

Original Article

Relationship of highly sensitive C-reactive protein and lipid levels in adolescents with type 1 diabetes mellitus

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Background: Atherosclerosis appears to begin in youth with type 1 diabetes mellitus (T1DM). Highly sensitive C-reactive protein (hsCRP) is an independent marker of cardiovascular disease (CVD) risk in adults, but its relation to dyslipidemia and other CVD risk factors in adolescents with T1DM is unknown.

Objective: To study the association between lipids and hsCRP in youth with T1DM.

Design: Cross-sectional cohort.

Methods: hsCRP and fasting lipids were measured in 74 patients with T1DM, mean age 16.2 ± 2.62 yr, mean duration of diabetes 7.3 ± 4.0 yr, and mean hemoglobin A1c (HbA1c) $8.5 \pm 1.3\%$. According to the American Heart Association/Centers for Disease Control recommendations, hsCRP values were divided into three groups: group 1: <1.0 mg/L, low CVD risk; group 2: 1.0 – 3.0 mg/L, average CVD risk; and group 3: >3 mg/L, high CVD risk. Univariate linear regression between hsCRP and lipid and clinical parameters was used, with adjustment for age.

Results: hsCRP was significantly associated with triglycerides (Tg), apoB, systolic blood pressure (SBP), and diastolic blood pressure (DBP). Subjects in the high CVD risk group had no further worsening of lipids or BP, except for a higher Tg level. ApoB, SBP, and DBP were elevated in females with hsCRP ≥ 1 compared with the low-risk group, and high-density lipoprotein was decreased. In males, this difference was only significant for SBP.

Conclusions: Elevation of hsCRP to a level ≥ 1.0 mg/L appears to be associated with elevated lipid levels in adolescents with T1DM, particularly in females. hsCRP is a marker in youth that clusters with dyslipidemia and may indicate an increased CVD risk in youth with T1DM.

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Cardiovascular disease (CVD) is a major cause of morbidity and mortality in patients with type 1 diabetes mellitus (T1DM) (1, 2). Numerous studies indicate that atherosclerosis found in youth is related to dyslipidemia. In both the Bogalusa Heart Study (3–5) and the Pathobiological Determinants of Atherosclerosis in Youth studies (6, 7), atherosclerosis was related to lipid values among other risk factors. In the Pittsburgh Epidemiology of Diabetes Complications

Study (8), young adults with T1DM were found to have elevated low-density lipoprotein (LDL) as an independent risk factor for CVD and mortality. In addition, we have previously shown that carotid artery intima-media thickness (IMT), a non-invasive marker of subclinical atherosclerosis, is increased in youth with T1DM in association with abnormal high-density lipoprotein (HDL) levels and LDL/HDL ratios (9).

Atherosclerosis is characterized by elevated levels of circulating inflammatory markers, such as highly sensitive C-reactive protein (hsCRP) (10, 11). Measurement of hsCRP is done in adults as part of a global risk assessment and as a means of identifying patients at high risk for cardiac events (12). However, there are little data determining if elevated hsCRP is associated with dyslipidemia in pediatric subjects who may be at risk for CVD. As previous studies have shown that dyslipidemia is a risk factor for CVD in youth with T1DM, this study was conducted to delineate the relationship between serum lipid concentrations and hsCRP in a cohort of adolescents and young adults with T1DM.

Methods

Study population

Seventy-four subjects (37 male and 37 female) with a mean age of 16.2 ± 2.62 yr (range 12–25 yr), a mean duration of T1DM of 7.3 ± 4.04 yr, and a mean hemoglobin A1c (HbA1c) of $8.46 \pm 1.29\%$ participated. Subjects were recruited from the clinical population of the Childrens Hospital Los Angeles (CHLA) Comprehensive Diabetes Center between 2001 and 2004 and were representative of our total diabetes patient population of ~940 patients. Inclusion criteria were duration of T1DM >1 yr, willingness of parent/patient to sign consent and assent, and insulin administration since diabetes diagnosis. Exclusion criteria were a clinical history and/or evidence of relevant systemic disease (such as untreated hypothyroidism, systemic lupus erythematosus, other rheumatologic disease, liver disease, pregnancy, and acute or chronic infection), history of preexisting heart disease or history of chronic use of medications, such as glucocorticoids, that could affect the hsCRP or lipid determinations. The study was approved by the CHLA Committee on Clinical Investigations.

Blood was obtained after an overnight 8-h fast and prior to insulin administration and food in the study cohort. Samples from patients on insulin pumps were obtained prior to breakfast bolus dose administration. Fasting laboratory measurements included total cholesterol, LDL, HDL, triglycerides (Tg), apoB, lipoprotein (a) [lp(a)], HbA1c, and hsCRP. Anthropometric data, i.e., weight (kg), height (cm), and body mass index (BMI, kg/m^2), systolic blood pressure (SBP), and diastolic blood pressure (DBP) were calculated.

Laboratory methods

HbA1c (%) was measured with a calibrated DCA 2000 (Bayer, Tarrytown, NY, USA). Total cholesterol and Tg were measured by enzymatic assay; HDL by

quantitative enzyme immunoassay; apoB by fixed rate time nephelometry; lp(a) by immunoprecipitation; and hsCRP by nephelometry. LDL was calculated by the Friedewald formula. Lp(a) and apoB were measured at Quest Diagnostics (San Juan Capistrano, CA, USA). The remainder of the laboratory tests was performed in the CHLA clinical laboratories.

Statistical analyses

The study cohort was cross-sectional. hsCRP values were log transformed to improve the normality of the distribution. Univariate linear regression was performed for each measured parameter vs. log(hsCRP) and adjusted for age. hsCRP results were originally divided into three groups according to the American Heart Association/Centers for Disease Control (AHA/CDC) recommendations for CVD risk assessment: group 1: <1.0 mg/L, low CVD risk; group 2: 1.0–3.0 mg/L, average CVD risk; and group 3: >3.0 mg/L, high CVD risk. However, after finding that there were no differences in any of the study variables between the average- and high-risk groups, these two groups were combined. The study variables of the hsCRP <1 group were then compared with those of the hsCRP ≥ 1 group using a logistic non-parametric regression, stratified by gender and BMI. Log-transformed data were used in this analysis because of data skewness. The level of significance was prespecified as <0.05.

Results

Characteristics of the study population are presented in Table 1. The overall ethnic distribution was 62% non-Hispanic white, 32% Hispanic, 1% African American, and 4% Asian. Mean hsCRP was 2.76 ± 3.8 mg/L. Mean lipid concentrations (normal range for age and assay) for the subjects in the study were as follows: cholesterol 184 ± 38 mg/dL, HDL 55.1 ± 12.0 mg/dL, LDL 107.5 ± 28.9 mg/dL, Tg 105.6 ± 99.5 mg/dL, lp(a) 36.2 ± 48.2 mg/dL, and apoB 87.4 ± 25.0 mg/dL. Univariate linear regression showed that TG, apoB, and SBP and DBP were all significantly correlated with the hsCRP, after adjusting for age (Table 2). Total cholesterol and HDL appeared to have positive and negative correlations, respectively, with hsCRP, although these correlations did not quite reach statistical significance. Years since diagnosis, HbA1c, LDL, and lp(a) showed no significant correlation with hsCRP.

To help evaluate the prognostic value of hsCRP, comparisons were made between the subjects with hsCRP <1 ('low risk' by the AHA/CDC guidelines), hsCRP between 1 and 3 ('average risk'), and hsCRP >3 ('high risk') for the study variables with significant or borderline significant linear regressions (Fig. 1). Because there were no significant differences between the average- and high-risk groups, these two groups

Table 1. Clinical characteristics of subjects with type 1 diabetes mellitus

hsCRP group	n = 74	Age (yr)	Gender (% males)	Diabetes duration	HbA1c (%)	BMI (kg/m ²)	SBP (mmHg)	DBP (mmHg)
Low risk (<1 mg/L)	33	15.9 ± 2.5	64	7.2 ± 4.4	8.2 ± 1.2	22.4 ± 2.9	109.1 ± 11.2	60.8 ± 6.0
Average risk (1–3 mg/L)	18	16.1 ± 3.7	44	7.8 ± 4.4	8.5 ± 1.6	25.4 ± 3.3	120.1 ± 9.8	66.5 ± 6.2
High risk (>3 mg/L)	23	16.5 ± 2.5	35	7.2 ± 3.3	8.8 ± 1.2	27.3 ± 6.3	117.2 ± 9.3	66.0 ± 6.3

BMI, body mass index; DBP, diastolic blood pressure; HbA1c, hemoglobin A1c; hsCRP, highly sensitive C-reactive protein; SBP, systolic blood pressure. Data are mean ± SD.

were combined for statistical analysis (Table 3). Subjects with hsCRP ≥1 had significantly higher Tg, cholesterol, apoB, SBP, and DBP compared with those with hsCRP <1. HDL was not significantly different between these two groups. Stratification by gender showed that the differences in these lipid levels and BP between the two hsCRP groups were greater and more statistically significant in the females compared with the males.

Discussion

The results of our study show that 24% of our patients had an hsCRP value in the average-risk CVD range, 31% had an hsCRP level in the high-risk CVD range, and 45% had a hsCRP level in the low-risk CVD range. Our data support previous studies that have shown that CRP levels are elevated in youth with diabetes (13). Mangge et al. (13) showed that hsCRP levels were significantly increased in a cohort of 148 children with T1DM compared with normal controls. In a small subset of their subjects, they showed an increase in carotid IMT, suggesting an association between hsCRP, low-grade inflammation, and early atherosclerosis. Similarly, Hayaishi-Okano et al. (14) reported elevated hsCRP concentrations that correlated with carotid IMT in a study of 55 young patients (mean age 22.1 ± 3.6 yr) with T1DM compared with healthy controls.

Table 2. Non-parametric Spearman correlations of study parameters vs. highly sensitive C-reactive protein

Parameter	Spearman coefficient (r)	p Value
Triglycerides (mg/dL)	0.467	0.001
Cholesterol (mg/dL)	0.225	0.057
HDL (mg/dL)	-0.230	0.051
LDL (mg/dL)	0.200	0.092
Lp(a) (mg/dL)	-0.031	0.812
ApoB (mg/dL)	0.357	0.002
Years since diagnosis	0.007	0.947
HbA1c (%)	0.165	0.170
SBP (mmHg)	0.447	0.004
DBP (mmHg)	0.390	0.002

DBP, diastolic blood pressure; HDL, high-density lipoprotein; HbA1c, hemoglobin A1c; LDL, low-density lipoprotein; Lp(a), lipoprotein (a); SBP, systolic blood pressure.

Our study showed an association between hsCRP and abnormal lipid levels. This is in contrast to the findings of Kilpatrick et al. (15). They determined risk factors associated with CVD and their relationship to raised CRP in a population of patients with T1DM between the ages of 13 and 67 yr, with a mean age of 30 yr. They demonstrated that, in subjects without overt CVD, CRP was associated with HbA1c, age, BMI, female gender, and family history of CVD.

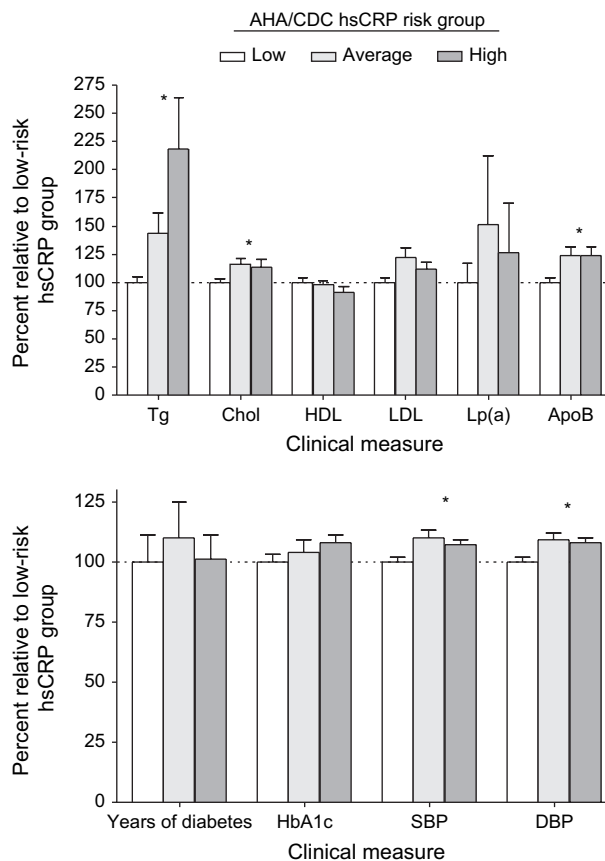


Fig. 1. Relative change in lipid (top panel) and clinical (bottom panel) parameters by hsCRP risk group. *p < 0.05 of combined average- and high-risk groups compared with low-risk group, adjusted for age, gender, and body mass index. AHA/CDC, American Heart Association/Centers for Disease Control; chol, cholesterol; DBP, diastolic blood pressure; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein; hsCRP, highly sensitive C-reactive protein; LDL, low-density lipoprotein; Lp(a), lipoprotein (a); SBP, systolic blood pressure; Tg, triglycerides.

Table 3. Logistic regression of hsCRP <1 or \geq 1 for the variables stratified by gender and adjusted for body mass index <25 or \geq 25

	Females			Males		
	hsCRP <1	hsCRP \geq 1	p	hsCRP <1	hsCRP \geq 1	p
Tg (mg/dL)	63 \pm 16	105 \pm 43	0.049	73 \pm 11	80 \pm 34	0.101
Cholesterol (mg/dL)	179 \pm 26	198 \pm 20	0.027	164 \pm 17	165 \pm 35	0.356
HDL (mg/dL)	59 \pm 10	54 \pm 6	0.738	49 \pm 5	51 \pm 14	0.682
ApoB (mg/dL)	73 \pm 16	94 \pm 15	0.013	75 \pm 12	81 \pm 21	0.376
SBP (mmHG)	101 \pm 7	113 \pm 6	0.025	108 \pm 6	122 \pm 7	0.076
DBP (mmHG)	59 \pm 7	70 \pm 4	0.026	61 \pm 5	67 \pm 4	0.503

DBP, diastolic blood pressure; HDL, high-density lipoprotein; hsCRP, highly sensitive C-reactive protein; SBP, systolic blood pressure.

Only those parameters that were found to have significant or borderline significant correlations with hsCRP were analyzed. Data are median \pm semi-interquartile range. The p values are based on using the log transformation of the variables because of skewness.

However, they did not find a significant association with dyslipidemia or with other parameters such as duration of diabetes, smoking status, presence of microvascular complications, or hypertension. In contrast, our findings are comparable to those reported in the EURODIAB Prospective Complications Study (16). In this study, CRP was strongly associated with the levels of Tg and HDL (inversely). LDL correlated with CRP in crude, but not in adjusted, analyses, possibly as a result of adjustment for HDL and Tg. Furthermore, individuals in the study with vascular complications had significantly higher levels of cholesterol, LDL, and Tg compared with those without vascular complications. In a subsequent analysis (17), the EURODIAB investigators showed an independent association of CRP with microvascular complications and CVD in this cohort of subjects with T1DM, supporting the hypothesis that endothelial dysfunction leads to alterations of normal vascular homeostatic properties and may be the initial step in the development of atherosclerosis. Finally, a recent report by Ladeia et al. demonstrated a correlation between hsCRP and Tg in adolescent boys, though not with other lipids or HbA1c (18).

The association between hsCRP and lipid levels was stronger in females than in males, and in fact, a higher proportion of females in our cohort were in the average- and high-risk hsCRP group than males. Other studies have found higher CRP levels in females compared with males, though to our knowledge, this is the first report showing that hsCRP is more closely related to other traditional CVD risk factors in female adolescents with T1DM. Clearly, this finding deserves further investigation before conclusions regarding the relationship between inflammation and lipid metabolism in the two genders can be made.

In our study, we did not find a significant association between glycemic control, measured as HbA1c at the time of the study, and hsCRP. This is in contrast to the findings in adult studies. Wu et al. (19), in a study excluding adults with diabetes, found an association between CRP and HbA1c levels. King et al.

(20), in a sample of adult respondents with diabetes from the National Health and Nutrition Examination Survey III, found that the likelihood of elevated CRP concentrations increased with rising HbA1c levels. The mean HbA1c in our population was $8.46 \pm 1.29\%$, which is comparable to levels from adolescents participating in the Epidemiology of Diabetes Interventions and Complications (EDIC) study (mean HbA1c 8.4% after study end) (21). In the EDIC T1DM adult cohort, the HbA1c measured in the Diabetes Control and Complications Trial at the 1-yr follow-up visit did not correlate with the degree of atherosclerosis measured by carotid IMT, but there was a significant correlation at the 6-yr visit (21, 22). Our results support the EDIC hypothesis that the effects of poor glycemic control on the endothelial inflammatory process and development of atherosclerosis may be a gradual process that does not fully manifest its influence on inflammatory markers for a period of years.

One of the limitations of our study was that hsCRP and lipid parameters were measured only once, and so could reflect random fluctuations and intra-individual variations. Furthermore, subclinical infection could cause elevations in hsCRP, which would increase the variability in this measure and decrease our power to detect associations between hsCRP and lipid levels. However, because assays for hsCRP and lipids were performed on the same plasma sample, they are compatible with the suggested utility of simultaneous assessment of inflammatory markers and lipid parameters as a method for risk detection. The hsCRP cut points for low, average, and high CVD risk used in our study corresponded with the approximate tertiles of hsCRP in the adult population. The high-risk tertile has an approximately twofold increase in relative risk compared with the low-risk tertile, based on the distributions of hsCRP samples from >15 populations involving >40 000 adults referenced in the AHA/CDC Workshop on Inflammatory Markers (12). In our study, we demonstrated that hsCRP

elevation at and above 1 mg/L correlated significantly with the elevations of lipid levels. We did not, however, find a further dose–response relationship related to the degree of hsCRP elevation above the threshold value of 1 mg/L. The reason for this threshold effect of hsCRP is unknown. Data are needed in children to allow for adequate definition of the hsCRP distribution in the pediatric population. Because of the cross-sectional design of the study, we cannot infer from these results a cause-and-effect relationship between hsCRP and dyslipidemia. We hypothesize that inflammation and dyslipidemia cluster. Longitudinal tracking of lipid levels, as well as their temporal relationship to inflammatory markers and glycemic control, are needed in children with T1DM. Furthermore, the association of dyslipidemia during childhood and adolescence with other diabetes complications as well as its relationship to non-invasive markers of subclinical atherosclerosis, such as ultrasound measurement of the carotid IMT, should be explored. Because the primary prevention of CVD in youth with T1DM is an imperative goal, further studies are required to determine the efficacy of aggressive treatment of dyslipidemia as well as other interventions aimed at improving impaired endothelial function in this high-risk population.

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