Increased circulating osteopontin levels in adult patients with type 1 diabetes mellitus and association with dysmetabolic profile

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Abstract

Objective: Osteopontin (OPN) is a sialoprotein implicated in different immunity and metabolic pathways. Capable of activating dendritic cells and inducing Th1-Th17-mediated tissue damage, OPN plays a significant role in the development/progression of several autoimmune diseases; interestingly, it was also shown that OPN participates in the acute pancreatic islets response to experimentally induced diabetes in non-obese diabetic (NOD) mice. Furthermore, OPN promotes adipose tissue dysfunction, systemic inflammation and insulin resistance. Our aims of this study were to evaluate circulating OPN levels in adult patients with type 1 diabetes mellitus (T1DM) compared to non-diabetic control participants and to unravel clinical and biochemical correlates of OPN concentration.

Design: Case–control study.

Methods: We enrolled 54 consecutive T1DM patients referred to our diabetes outpatient clinic at Sapienza University of Rome and 52 healthy sex and age-comparable controls. The study population underwent clinical evaluation, blood sampling for biochemistry and complete screening for diabetes complications. Serum OPN levels were measured by MILLIPLEX Multiplex Assays Luminex.

Results: T1DM patients had significantly higher serum OPN levels than controls (17.2 ± 12.9 vs 10.5 ± 11.6 mg/ml, P = 0.009). OPN levels correlated with T1DM, higher blood pressure, BMI, creatinine, γ-GT, ALP and lower HDL; the association between high OPN levels and T1DM was independent from all confounders. No correlation was shown between OPN and HbA1c, C-peptide, insulin requirement, co-medications and diabetes duration.

Conclusions: This study demonstrates for the first time in a case–control study that adults with T1DM have increased serum OPN levels, and that higher OPN concentrations are associated with an unfavorable metabolic profile in these patients.

Background

Osteopontin (OPN), or early T-lymphocyte activation-1, is a sialoprotein originated by the bone and by a large number of other tissues and cells. OPN has been described as a secreted protein involved in a wide spectrum of physiopathological processes, as OPN expression was demonstrated in the nucleus and cytoplasm of several different cells. The term ‘osteopontin’ takes origin from the role of this protein in bone metabolism, as OPN exerts a major function in controlling biomineralization and stimulating adhesion, migrations and bone resorption by osteoclasts (1, 2, 3).

Among its non-bone related functions, OPN plays a pivotal role in the regulation of immune cell functions including monocyte adhesion, migration, differentiation and phagocytosis (4, 5, 6, 7). It also exerts an influence on T-helper (Th) cells polarization to Th1 or Th2 phenotypes, a critical aspect of cell-mediated
immunity, by enhancing Th1 and inhibiting Th2 cytokine expression (8).

OPN was also demonstrated to induce adipose tissue inflammation, increase pro-inflammatory cytokines release in the bloodstream and, consequently, promote the development of insulin resistance (IR)-related conditions (9, 10, 11). Increased OPN levels were found in obese participants (12, 13) and predicted coronary calcification, nephropathy and coronary artery disease in patients with type 2 diabetes, independent of traditional risk factors (14, 15).

For its action in the regulation of immune system, OPN has been recognized to have a role in the development/progression of several autoimmune diseases, such as multiple sclerosis (16, 17, 18, 19) rheumatoid arthritis (20, 21), psoriasis (22) and Graves’ disease (23).

Interestingly, OPN was shown to influence the acute pancreatic islets’ response to experimentally induced diabetes in non-obese diabetic (NOD) mice, and genetic studies of single nucleotide polymorphisms (SNPs) in humans suggest that the OPN encoding gene might be associated with an increased susceptibility to the development of type 1 diabetes mellitus (T1DM) (24, 25). Moreover, serum OPN levels were demonstrated to strongly predict incipient diabetic nephropathy (DN), cardiovascular events and all-cause mortality in patients with T1DM (26) and were associated with renal failure and left ventricular hypertrophy in patients affected by systemic hypertension (27, 28). Furthermore, in a recent study, obese individuals exhibited significantly increased blood OPN levels and higher adipose tissue/peripheral blood mononuclear cells OPN expression compared with lean individuals; OPN also correlated with fasting blood glucose (FBG) and BMI (13).

However, so far no study has investigated whether OPN serum levels in adult T1DM patients are increased compared with non-diabetic controls or which variables may be associated with increased OPN levels.

Therefore, the aims of this study were to evaluate circulating OPN levels in an adult population of T1DM patients compared to non-diabetic participants and to explore clinical and biochemical correlates of OPN concentration.

**Subjects and methods**

This is an observational case–control study. For our purposes, we enrolled 54 consecutive patients with T1DM (M/F: 36/18, mean ± s.d. age: 36.2 ± 12 years, mean ± s.d. diabetes’ duration: 13.2 ± 13.3 years) among those referring to our diabetes outpatient clinics at Sapienza University of Rome and 52 control participants comparable for sex and age (M/F: 33/19, mean ± s.d. age: 39.3 ± 7.4 years, P value: 0.87 and 0.16 respectively) without T1DM or other chronic diseases selected among Sapienza University employees undergoing clinical evaluation for the Occupational Medicine Service. Participants’ recruitment took place between June 2013 and February 2014.

Each participant underwent a medical history collection and physical examination (height, weight, waist circumference, systolic and diastolic blood pressure (SBP, DBP, mmHg) measurement and BMI (kg/m²) calculation) as well as, where appropriate, a clinical/instrumental assessment for diabetes complications, daily insulin requirement (IU/kg per day) and concomitant medications at the time of study enrollment (statins, angiotensin converting enzyme inhibitors (ACE-I)).

Ophthalmoscopy was performed on T1D patients by the same ophthalmologist experienced in diabetes. One drop of atropine was put in each eye and left for 20–30 min for the pupil to dilate. Ophthalmoscopy was followed by retinal fluorangiography, when indicated. Retinal examination was used to identify and quantify diabetic retinopathy (DR) according to the International Clinical Diabetic Retinopathy Disease Severity Scale (29).

DN was defined as persistent microalbuminuria (30–300 mg/day) or macroalbuminuria (> 300 mg/day) in at least two of three urine samples collected over 24 h.

All study participants underwent blood sampling for biochemistry after an overnight fasting. FBG (mg/dl), HbA1c (% – mmol/mol), C-peptide (ng/ml), total cholesterol (mg/dl), HDL-cholesterol (mg/dl), triglycerides (mg/dl), blood urea nitrogen (BUN, mg/dl), creatinine (mg/dl), aspartate aminotransferase (AST, IU/l), alanine aminotransferase (ALT, IU/l), alkaline phosphatase (ALP, IU/l) and gamma-glutamyl transpeptidase (γ-GT, IU/l) were measured by standard laboratory methods after an overnight fasting. LDL-cholesterol (mg/dl) value was obtained using Friedewald formula. The glomerular filtration rate (GFR, ml/min) ‘was estimated by means of Cockcroft-Gault formula. Serum parathyroid hormone (PTH, pg/ml) and OPN (µg/l) levels were measured by MILLIPLEX Multiplex Assays Luminex in sera frozen immediately after separation and stored at −25 °C for two weeks.

**Statistical analysis**

SPSS version 17 statistical package was used to perform the analyses. Student’s t test for continuous variables and χ²
test for categorical variables were used to compare mean values between two independent groups. As OPN, BMI, triglycerides, AST, ALT, BUN, HDL, FBG, HbA1c, ALP, PTH and γ-GT were skewed variables, we used natural logarithmic transformation to normalize the distribution of these parameters before all analyses. For statistical analyses, the presence of DR was categorized as follows: \(^0\) = absence of DR, \(^1\) = non-proliferative DR, \(^2\) = proliferative DR; DN was considered on the basis of the absence (DN = 0) or the presence of persistent microalbuminuria (DN = 1) or macroalbuminuria (DN = 2) in at least two of three urine samples collected over 24 h.

Bivariate and multivariate linear regression analyses were used to detect the association between serum OPN levels, considered as a continuous variable, and all possible determinants. Correlations between continuous variables were calculated by Pearson’s coefficient, whereas Spearman’s coefficient was used for dichotomic/ordinal parameters. A multiple linear regression analysis, including all variables significantly associated with OPN levels at the bivariate analyses, was performed to confirm the independence of the association between OPN (considered as dependent variable) and the diagnosis of T1DM.

Data are shown as mean ± S.D., median (min–max) or as percentage in both text and tables, as appropriate. For all of the above, \(P < 0.05\) was considered statistically significant.

This study was approved by the local ethical committee, Sapienza University of Rome, functioning according to the 3rd edition of the Guidelines on the Practice of Ethical Committees in Medical Research issued by the Royal College of Physicians of London. Written consent was obtained from each patient and control participant after a full explanation of the purpose and nature of all procedures used.

**Results**

Clinical and biochemical characteristics of study cohorts are shown in Tables 1 and 2. Patients with T1DM had significantly higher serum OPN levels compared with controls (mean ± S.D.: 17.2 ± 12.9, (median (min–max): 13.6 (1.4–62.9)) μg/l vs mean ± S.D.: 10.5 ± 11.6, (median (min–max): 5.7 (0.2–76.89) μg/l, \(P = 0.009\)); they also showed lower BMI, waist circumference, triglycerides, higher FBG and ALP than the control group.

In the overall study population (\(n = 106\)) circulating OPN levels correlated with higher SBP (correlation coefficient: 0.35, \(P = 0.001\)), DBP (correlation coefficient: 0.23, \(P = 0.03\)), BMI (correlation coefficient: 0.22, \(P = 0.02\)), BUN (correlation coefficient: 0.26, \(P = 0.02\)), serum creatinine (correlation coefficient: 0.25, \(P = 0.02\)), γ-GT (correlation coefficient: 0.31, \(P = 0.006\)), ALP (correlation coefficient: 0.27, \(P = 0.04\)), PTH (correlation coefficient:

**Table 1** Clinical and biochemical characteristics of T1D patients and control participants. Values are expressed by mean ± S.D., median (min–max) or rate of subjects, as appropriate.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>T1DM ((n = 54))</th>
<th>Controls ((n = 52))</th>
<th>(P) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>36.2 ± 12</td>
<td>39.3 ± 7.4</td>
<td>0.16</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>36/18</td>
<td>33/19</td>
<td>0.87*</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>22.9 (17.4–35.9)</td>
<td>24.4 (18.6–40.2)</td>
<td>0.04</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>77.5 ± 10.5</td>
<td>87.5 ± 16.4</td>
<td>0.01</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>119.4 ± 12.7</td>
<td>120.7 ± 14.6</td>
<td>0.66</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>77.9 ± 10.4</td>
<td>77.1 ± 8.7</td>
<td>0.74</td>
</tr>
<tr>
<td>FBG (mg/dl)</td>
<td>116.5 (86–385)</td>
<td>91 (74–120)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BUN (mg/dl)</td>
<td>38 (9–61)</td>
<td>34 (19–65)</td>
<td>0.78</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.96 ± 0.3</td>
<td>0.92 ± 0.2</td>
<td>0.42</td>
</tr>
<tr>
<td>GFR (ml/min)</td>
<td>122 ± 37.3</td>
<td>135.2 ± 31.2</td>
<td>0.056</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>187.4 ± 32.7</td>
<td>195.8 ± 39.4</td>
<td>0.27</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>54 (31–93)</td>
<td>54.5 (31–79)</td>
<td>0.81</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>115.9 ± 28.2</td>
<td>116.7 ± 35.2</td>
<td>0.90</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>75 (29–323)</td>
<td>83 (35–295)</td>
<td>0.02</td>
</tr>
<tr>
<td>AST (IU/l)</td>
<td>19 (12–50)</td>
<td>17.5 (11–35)</td>
<td>0.056</td>
</tr>
<tr>
<td>ALT (IU/l)</td>
<td>20.5 (7.59)</td>
<td>18 (10.50)</td>
<td>0.047</td>
</tr>
<tr>
<td>ALP (IU/l)</td>
<td>73.5 (56–354)</td>
<td>62.5 (10–109)</td>
<td>0.006</td>
</tr>
<tr>
<td>γ-GT (IU/l)</td>
<td>15 (8–244)</td>
<td>18 (6–62)</td>
<td>0.31</td>
</tr>
<tr>
<td>PTH (pg/ml)</td>
<td>25.7 (3.6–443)</td>
<td>52.5 (1–287)</td>
<td>0.68</td>
</tr>
<tr>
<td>OPN (μg/l)</td>
<td>13.6 (1.4–62.9)</td>
<td>5.7 (0.2–76.8)</td>
<td>0.009</td>
</tr>
</tbody>
</table>

\(t\) test for independent samples test applied. \(\chi^2\) test applied. \(P\) values <0.05 are considered statistically significant.
Our study demonstrates that circulating OPN levels are significantly higher in adult patients with T1DM compared to healthy participants and that the association between diabetes and increased OPN levels is independent from clinical and biochemical confounders. Furthermore, circulating OPN levels directly correlate with several cardio-metabolic risk factors, such as higher BMI, SBP and DBP and lower HDL, but not with diabetes complications, IR, C-peptide and disease duration.

Recently, increased OPN levels were observed in sera of pediatric patients with T1DM compared with healthy children in an Iranian study conducted by Karamizadeh et al. (30), but the presence of clinical and biochemical determinants of high OPN levels were not explored.

Moreover, in a large population of T1DM patients from the FinnDiane study cohort with a median disease duration of 20 years, Gordin et al. (26) demonstrated that serum OPN concentration was an independent predictor of DN, cardiovascular events and all-cause mortality after a 10-year follow-up. These authors also found an association between higher OPN levels and micro- and macroalbuminuria at baseline and, in agreement with our results, did not find any relationship between OPN levels and glycemic control. However, in this study (26) OPN levels at baseline were not compared with a control population, thus no inference can be made if the range of OPN levels detected in T1DM patients are comparable to those present in normal individuals. In our study sample, the median diabetes duration was 7 years and the prevalence of DN was 20%; among patients with stable albuminuria, 30% had macroalbuminuria. Indeed, we observed a direct correlation between OPN levels and serum creatinine, but this association disappeared after adjusting for other confounders at multivariate analysis.

Interestingly, in our study population, higher OPN levels correlated with a dysmetabolic profile. Our findings are in line with data from type 2 diabetic patients in which elevated circulating OPN levels were associated with the presence and development of coronary artery disease and artery calcification (14, 15). Several studies described OPN

### Table 2 Characteristics of T1DM patients. Values are expressed by mean ± s.d., range or number/percentage, as appropriate.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disease duration (years)</td>
<td>13.2 ± 13.3</td>
</tr>
<tr>
<td>Median (min-max; years)</td>
<td>7 (&lt;1–40)</td>
</tr>
<tr>
<td>HbA1c (mmol/mol, %)</td>
<td>54 ± 10 to 7.1 ± 1.7</td>
</tr>
<tr>
<td>IR (U/kg per day)</td>
<td>0.45 ± 0.5</td>
</tr>
<tr>
<td>Fasting C-peptide (ng/ml; (range))</td>
<td>0.1 ± 0.3 (0–1)</td>
</tr>
<tr>
<td>Microalbuminuria (mg/day; (range))</td>
<td>9.6 ± 16.5 (0–76)</td>
</tr>
<tr>
<td>Prevalence of DR % (n)</td>
<td>20% (11)</td>
</tr>
<tr>
<td>Prevalence of DN % (n)</td>
<td>20% (11)</td>
</tr>
<tr>
<td>Microalbuminuria</td>
<td>73% (8)</td>
</tr>
<tr>
<td>Macroalbuminuria</td>
<td>27% (3)</td>
</tr>
<tr>
<td>Use of statins % (n)</td>
<td>22% (12)</td>
</tr>
<tr>
<td>Use of antihypertensive agents % (%)</td>
<td>35% (19)</td>
</tr>
</tbody>
</table>

0.37, *P* < 0.001), lower HDL (correlation coefficient: −0.25, *P*=0.02) and the diagnosis of T1DM (correlation coefficient: 0.32, *P* < 0.001). No correlation was found between OPN levels and HbA1c, C-peptide, GFR, microalbuminuria, IR, diabetes complications and disease duration in T1DM patients.

To rule out an influence of chronic ACE-I treatment on OPN levels, as previously suggested by studies in experimental models, circulating OPN levels were also evaluated separately in T1DM patients according to the use of ACE-Is and no difference was detected between T1DM patients with (*n*= 19) and without (*n*= 35) ACE-I therapy (T1DM treated with ACE-I OPN: 15.9 ± 11.3 μg/l, T1DM participants without ACE-I OPN: 17.3 ± 18.5 μg/l, *P*=0.98).

Finally, the multivariate linear regression analysis demonstrated that higher circulating OPN levels were associated with the diagnosis of T1DM independent of all possible confounders (*P*=0.03; Table 3).

### Table 3 Multivariate linear regression analysis. OPN is considered as the dependent variable of the model.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Non-standardized β</th>
<th>Standard error</th>
<th>Standardized β</th>
<th>t</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>−26.477</td>
<td>20.909</td>
<td>−</td>
<td>1.266</td>
<td>0.213</td>
</tr>
<tr>
<td>PTH</td>
<td>−0.005</td>
<td>0.023</td>
<td>−0.033</td>
<td>0.220</td>
<td>0.827</td>
</tr>
<tr>
<td>HDL</td>
<td>−0.054</td>
<td>0.125</td>
<td>−0.064</td>
<td>0.431</td>
<td>0.669</td>
</tr>
<tr>
<td>Creatinine</td>
<td>10.159</td>
<td>8.895</td>
<td>0.170</td>
<td>1.142</td>
<td>0.261</td>
</tr>
<tr>
<td>DBP</td>
<td>−0.294</td>
<td>0.368</td>
<td>−0.205</td>
<td>0.799</td>
<td>0.429</td>
</tr>
<tr>
<td>SBP</td>
<td>0.042</td>
<td>0.265</td>
<td>0.011</td>
<td>0.037</td>
<td>0.971</td>
</tr>
<tr>
<td>γ-GT</td>
<td>0.003</td>
<td>0.073</td>
<td>0.011</td>
<td>0.037</td>
<td>0.971</td>
</tr>
<tr>
<td>ALP</td>
<td>−0.040</td>
<td>0.100</td>
<td>−0.126</td>
<td>0.396</td>
<td>0.695</td>
</tr>
<tr>
<td>T1D (yes/no)</td>
<td>10.996</td>
<td>5.038</td>
<td>0.370</td>
<td>2.183</td>
<td>0.035</td>
</tr>
</tbody>
</table>
as a key regulator of adipose tissue inflammation and IR; notably, both serum levels and adipose tissue expression of the number of pro-inflammatory cytokines, such as IL6, TNFz, MCP-1 and iNOS, were significantly reduced in experimental models of mice lacking the OPN gene (11). In these studies, OPN deficiency led to reduced adipose tissue inflammation and increased insulin sensitivity (10, 11).

In agreement with the results obtained in our study, circulating OPN levels were also associated with a dysmetabolic profile in other autoimmune diseases, such as psoriasis and rheumatoid arthritis (22, 31); in these cohorts, higher OPN concentrations correlated with hypertension and increased arterial stiffness. The hypothesis of a direct role of OPN in atherosclerosis is supported by the occurrence of more severe cardiovascular disease and restenosis after percutaneous coronary intervention in patients with elevated OPN levels, independent of traditional cardiovascular risk factors (32, 33).

In conclusion, this study shows for the first time in a cross-sectional setting that increased OPN levels are independently associated with T1DM and identify patients with an unfavorable metabolic profile. Therefore, our results provide further support to the hypothesis that OPN may have a role in the prediction of micro- and macro-vascular diabetes complications. Future studies are warranted to evaluate OPN as a possible novel marker/mediator of increased cardiovascular risk and a useful tool for risk stratification in T1DM patients.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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