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

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Running title: Osteopontin and type 1 diabetes

Word count: 1946

Key-words: OPN, T1DM, inflammation, cardiovascular risk

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ABSTRACT

Objective. Osteopontin (OPN) is a sialoprotein implicated in different immunity and metabolic pathways. As 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 to participate in the acute pancreatic islets response to experimentally-induced diabetes in NOD mice. Furthermore, OPN promotes adipose tissue dysfunction, systemic inflammation and insulin resistance. Aims of this study were to evaluate circulating OPN levels in adult patients with type 1 diabetes mellitus (T1DM) compared to non-diabetic subjects 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 outpatients clinic at Sapienza University of Rome, and 52 healthy sex and age-comparable controls. 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, comedication 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 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 the cytoplasm of several different cells. The term osteopontin takes origin from the role of this protein in bone metabolism, as OPN exert a major function in controlling biomineralization and stimulating adhesion, migrations and bone resorption by osteoclasts [1-3].

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 [4-7]. 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, increasing pro-inflammatory cytokines release in the bloodstream and, consequently, promoting the development of insulin resistance-related conditions [9-11]. Increased OPN levels were found in obese subjects [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 [16-19], 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 mice and genetic studies of 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, 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 [29].

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

Therefore, aims of this study were to evaluate circulating OPN levels in an adult population of T1DM patients compared to non-diabetic subjects 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 ± SD age: 36.2 ± 12 years, mean ± SD diabetes’ duration: 13.2 ± 13.3 years) among those referring to our Diabetes outpatients clinics at Sapienza University of Rome and 52 control subjects comparable for sex and age (M/F: 33/19, mean ± SD age: 39.3 ± 7.4 years, p-value: 0.87, 0.16 respectively) and without T1DM or other chronic diseases, selected among Sapienza University employees undergoing clinical evaluation for the Occupational Medicine Service. Subjects’ recruitment took place between June 2013 and February 2014.

Each subject underwent medical history collection and physical examination [height, weight, waist circumference, systolic and diastolic blood pressure (SBP, DBP, mmHg) measurement and Body Mass Index (BMI, Kg/m²) calculation] as well as, where appropriate, clinical/instrumental assessment for diabetes’ complications, daily insulin requirement (IU/Kg/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 [30].

Diabetic nephropathy (DN) was defined as persistent microalbuminuria (30-300 mg/day) or macroalbuminuria (>300 mg/day) in at least 2 of 3 urine samples collected over 24 hours.

All study subjects 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 ureic 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 Fiedwald 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® on sera frozen immediately after separation and stored at -25°C for few days.

Statistics

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 follow: 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 2 of 3 urine samples collected over 24 hours.

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 ± standard deviation, median (min-max) or as percentage in both text and tables, as appropriate. For all the above, a p-value < 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 subject after 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±SD: 17.2 ± 12.9, [median(min-max): 13.6(1.4-62.9)] µg/L vs mean±SD: 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 alkaline phosphatase than control group.

In the overall study population (\( n=107 \)) 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, \( p=0.02 \)), \( \gamma \)-GT (correlation coefficient: 0.31, \( p=0.006 \)), ALP (correlation coefficient: 0.27, \( p=0.04 \)), PTH (correlation coefficient: 0.37, \( p<0.001 \)), lower HDL (correlation coefficient: -0.25, \( p\text{-value}=0.02 \)) 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-Is 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-Is OPN: 15.9±11.3 µg/L, T1DM subjects without ACE-Is 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).

**Discussion**

Our study demonstrates that circulating OPN levels are significantly higher in adult patients with T1DM compared to healthy subjects 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, 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 Z et al. [31], but the presence of clinical and biochemical determinants of high OPN levels was not explored.

Moreover, Gordin D et al. [26] demonstrated in a large population of T1DM patients from the FinnDiane study cohort, with a median disease duration of 20 years, that serum OPN concentration was an independent predictor of diabetic nephropathy, 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 to 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, less than 30% had macroalbuminuria. Indeed, we observed a direct correlation between OPN levels, BUN 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 as a key regulator of adipose tissue inflammation and insulin resistance; notably, both serum levels and adipose tissue expression of a number of pro-inflammatory cytokines, such as IL-6, TNF-α, 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 associated with a dysmetabolic profile also in other autoimmune diseases, such as psoriasis and rheumatoid arthritis [22,32]; 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 [33,34].

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 macrovascular 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.

Disclosure

This work was funded by research grants from the Sapienza Ateneo Scientific Research 2010 to CA, MDF, MGC and from the Regione Autonoma della Sardegna RAS 2007 (number CRP-59453) to MGB. There is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.
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Table 1. Clinical and biochemical characteristics of T1D patients and controls.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>T1D (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/m²)</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. * χ² test applied. Values are expressed by mean±SD, median (min-max) or rate of subjects, as appropriate. P-values <0.05 are considered statistically significant.
Table 2. Characteristics of T1DM patients.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</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 - 7.1±1.7</td>
</tr>
<tr>
<td>IR (U/Kg/day)</td>
<td>0.45±0.5</td>
</tr>
<tr>
<td>Fasting C-peptide (ng/ml)</td>
<td>0.1±0.3</td>
</tr>
<tr>
<td>Range</td>
<td>0-1</td>
</tr>
<tr>
<td>Microalbuminuria (mg/day)</td>
<td>9.6±16.5</td>
</tr>
<tr>
<td>Range</td>
<td>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 % (n)</td>
<td>35% (19)</td>
</tr>
</tbody>
</table>

Values are expressed by mean±SD, range or number/percentage, as appropriate.
<table>
<thead>
<tr>
<th>Non standardized Beta</th>
<th>Standard deviation Error</th>
<th>Standardized Beta</th>
<th>t</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Constant)</td>
<td>-26.477</td>
<td>20.909</td>
<td>-</td>
<td>-1.266</td>
</tr>
<tr>
<td>PTH</td>
<td>-0.005</td>
<td>0.023</td>
<td>-0.033</td>
<td>-0.220</td>
</tr>
<tr>
<td>HDL</td>
<td>-0.054</td>
<td>0.125</td>
<td>-0.064</td>
<td>-0.431</td>
</tr>
<tr>
<td>Creatinine</td>
<td>10.159</td>
<td>8.895</td>
<td>0.170</td>
<td>1.142</td>
</tr>
<tr>
<td>DBP</td>
<td>-0.294</td>
<td>0.368</td>
<td>-0.205</td>
<td>-0.799</td>
</tr>
<tr>
<td>SBP</td>
<td>0.472</td>
<td>0.265</td>
<td>0.471</td>
<td>1.777</td>
</tr>
<tr>
<td>γ-GT</td>
<td>0.003</td>
<td>0.073</td>
<td>0.011</td>
<td>0.037</td>
</tr>
<tr>
<td>ALP</td>
<td>-0.040</td>
<td>0.100</td>
<td>-0.126</td>
<td>-0.396</td>
</tr>
<tr>
<td>T1D (yes/no)</td>
<td><strong>10.996</strong></td>
<td><strong>5.038</strong></td>
<td><strong>0.370</strong></td>
<td><strong>2.183</strong></td>
</tr>
</tbody>
</table>

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