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The urinary inflammatory profile in gluten free diet—adherent adolescents with type 1 diabetes and celiac disease

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ABSTRACT

Aims: Our objective was to characterize urinary cytokine/chemokine excretion in adolescents with type 1 diabetes (T1D) and celiac disease (CD) adhering to gluten free diet (GFD) compared to matched T1D patients and healthy control (HC) group from an existing cohort.
Methods: Eighteen T1D + CD + GFD patients aged 10–16 years were identified and matched 2:1 for age, sex, diabetes duration and glycated hemoglobin to 36 T1D subjects and 36 HC. T1D + CD + GFD patients were adherent with a GFD. Urine and serum levels of cytokines/chemokines as well as baseline clinical and laboratory variables were assessed.
Results: T1D + CD + GFD patients exhibited lower levels of urinary IL-1B, IL-4, IL-5 ($p < 0.05$) and IFN-γ, IL-8 and G-CSF levels ($p < 0.07$) compared with T1D patients. Urinary biomarker levels between T1D + CD + GFD and HC were mostly similar. In contrast, urinary FGF-2, Flt-3, IL-1B, IL-1RA, IL-4, IL-5, IL-9, IL-10, IL-12p40, IL-15, MIP-1β, and TNF-β ($p < 0.05$) were higher in T1D patients compared to HC. Similar levels of inflammatory markers were seen in the serum for all 3 groups.
Conclusions: T1D + CD + GFD patients demonstrated decreased urinary inflammatory cytokine/chemokines compared to T1D and some similar to HC, which is suggestive of a potential modulatory role of treated CD on urinary markers.

1. Introduction

Recent data support a role for celiac disease (CD) in type 1 diabetes (T1D) as a risk factor in the progression of microvascular complications. In a recent large cohort of childhood-onset T1D and CD patients, increased risk for microvascular complications with earlier age of onset was seen in dual diagnosis patients compared to T1D alone patients (Rohrer et al., 2015). In addition, patients with unrecognized or untreated CD and T1D had higher rates of retinopathy, nephropathy and neuropathy (Leeds, Hopper, Hadjivassiliou, Tesfaye, & Sanders, 2011). Furthermore, population-based studies have described increased morbidity and mortality in adults with longer duration of CD (Mollazadegan, Kugelberg, et al., 2013; Mollazadegan, Sanders, et all., 2013). However, these data do not provide insight into the impact of adherence to the gluten free diet (GFD), which remains the only effective treatment for CD, on diabetes-related complications. Recent data in adolescent patients with both CD and T1D are suggestive of a renoprotective role for the GFD (Malalasekera, Cameron, Grixti, & Thomas, 2009) and cohort studies comparing T1D with CD to T1D alone patients have reported lower rates of microalbuminuria progression and advanced glycation end-products (AGE) in diabetes patients following a GFD (Gopee, van den Oever, Cameron, & Thomas, 2013; Pham-Short et al., 2014). Additional evidence has shown that adoption of a GFD in CD subjects reduces markers of oxidative stress (Ferretti, Bacchetti, Masciangelo, & Saturni, 2012) and alters the urine metabonomic profile (Bertini et al., 2009).

Unfortunately, data related to mechanisms of renal injury due to CD in patients with T1D remain limited. Since diabetic nephropathy (DN) related to T1D is characterized by a long, clinically silent phase, it is

Conflict of interest: The results presented in this paper have not been published previously in whole or in part. These data were presented in abstract form to the American Diabetes Association Meeting, June 2014.

The study was approved by the Research Ethics Board of the Hospital for Sick Children, Toronto, ON, Canada. The study protocol was conducted in accordance with the guidelines of the Declaration of Helsinki of 1975, as revised in 2008 and informed consent was signed by all participants and their parents.

The authors declare that they have no conflict of interest.

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important to elucidate potential "pre-clinical" pathways that promote renal injury, including the interaction between hyperglycemia, increased intraglomerular pressure and inflammation (Cherney, Scholey, Sochett, Bradley, & Reich, 2011; Har et al., 2013). For example, it has been previously demonstrated that changes in urinary cytokine/chemokine excretion occur in patients with T1D prior to clinical nephropathy manifestations such as microalbuminuria (Cherney et al., 2012). Moreover, urinary inflammatory markers correlate with declining of renal function in T1D patients (Blazquez-Medela et al., 2014; Wolkow et al., 2008). The relationship between urinary cytokines/chemokines, T1D and CD, with or without GFD, is not currently known.

Given the critical role of inflammation and immune-mediated injury that occurs in the pathogenesis of early DN, we identified young patients with T1D and CD treated with GFD from an existing diabetes cohort study and measured urinary cytokine/chemokine excretion. Comparisons were made to patients diagnosed with T1D without CD and a healthy control (HC) group. The aim of this pilot study was to evaluate the urinary excretion of these inflammatory factors in a cohort of adolescent patients with T1D and CD adherent to a GFD. We hypothesized that the adherent T1D and CD group may represent a distinct group with altered urinary immune markers in relation to subjects with and without diabetes.

2. Subjects, materials and methods

2.1. Participants

Patients were recruited from an existing multicenter trial for which assessment of inflammatory markers was being evaluated (Har et al., 2013) and HC were recruited through local advertisements, friends and family.

Inclusion criteria for patients with T1D were: age 10–16 years, duration of T1D ≥1 year, no history of hypertension, renal disease or vascular disease. Of 388 participants we identified 20 patients diagnosed with CD confirmed by duodenal biopsy. Two patients were excluded from analysis, as they did not provide a urine specimen. Eighteen CD positive patients (T1D + CD + GFD) were matched for age, sex, T1D duration and glycated hemoglobin (HbA1c) to 36 T1D without CD (T1D) subjects and to 36 healthy controls (HC) for age and sex. All CD positive T1D patients were compliant with a GFD as per annual testing with negative anti-tissue transglutaminase (TTG).

Demographic data were collected from the baseline visit for each subject including: height, weight, waist circumference, blood pressure, and medical history. A fasting venous sample was also collected for testing total cholesterol, HDL, LDL, triglycerides and HbA1c.

2.2. Urinary and serum proteomics

Participants provided early morning urines in a 50 ml sterile container, as per our previously published protocol (Cherney et al., 2011; Cherney et al., 2012). In brief, urine specimens were then centrifuged at 1500 g for 15 minutes to remove cells, separated into 1 ml aliquots and frozen at −80 °C. Serum was collected on the same morning and stored at −80 °C. We used Luminex xMAP technology for multiplexed quantification of 41 human cytokines, chemokines, and growth factors: EGF, Eotaxin, FGF-2, Flt-3 ligand, Fractalkine, G-CSF, GM-CSF, GRO, IFN-α2, IFN-γ, IL-10, IL-12 (p40), IL-12 (p70), IL-13, IL-15, IL-17A, IL-1ra, IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IP-10, MCP-1, MCP-3, MDC (CCL22), MIP-1α, MIP-1β, PDGF-AA, PDGF-AB/BB, RANTES, TGFα, TNF-α, TNF-β, VEGF, sCD40L. The forty-one markers were simultaneously measured in the samples using a MILLIPLEX Human Cytokine/Chemokine 41-plex kit (Millipore, St. Charles, MO, USA). The multiplexing analysis was performed using the Luminex™ 100 system (Luminex, Austin, TX, USA) by Eve Technologies Corp. (Calgary, Alberta). The investigator performing data analysis was blinded to all study parameters. In addition, we determined the urinary albumin to creatinine ratio (ACR) by immunoturbidimetry on urine collected on the day of the baseline, therefore adjusting for creatinine.

2.3. Statistical analysis

Descriptive statistics mean and standard deviation for continuous variables; and frequency and percentages for categorical variables were used to describe the sample. Continuous measures were log transformed to stabilize their variations. Analysis of variance and analysis of covariance were used to compare HC, T1D, and T1D + CD + GFD groups. Random effect method was used to take into account the matching of the data. When comparing differences between all three groups, we used *pairwise comparisons* and adjusted for false discovery rate (FDR), to control for multiple comparisons, by setting an overall significance at $p < 0.05$. After significant FDR ($p < 0.05$), individual p values ($p < 0.05$) were considered for single pairwise comparisons between groups (T1D vs. T1D + CD + GFD, T1D vs. HC, T1D + CD + GFD vs. HC). The statistical package SAS 9.3 (SAS Institute, North Carolina, USA) was used for data analysis.

3. Results

3.1. Baseline characteristics

Clinical characteristics are shown in Table 1. T1D patients had higher body mass index and ACR compared to HC. Among subjects with T1D, T1D + CD + GFD and HC, no statistically significant differences were detected in systolic and diastolic blood pressure, creatinine, total cholesterol, LDL or HDL cholesterol between the groups.

With regard to microvascular complications, none of the diabetes patients (T1D or T1D + CD + GFD) had macroalbuminuria, retinopathy or neuropathy.

3.2. Serum cytokines/chemokines

Assessment of serum cytokines was evaluated to determine differences in systemic inflammation between the groups and if

Table 1
Clinical characteristics of study groups (mean ± standard deviation).

Variables	T1D	T1D + CD + GFD	HC
n	36	18	36
Age (years)	14.5 ± 1.4	14.0 ± 1.3	14.0 ± 1.8
Male (%)	27.8	27.8	36.1
Age of diabetes onset (years)	6.5 ± 3.7	6.5 ± 4.2	–
Diabetes duration (years)	8.0 ± 3.4	8.3 ± 3.9	–
Celiac disease duration (years)	–	3.8 ± 2.4	–
HbA1c (%) (mmol/mol)	8.5 ± 1.1 (69 ± 6.8)[a]	8.7 ± 1.6 (72 ± 9.9)[b]	5.4 ± 0.3 (36 ± 1.9)
Waist (cm)	77.7 ± 11.8	74.6 ± 9.3	73.0 ± 13.1
Height (cm)	163.0 ± 8.8	162.6 ± 8.4	162.2 ± 11.2
Weight (kg)	62.9 ± 13.7	58.7 ± 11.6	56.0 ± 14.3
Body mass index (kg/m^2)	23.6 ± 4.9[a]	22.2 ± 4.2	21.1 ± 4.4
Systolic blood pressure (mmHg)	116.1 ± 12.4	111.9 ± 9.9	110.7 ± 11.2
Diastolic blood pressure (mmHg)	67.8 ± 7.0	64.8 ± 7.2	67.2 ± 7.2
Creatinine (μmol/L)	53.0 ± 7.3	52.8 ± 8.7	56.1 ± 11.6
Total cholesterol (mmol/L)	4.2 ± 0.9	4.3 ± 1.1	4.4 ± 0.9
HDL cholesterol (mmol/L)	1.57 ± 0.4	1.54 ± 0.4	1.48 ± 0.3
LDL cholesterol (mmol/L)	2.26 ± 0.7	2.39 ± 0.9	2.46 ± 0.8
Triglyceride (mmol/L)	0.84 ± 0.4	0.94 ± 0.4	0.99 ± 0.4
Albumin to creatinine ratio (mg/mmol)	2.3 ± 4.4[a]	0.92 ± 0.9	0.49 ± 0.3

[a] $p < 0.05$ for T1D vs. HC.
[b] $p < 0.05$ for T1D + CD + GFD vs. HC.

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