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Inflammation in childhood type 1 diabetes; influence of glycemic control

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ABSTRACT

Objective: Patients with type 1 diabetes have increased mortality from cardiovascular disease, and inflammation is important in the development of atherosclerosis. Our aim was to evaluate the extent of inflammation and the influence of glycemic control in the early phases of atherosclerosis in childhood type 1 diabetes. **Materials and methods**: A population based cohort representative of all children with type 1 diabetes in Norway was studied. Diabetes patients ($n = 314$) were compared to healthy controls ($n = 120$), aged 8–18 years. Circulating levels of VCAM-1, ICAM-1, E-selectin, P-selectin, TNFα, IL-6, CRP, MCP-1, IL-18, MMP-9 and TIMP-1 were measured by immunoassays. **Results**: The diabetes patients had a mean age of 13.7 (SD = 2.8) years, disease duration of 5.5 (SD = 3.4) years and HbA1c of 8.4 (SD = 1.2) % (68 mmol/mol, SD = 13.1). The levels of most of the measured markers were significantly increased in the diabetes group compared to controls. In the diabetes group, all except MCP-1 and MMP-9 were significantly correlated to HbA1c, albeit the relation to VCAM-1 was inverse. There were no significant correlations in the control group. The measured markers were only to a limited degree associated with traditional risk factors. CRP showed the most pronounced difference between diabetes patients and controls and the strongest correlation with HbA1c. The use of oral contraceptives profoundly increased CRP levels, independent of the presence of diabetes. **Conclusions**: Our results indicate that inflammation may play an important role in the accelerated atherosclerosis in early type 1 diabetes, and that this process seems primarily driven by hyperglycemia.

1. Introduction

Well known consequences of type 1 diabetes are accelerated atherosclerosis and increased cardiovascular mortality [1,2]. It is currently acknowledged that atherosclerosis is a chronic inflammatory condition [3]. Although the atherosclerotic process starts early in life, only a few studies have investigated the relationship between diabetes, atherosclerosis and inflammation in children and adolescents [4–9]. Except for Snell-Bergeon's study from 2010 [9], these previous studies included a limited number of both patients and markers of inflammation. Thus, further investigations are warranted, as several inflammatory biomarkers are involved in different stages of the atherosclerotic process.

Vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), E-selectin, P-selectin and monocyte chemoattractant protein-1 (MCP-1) are all involved in the recruitment of inflammatory cells from the blood stream into the vessel wall, and circulating levels have been associated with atherosclerosis [10–14]. Tumor necrosis factor alpha (TNFα) is a proinflammatory molecule, and circulating levels are associated with degrees of early atherosclerosis [15]. Elevated levels of interleukin-6 (IL-6) have been associated with an increased risk of

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cardiovascular death [16]. It is also an important stimulant for production of C-reactive protein (CRP), the most extensively studied marker of inflammation. CRP, as well as the proinflammatory cytokine interleukin-18 (IL-18), have been shown to be powerful predictors of cardiovascular disease [17–19]. Matrix metalloproteinase-9 (MMP-9) and its inhibitor, tissue inhibitor of metalloproteinase-1 (TIMP-1), are involved in remodeling the extracellular matrix of the arterial wall, and circulating MMP-9 levels predict cardiovascular mortality in patients with coronary artery disease [20]. In combination, these inflammatory biomarkers reflect several aspects of inflammation in atherosclerosis, and have not previously been studied collectively.

The aim of this study was to investigate the influence of childhood type 1 diabetes and glycemic control on a comprehensive selection of markers of inflammation reflecting the early stages of atherosclerosis.

2. Materials and methods

2.1. Study population

The study cohort includes 314 patients and 120 healthy controls, aged 8–18 years. It is population based and representative of all children with type 1 diabetes in Norway. The control subjects were primarily classmates of the diabetes patients. The details of the cohort have previously been described [21]. The study was conducted according to the Declaration of Helsinki, and the protocol was approved by the Norwegian Regional Committee for Research Ethics. All participants and their parents gave their written informed consent.

2.2. Laboratory methods

Overnight fasting blood samples were collected between 7.30 and 10 am. This was followed by a clinical examination. Within 1 h after venipuncture, blood samples were separated by sentrifugation at 2500 × g for 10 min and kept frozen at −80 °C until analysis. All markers of inflammation were measured in serum.

CRP was determined using an enzyme-linked immunosorbent assay (DRG Instruments GmbH, Germany) with a detection limit of 0.1 mg/L. The enzyme-linked immunosorbent assay method from Medical Biological Laboratories (Naku-ku, Nagoya, Japan) was used for analysis of IL-18. ICAM-1, VCAM-1, E-selectin, P-selectin, TNFα, IL-6, MCP-1, MMP-9 and TIMP-1 were measured by enzyme immunoassays from R&D Systems Europe (Abingdon, Oxon, UK). In our laboratory, the inter-assay coefficients of variation (CV) were: CRP < 5%, IL-18 6.5%, ICAM-1 6.6%, VCAM-1 5.3%, E-selectin 5.2%, P-selectin 7.2%, TNFα 8.5%, IL-6 10.7%, MCP-1 9.2%, MMP-9 7.3% and TIMP-1 4.4%.

HbA1c was measured at a DCCT-standardized laboratory using high performance liquid chromatography (Variant; Bio-Rad, Richmond, CA, USA), CV < 3%. Other routine laboratory analyses were performed by conventional methods.

2.3. Statistical analysis

Demographic and clinical data are presented as either proportions, means with their standard deviations (SD) or medians with the 25th and 75th percentile. Differences in continuous variables between groups were tested with the Student *t*-test for normally distributed data and the Mann–Whitney *U*-test for non-normally distributed data. Correlation analyses between continuous variables were performed using Pearson's correlation coefficient (r) and Spearman's rho (ρ) when appropriate. The χ^2-test for contingency tables with different degrees of freedom was used to detect associations between categorical independent variables. CRP was polytomized according to the 25%, 50%, and 75% quartiles. The association and gradient effect was estimated using the Mantel–Haenzel test of linear trend. Univariate linear regression analysis was performed to study the association between traditional risk factors as exposure variables with markers of inflammation as outcome variables. CRP was $\log_e$-transformed to achieve normally distributed residuals. To identify possible confounders, we studied all variables that could influence the markers of inflammation. Only variables with significant relationships with both the exposure and the outcome variables were considered as possible confounders and included in a multivariate analysis. Adjustment for multiple confounding factors was done using multivariate linear regression analysis with a manual backward elimination procedure. A significance level of 5% was used. All statistical analyses were performed using the SPSS software package for Mac, version 19.0 (SPSS, Chicago, IL).

3. Results

Almost all (97%) of the diabetes patients were on intensive insulin treatment with more than four daily insulin injections or using pumps (60%). The patients had a mean duration of diabetes of 5.5 years (SD = 3.4) and a mean HbA1c of 8.4% (SD = 1.2) (68 mmol/mol, SD = 13.1). The clinical and metabolic characteristics of the participants are shown in Table 1. The included patients constituted a representative sample of the young type 1 diabetes population in Norway regarding HbA1c, lipid status, blood pressure, gender and stage of puberty [21]. Only patients above the age of 8 years were included in the study, however, so they were slightly older, with marginally longer diabetes duration, higher body mass index (BMI) and were more frequently pump users than the rest of the diabetic children and adolescents in Norway.

Compared to the control subjects, the diabetes patients were taller, heavier and had higher BMI and waist circumference. They also had increased systolic and diastolic blood pressure, total cholesterol, HDL-cholesterol, LDL cholesterol, apolipoprotein B and apolipoprotein A1 (Table 1).

Table 1
Clinical and metabolic characteristics.

	Diabetes patients	Controls	*p*-value
n	314	120	
Age (years)	13.7 (2.8)	13.2 (2.6)	NS
Girls, *n* (%)	158 (50.3)	68 (56.7)	NS
Height (cm)	160.4 (14.4)	157.0 (13.5)	.019
Weight (kg)	54.9 (16.7)	48.0 (13.4)	<.001
BMI (kg/m^2)	20.8 (3.9)	19.1 (3.1)	<.001
Waist circumference (cm)	71.2 (10.0)	66.6 (6.6)	<.001
Systolic blood pressure (mmHg)	101.0 (10.1)	98.1 (10.2)	.009
Diastolic blood pressure (mmHg)	60.5 (8.3)	58.3 (7.5)	.012
Smokers, *n* (%)	11 (3.5)	2 (1.7)	NS
HbA1c (%) [mmol/mol, SD]	8.4 (1.2) [68, 13.1]	5.3 (0.3) [34, 3.3]	<.001
Total Cholesterol (mmol/L)	4.6 (0.8)	4.3 (0.8)	.001
HDL (mmol/L)	1.8 (0.4)	1.7 (0.4)	.042
LDL (mmol/L)	2.5 (0.7)	2.3 (0.7)	.022
Triglycerides (mmol/L)[a]	0.7 (0.5, 0.9)	0.6 (0.5, 0.9)	NS
Apolipoprotein B (g/L)	0.74 (0.19)	0.67 (0.17)	<.001
Apolipoprotein A1 (g/L)	1.55 (0.28)	1.44 (0.32)	.003
ApoB/ApoA1	0.5 (0.2)	0.5 (0.3)	NS
Urine Albumin/Creatinine (mg/mmol)[a]	0.70 (0.40, 1.33)	0.61 (0.37, 1.35)	NS

Mean values (SD).
[a] Median (25th and 75th percentile).