentrations are associated with liver histology in patients with nonalcoholic fatty liver disease.

Methods: Serum levels of osteopontin were measured by enzyme-linked immunosorbent assay in 179 well-characterized patients with nonalcoholic fatty liver referred for liver histology and 123 control subjects.

Results: Serum osteopontin levels were markedly higher in patients with nonalcoholic fatty liver disease than in controls (p < 0.001). Multivariable analysis showed that osteopontin levels were strongly and independently associated with both portal inflammation (β = 0.294, p < 0.01) and serum aminotransferase levels (aspartate aminotransferase: β = 0.295, p < 0.01; alanine aminotransferase: β = 0.285, p < 0.01).

Conclusion: In summary, these data demonstrate that serum levels of osteopontin are elevated in non-alcoholic fatty liver disease and are a significant independent predictor of portal inflammation in this clinical entity.

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1. Introduction

Over the last two decades, the rise in the prevalence rates of overweight and obesity may explain the emergence of nonalcoholic fatty liver disease (NAFLD) as one of the leading cause of liver disease worldwide [1,2]. NAFLD comprises a disease spectrum ranging from simple steatosis to nonalcoholic steatohepatitis (NASH) [3,4]. NAFLD is strongly associated with insulin resistance, and is now regarded as the liver manifestation of the metabolic syndrome [5,6].

Osteopontin (OPN) is a multifunctional protein expressed by a variety of cell types that has been implicated in various physiologic and pathologic processes [7,8]. In recent years, it has emerged as a potential biomarker and mediator of angiogenesis in hepatocellular carcinoma [9,10]. Because of its pro-inflammatory actions and its effects on macrophages, OPN has been also implicated in the pathogenesis of hepatic inflammation [11–13]. Indeed, Lima-Cabello et al. [14] demonstrated that the OPN gene is abundantly expressed in the liver of patients with NAFLD and chronic hepatitis C. Using a transgenic mouse model, Syn et al. [15] reported that OPN directly promotes fibrosis progression in NASH. Consistent with its putative role in NAFLD, genetic OPN deficiency has been recently shown to protect from obesity-induced hepatic steatosis by downregulating hepatic triacylglycerol synthesis in the mice [16]. However, despite its purported role in hepatic steatosis, the clinical relevance of OPN in human NAFLD remains unknown. Accordingly, the objective of the current study was to investigate plasma OPN levels in patients...
with biopsy-proven NAFLD and to assess their potential association with liver histology.

2. Methods

The present study represents part of a larger project aimed at identifying novel knowledge-based biochemical markers of NAFLD conducted in our hospital-based specialized outpatient clinics [17–20]. Written informed consent was obtained from all participants, and the study was approved by the local Ethics Committees. Briefly, 179 well-characterized patients with NAFLD who were consecutively referred for liver histology and 123 control subjects were enrolled in the study. Enrolment took place in a longitudinal fashion. All NAFLD patients had >5% macrovesicular steatosis as evaluated by light microscopic examination of a haematoxylin–eosin-stained liver section (4–5 μm thick) under a 10× objective lens [21]. In addition, all of them showed a daily alcohol intake lower than 20 g, persistent serum aminotransferase abnormalities, dyslipidaemia, or a positive history of liver steatosis. Patients with chronic viral hepatitis, autoimmune hepatitis, hereditary haemochromatosis, Wilson’s disease and drug-induced liver disease, or presenting with clinical or imaging evidence of decompensated cirrhosis were excluded. All controls were judged to be in good health, with serum aminotransferases in the normal range, and normal liver on ultrasound.

2.1. Liver histology

Liver biopsies were processed as previously described [17–20] and scored according to the current recommendations for the design of clinical trials for nonalcoholic steatohepatitis [21], resulting in four potential diagnostic categories: definite steatohepatitis, borderline steatohepatitis zone 3, borderline steatohepatitis zone 1, not steatohepatitis with steatosis. For the purpose of analysis, borderline steatohepatitis zone 3 and borderline steatohepatitis zone 1 were grouped together in a unique group (borderline steatohepatitis). The NAFLD activity score (NAS) developed by the Nonalcoholic Steatohepatitis Clinical Research Network (NASH CRN) was used to evaluate disease activity, including the scores for steatosis grade (0–3), lobular inflammation (0–3), chronic portal inflammation (0–2) and ballooning (0–2) [22]. Fibrosis was staged as follows: stage 0: none; stage 1: perisinusoidal or periportal fibrosis; stage 1a: mild perisinusoidal; stage 1b: moderate perisinusoidal; stage 1c: portal/periportal; stage 2: perisinusoidal and portal/periportal fibrosis; stage 3: bridging fibrosis; stage 4: cirrhosis [22].

2.2. Data collection

The following data were collected in all participants: clinical history; age; sex; blood pressure (defined as the mean of the second and third reading of 3 consecutive blood pressure measurements); body mass index (BMI); serum aminotransferases; total, low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol; triglycerides; and high-sensitivity C-reactive protein (hs-CRP). Diabetes mellitus was diagnosed according to American Diabetes Association criteria [23]. The metabolic syndrome was diagnosed using the Adult Treatment Panel III criteria [24], based on the presence of 3 or more of the following criteria: (1) fasting glucose ≥100 mg/dL or treated for diabetes, (2) central obesity (waist circumference ≥102 cm (men) and ≥88 cm (women), (3) arterial pressure ≥130/85 mmHg or treated for hypertension, (4) triglyceride levels ≥150 mg/dL or current use of fibrates, (5) HDL-cholesterol <40 mg/dL (men) and <50 mg/dL (women). Insulin resistance was defined according to the homeostatic metabolic assessment-insulin resistance (HOMA-IR), using the following formula: insulin resistance = fasting plasma insulin (in microunits per millilitre) × fasting plasma glucose (in millimoles per litre)/22.5.

2.3. Osteopontin assay

For the determination of OPN in blood, venous blood samples were drawn after an overnight fast. Separated serum was frozen in aliquotes at −20 °C. Serum OPN was measured by enzyme-linked immunosorbent assay (Human Osteopontin Quantikine kit, R&D Systems, Minneapolis, MN, USA) according to the manufacturer’s protocol. The intra- and interassay coefficients of variation of this assay during this study were less than 7% and less than 9%, respectively. Each sample was analysed in duplicate, and the mean value of the two measures was used for the analyses.

2.4. Data analysis

Summary statistics for the continuous variables were presented as means ± standard deviations or medians with 25th and 75th percentiles, and comparisons between the two groups were performed with the Student’s t-test or the Mann–Whitney U test, as appropriate. Log transformation was applied to nonparametric variables to decrease the skewness of the data. Differences in OPN levels across the histological spectrum of NAFLD (definite NASH, borderline NASH and simple steatosis) were determined using the Kruskall–Wallis test, followed by Dunn’s multiple-comparison post hoc analysis. Categorical data were summarized as frequencies, and comparisons between groups were performed with Pearson’s chi-square test. Spearman’s rho correlations were used to examine the linear relationship between the study variables. Multivariable stepwise linear regression analyses were performed to identify independent predictors of serum OPN levels in patients with NAFLD; the covariates included in these models were all the variables listed in Table 1. All analyses used two-sided tests with an overall significance level of alpha = 0.05. All calculations were performed using SPSS (version 14.0) statistical software package (SPSS Inc., Chicago, IL, USA).

3. Results

The general characteristics of the study participants are shown in Table 1. The two study groups did not differ in terms of age, sex and HDL cholesterol. Patients with NAFLD showed significant differences in terms of body mass index, systolic and diastolic blood pressure, HOMA-IR, AST, ALT, total cholesterol, LDL cholesterol and triglycerides compared with controls. The prevalence of diabetes and the metabolic syndrome was higher in patients with NAFLD than in controls.

3.1. Serum osteopontin, NAFLD, and liver histology

Serum OPN levels were significantly higher in patients with NAFLD (median: 58 μg/L; interquartile range: 33–78 μg/L) than in controls (median: 33 μg/L; interquartile range: 25–50 μg/L; p = 0.001, Fig. 1). The histological features (according to the current recommendations for the design of clinical trials for nonalcoholic steatohepatitis [21]) of patients with NAFLD are shown in Table 2. As assessed by the Kruskall–Wallis test, serum OPN levels did not differ significantly across the histological spectrum of NAFLD (p = 0.55). Specifically, the Dunn’s multiple comparison post hoc test indicated that serum OPN levels were similar in patients with definite NASH (n = 137; median: 55 μg/L; interquartile range: 32–77 μg/L), borderline NASH (n = 26, median: 53 μg/L; interquartile range: 28–81 μg/L), and not NASH, with steatosis (n = 16, median: 63 μg/L; interquartile range: 45–87 μg/L). We then
evaluated the univariate correlation coefficients of serum OPN with clinical, biochemical and histological parameters (Table 3). Serum OPN levels were significantly associated with sex, AST, ALT, hs-CRP and portal inflammation. However, there were no significant associations of serum OPN levels with neither the histological NASH score nor with the fibrosis score (Table 3). Serum OPN concentrations did not differ significantly in NAFLD patients with a BMI > 30 kg/m² (n = 99, median: 54 μg/L; interquartile range: 27–77 μg/L) compared with those with a BMI ≤ 30 kg/m² (n = 80, median: 60 μg/L; interquartile range: 42–80 μg/L, p = 0.22).

3.2. Multivariable analysis

When all variables listed in Table 1 were entered into a multivariable regression model as covariates with OPN as the dependent variable, serum OPN levels were strongly and independently associated with portal inflammation (β = 0.294, p < 0.01) and serum aminotransferase levels (AST: β = 0.295, p < 0.01; ALT: β = 0.285, p < 0.01).

4. Discussion

There is growing evidence suggesting that OPN may play an important role in the pathogenesis of liver diseases. For example, a recent paper has shown that hepatic OPN expression in alcoholics correlates with hepatic inflammation, fibrosis, TGF-β expression, neutrophils accumulation and serum OPN level [25]. Another manuscript has reported an association between plasma OPN and both hepatic OPN expression and fibrosis severity [26]. In addition, serum OPN can actually derive from adipose tissue macrophages, which have been directly linked to hepatic injury in metabolic liver diseases [27]. The most significant finding of our study was that serum levels of OPN were increased in patients with NAFLD and were independent predictors of both portal inflammation and liver enzymes. However, no association of OPN concentrations with hepatic fibrosis and the histological severity of NAFLD was
Table 3  
Correlations between serum osteopontin and other variables in 179 patients with biopsy proven nonalcoholic fatty liver disease.

<table>
<thead>
<tr>
<th></th>
<th>r</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>−0.123</td>
<td>0.028</td>
</tr>
<tr>
<td>Age</td>
<td>−0.046</td>
<td>0.448</td>
</tr>
<tr>
<td>Body mass index</td>
<td>0.043</td>
<td>0.491</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>0.058</td>
<td>0.394</td>
</tr>
<tr>
<td>Metabolic syndrome</td>
<td>0.074</td>
<td>0.223</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>0.167</td>
<td>0.023</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>0.058</td>
<td>0.394</td>
</tr>
<tr>
<td>Diastolic blood pressure</td>
<td>0.051</td>
<td>0.401</td>
</tr>
<tr>
<td>ALT</td>
<td>0.223</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AST</td>
<td>0.243</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>0.063</td>
<td>0.306</td>
</tr>
<tr>
<td>HDL cholesterol</td>
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</tr>
<tr>
<td>LDL cholesterol</td>
<td>0.014</td>
<td>0.306</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>0.108</td>
<td>0.078</td>
</tr>
<tr>
<td>hs-CRP</td>
<td>0.180</td>
<td>0.003</td>
</tr>
<tr>
<td>Steatosis grade</td>
<td>0.023</td>
<td>0.761</td>
</tr>
<tr>
<td>Steatosis location</td>
<td>0.001</td>
<td>0.989</td>
</tr>
<tr>
<td>Chronic portal inflammation</td>
<td>0.251</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ballooning</td>
<td>0.122</td>
<td>0.104</td>
</tr>
<tr>
<td>Fibrosis</td>
<td>0.094</td>
<td>0.211</td>
</tr>
</tbody>
</table>

HOMA-IR, homeostasis model assessment of insulin resistance; AST, aspartate aminotransferase; ALT, alanine aminotransferase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; hs-CRP, high-sensitivity C-reactive protein; NASH, nonalcoholic steatohepatitis.

observed. To our knowledge, this is the first report on the clinical significance of OPN in patients with NAFLD.

The mechanism(s) by which OPN levels are increased in NAFLD is unknown and likely multifactorial. OPN is a secreted phosphorylated glycoprotein that is expressed by a variety of cell types and mediates numerous and diverse biological functions [7]. Although OPN is involved in normal physiological processes, there is a growing body of literature implicating it in the pathogenesis of a variety of disease states [8], including metabolic liver disorders [14–16]. In this regard, it has been suggested that OPN may play a role in hepatic fat accumulation by virtue of its effects on lipid metabolism, fibrosis and inflammation [14–16]. OPN has effects on a number of different cell types that may contribute to hepatic inflammation, such as neutrophils, T cells and macrophages [11–13]. The most relevant in this regard may relate to the macrophage. OPN is dramatically upregulated during macrophage differentiation and constitutes one of the major macrophage products [13]. OPN production by macrophages is induced by a variety of inflammatory cytokines, and once produced, OPN in turn serves as a potent chemoattractant for macrophages [11–13]. Indeed, OPN plays a key role in macrophage biology by regulating migration, survival, phagocytosis and pro-inflammatory cytokine production [11–13]. Although these biologic actions of OPN suggest a potential role for it in all phases of NAFLD [14–16], such pro-inflammatory effects of OPN do at least provide biologic plausibility for the observed increase of this protein in this syndrome. Furthermore, these broad proinflammatory actions of OPN may explain its utility as a biomarker of hepatocyte injury as reflected by its association with AST and ALT.

With respect to portal inflammation in particular, a study by Brunt et al. [28] has shown that this histological feature is significantly associated with an increased risk of progressive disease. Portal inflammation is chiefly the expression of an immunopathogenic process in the portal tracts [29]. Interestingly, OPN has been shown to be an important immune molecule in portal tracts in patients with primary biliary cirrhosis, where it contributes to the recruitment of mononuclear cells [30,31]. Further immunohistochemical studies are needed to investigate the site of OPN expression in the liver, portal chronic inflammation and the presence and progression of NAFLD. It is also important to emphasize that portal inflammation is not related to lobular inflammation or steatosis [28]; as a consequence, the lack of association with NASH itself is not surprising. However, given the potential role of OPN in recruiting innate immune cells from the blood [11–13,27], we believe that our finding of an association between OPN and portal inflammation is potentially relevant for our current understanding of NAFLD pathogenesis and suggestive of future venues of research.

There are several limitations to the present study. First, it was conducted exclusively in Turkish subjects, and as such, the results cannot be extrapolated to other populations. Second, the size of our population was relatively small. As such, the findings need to be confirmed in larger and prospectively designed studies. Finally, our study was cross-sectional and therefore does not elucidate the causal relationships between serum OPN and the presence of NAFLD. Therefore, we do not know whether circulating OPN levels could be mechanistically related to liver disease by reflecting OPN expression in the hepatic parenchyma. Given the biologic effects of OPN, it is unclear whether OPN represents a risk marker or a risk factor (or perhaps both) for the development of NAFLD.

In conclusion, we found that serum OPN levels are significantly increased and independently associated with liver enzymes and portal inflammation in a cohort of NAFLD patients referred for liver biopsy. Importantly, the findings from this clinical study are consistent with the known hepatic actions of OPN with respect to hepatic inflammation in particular. Furthermore, the present study is consistent with, and extends, the growing body of literature which suggests that OPN represents a novel and powerful biomarker in chronic liver diseases.

Source of support

This study was supported by grants from the Institute of Gastroenterology, Marmara University, Istanbul, Turkey.

Conflict of interest statement

None declared.

Acknowledgements

This study was supported by grants from the Institute of Gastroenterology, Marmara University, Istanbul, Turkey. The authors are grateful to Giovanni Musso (Gradeno Hospital, Turin, Italy) for helpful discussions.

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