Serum Monocyte Chemoattractant Protein-1 (MCP-1) Concentrations Associate with Diabetes Status but not Arterial Stiffness in Children with Type 1 Diabetes

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Objective: The relationship between circulating markers of inflammation and arterial stiffness in children with type 1 diabetes (T1D) is not well-studied. We tested whether inflammatory MCP-1 concentrations correlate with arterial stiffness or T1D status.

Research Design and Methods: MCP-1 concentrations and radial tonometry data were available for 98 children with T1D and 55 healthy controls. Arterial stiffness was calculated as heart rate-corrected augmentation index (AI75). Correlation between MCP-1 and AI75, and differences in MCP-1 concentrations between cases and controls were tested.

Results: MCP-1 was significantly higher in children with T1D than controls (p<0.001). However, there were no correlations between MCP-1 and AI75 in the overall sample or upon stratification by T1D status (range p=0.28-0.66).

Conclusion: Circulating MCP-1 was not associated with arterial stiffness but was significantly elevated in children with T1D, indicating a pro-inflammatory state as young as 10 years. The clinical significance MCP-1 elevation in T1D needs further investigation.
Type 1 diabetes (T1D) is associated with endothelial inflammation and arterial stiffness. We previously demonstrated arterial stiffness is apparent in T1D children as young as 10 years of age when compared to matched controls (1) but noted poor correlation with both traditional cardiovascular disease (CVD) risk factors (e.g., HbA1c, LDL-C, family history) and novel serum CVD risk factors (interleukin-6, tumor necrosis factor, c-reactive protein, superoxide dismutase, and nitric oxide) (1; 2). Notably, a genetic association with arterial stiffness was seen (3). We postulated that the lack of correlation between arterial stiffness and previously studied risk factors was likely representative of the low short term absolute risk for macrovascular events in our young T1D cohort. Given that the majority of CVD events in T1D patients are clustered amongst those with concurrent diabetic nephropathy, we sought to determine if Monocyte Chemoattractant Protein-1 (MCP-1), a serum marker with known correlation to CVD events and diabetic nephropathy, would correlate with arterial stiffness in children with T1D (4). As MCP-1 is stimulated by chronic hyperglycemia and is responsible for induction of superoxide anion, cytokine production, and adhesion molecule expression (5), exploration of potential correlation with global vascular dysfunction in children with T1D was warranted.

In this analysis, we sought to 1) determine whether MCP-1 concentrations correlate with arterial stiffness as measured by radial artery tonometry; and 2) validate previous associations between circulating MCP-1 concentrations and T1D status in a case-control analysis.

**RESEARCH DESIGN AND METHODS**

The study population and method for arterial stiffness measurement have been previously described (1). Briefly, children with T1D of at least 1 year duration were recruited from the Florida Diabetes Camp. Control children were recruited from general pediatrics practices in Gainesville, Florida. Eligible children had no CVD and no history of antihypertensive or lipid-lowering medication use. Blood was collected and augmentation index corrected to a heart rate of 75 (AI75) was measured by radial tonometry in children fasted for at least 8 hours as previously described (1). Serum lipids and cytokines, blood HbA1c, and plasma glucose were analyzed as previously reported (1). Serum MCP-1 concentrations were quantified by cytometric fluorescence detection (R&D Systems, Minneapolis, MN) and natural log(ln)-transformed prior to analyses. The study was approved by the Institutional Review Board of the University of Florida and children were enrolled after written consent and assent.

**RESULTS**

AI75 measurements and MCP-1 concentrations were available for 98 children with T1D and 55 healthy controls (Table 1). Both groups were well matched for age, heart rate, total- and LDL-cholesterol. Controls had significantly higher BMI and triglycerides and lower HDL-C. lnMCP-1 correlated with triglycerides in T1D subjects (r=0.2, p=0.04) but showed no correlation with age, heart rate, BMI, glucose, HbA1c, or other lipid parameters in controls or T1D subjects.

Overall (n=153), there was no correlation between lnMCP-1 concentrations and AI75 (r=0.04; p=0.66). Furthermore, there were no significant correlations between lnMCP-1 concentrations and AI75 when children were stratified by T1D status: T1D, r=-0.11 (p=0.28) and children without T1D, r=-0.12 (p=0.38). AI75 did not differ across tertiles of lnMCP-1 in either children with or without T1D. Among those with T1D, AI75
across lnMCP-1 tertiles were 6.38±9.47, 1.89±10.19, and 4.59±12.45 (p=0.20, p for trend=0.32). Among children without T1D, AI75 across lnMCP-1 tertiles were 0.14±5.91, -4.42±10.72, and -5.03±11.92 (p=0.26, p for trend=0.14).

Despite the lack of association between lnMCP-1 and AI75, lnMCP-1 concentrations differed between children with and without T1D. Ln MCP-1 concentrations were 5.75 ± 0.39 pg/ml and 5.36 ± 0.45 pg/ml in children with and without T1D, respectively (p<0.001).

CONCLUSIONS
Most studies of chemokines in adults with T1D have concentrated on their correlation with microvascular disease (6-8). Conversely, studies of chemokines in children with T1D have largely been performed to study the potential of chemokines to predict or explain developing or ongoing autoimmunity (9). Elevated MCP-1 concentrations have previously been documented in children with newly diagnosed T1D diabetes when compared to children at increased risk of developing diabetes and controls (10).

In this study we confirmed that serum MCP-1 concentrations are elevated in children with T1D when compared to matched controls. Nevertheless, MCP-1 levels failed to correlate with non-invasive measures of arterial stiffness, regardless of whether or not comparisons were made amongst T1D subjects, controls, or the entire study population. Interestingly, MCP-1 levels in our T1D population correlated with triglyceride levels. The observed correlation between serum MCP-1 and triglycerides in T1D subjects provides support for the concept of MCP-1 as a marker or potential mediator of CVD in the T1D population. As we failed to reject the null hypothesis, additional analyses were not performed to avoid statistical bias for this focused study. A larger study, designed to control for additional confounders (i.e. sleep apnea) and powered to evaluate the role of lipids, additional chemokines, acute glycemic changes, albuminuria, age, gender, pubertal status, duration of diabetes, and longitudinal HbA1c values would likely yield additional informative data on MCP-1’s role in T1D.

Given the strong associations between MCP-1 and CVD in large adult population studies, elevated MCP-1 levels likely reflect some component of overall lifetime macrovascular risk in T1D patients (11). The low absolute risk of near-term CVD in children with high long-term risk may account for the lack of correlation between MCP-1 and arterial stiffness in this relatively young cohort. Because systemic cytokine concentrations may be influenced by multiple factors not solely related to arterial stiffness per se (e.g., acute stress, time of day, metabolic status), serum MCP-1 concentrations at a single time-point may imprecisely correlate with the specific arterial stiffness phenotype. Rather, MCP-1 concentrations could more plausibly provide a global index of inflammatory burden of disease as was seen in the significant difference in MCP-1 by diabetes status in our analysis.

In summary, serum MCP-1 levels are higher in children with T1D than controls and correlate with triglycerides but not with arterial stiffness. Future efforts will explore the potential relationship between triglycerides and MCP-1 as we attempt to identify sensitive and specific serum markers predictive of long term risk CVD risk in children with T1D.

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REFERENCES


T1D-Control Comparisons and Correlations with ln MCP-1

<table>
<thead>
<tr>
<th>Variable</th>
<th>T1D Controls</th>
<th>Controls</th>
<th>T1D-Control Comparison P value</th>
<th>In MCP-1 Correlation T1D R (P-value)</th>
<th>In MCP-1 Correlation Controls R (P-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCP-1 (pg/ml)</td>
<td>234.9 ± 106.8</td>
<td>337.9 ± 122.1</td>
<td>&lt;0.001</td>
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<tr>
<td>ln MCP-1 (pg/ml)</td>
<td>5.75 ± 0.39</td>
<td>5.36 ± 0.45</td>
<td>&lt;0.001</td>
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<tr>
<td>Age (age)</td>
<td>12.9 ± 1.4</td>
<td>13.6 ± 2.3</td>
<td>0.084</td>
<td>-0.02 (0.84)</td>
<td>-0.06 (0.64)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.0 ± 3.5</td>
<td>24.0 ± 5.4</td>
<td>0.003</td>
<td>0.11 (0.29)</td>
<td>0.10 (0.46)</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>8.5 ± 1.2</td>
<td>5.27 ± 0.3</td>
<td>&lt;0.001</td>
<td>-0.05 (0.60)</td>
<td>-0.004 (0.97)</td>
</tr>
<tr>
<td>Heart Rate (beats/min)</td>
<td>78.6 ± 11.8</td>
<td>76.5 ± 11.7</td>
<td>0.22</td>
<td>-0.03 (0.77)</td>
<td>0.05 (0.70)</td>
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<tr>
<td>Fasting Glucose (mg/dL)</td>
<td>161.8 ± 70.4</td>
<td>85.1 ± 8.8</td>
<td>&lt;0.001</td>
<td>0.06 (0.52)</td>
<td>0.10 (0.46)</td>
</tr>
<tr>
<td>Total Cholesterol (mg/dL)</td>
<td>159.4 ± 32.3</td>
<td>158.7 ± 28.2</td>
<td>0.87</td>
<td>0.11 (0.29)</td>
<td>0.17 (0.22)</td>
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<tr>
<td>LDL Cholesterol (mg/dl)</td>
<td>88.1 ± 26.6</td>
<td>88.4 ± 24.5</td>
<td>0.98</td>
<td>0.10 (0.36)</td>
<td>0.15 (0.28)</td>
</tr>
<tr>
<td>HDL Cholesterol (mg/dl)</td>
<td>57.3 ± 11.3</td>
<td>51.6 ± 11.9</td>
<td>0.003</td>
<td>-0.17 (0.1)</td>
<td>-0.09 (0.51)</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>68.5 ± 61.2</td>
<td>94.9 ± 59.3</td>
<td>0.008</td>
<td>0.20 (0.04)</td>
<td>0.18 (0.55)</td>
</tr>
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