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C-reactive protein levels and prevalence of chronic infections in subjects with hypoalphalipoproteinemia

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Abstract

Low levels of high-density lipoprotein cholesterol (HDL-C) show a consistent relationship with the development of atherosclerosis. The underlying mechanisms are not well understood, but recent studies in subjects with primary hypoalphalipoproteinemia suggest that this could represent a proinflammatory condition. To better assess the link between HDL-C levels and C-reactive protein levels and the possible role of chronic infections as putative mediators of this relationship, we studied a population sample with nonselected causes of hypoalphalipoproteinemia. Eighty-six consecutive patients with HDL-C levels below 40 mg/dL who attend our lipid clinic and 86 control subjects with normal concentrations matched for gender, age, smoking habit, and weight were included in the study. Mean HDL-C levels were 34 ± 3.9 and 55.4 ± 8.8 mg/dL for subjects with hypoalphalipoproteinemia and control subjects, respectively. C-reactive protein concentrations were increased in case patients as compared with control subjects (2.13 ± 2.0 vs 1.52 ± 1.8 mg/L; $P = .025$). The prevalence of herpes simplex virus type 1, cytomegalovirus, *Chlamydia pneumoniae*, and *Helicobacter pylori* infections did not differ between the 2 groups. Although a possible confounding variable could be a degree of insulin resistance within the group of patients with low HDL-C levels, our results indicate that C-reactive protein levels are increased in subjects with nonselected hypoalphalipoproteinemia and that chronic infections do not appear to mediate this relationship.
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1. Introduction

Low concentrations of plasma high-density lipoprotein cholesterol (HDL-C) (hypoalphalipoproteinemia) are associated with an increased risk of cardiovascular disease. Data from the Framingham studies suggest that for each 1-mg/dL decrease in HDL-C, the risk of coronary heart disease (CHD) increases by 2% to 4% [1]. Isolated hypoalphalipoproteinemia, low levels of HDL-C and normal low-density lipoprotein cholesterol (LDL-C) concentrations, is the most common lipid abnormality found in young survivors of a myocardial infarction [2], and its prevalence is very high in patients with angiographically documented CHD [3]. The pathophysiological mechanisms involved in this association are not completely understood, but interactions between oxidant activity, reverse cholesterol transport, and, more recently, inflammatory responses have been suggested.

Levels of C-reactive protein (CRP), a sensitive marker of inflammation [4,5], have been shown to be increased in subjects with primary hypoalphalipoproteinemia, a condition characterized by very low levels of HDL-C. Inflammation [6] and high levels of CRP [7,8] have been shown to be related to the development of atherosclerosis and cardiovascular events. As such, the hypothesis that follows could be that the connection between hypoalphalipoproteinemia and cardiovascular disease may be mediated, at least in part, by inflammation.

Because most cases of hypoalphalipoproteinemia result from a mixture of secondary causes (eg, hypertriglyceridemia, obesity, sedentary lifestyle, smoking habit, prescribed drugs/medications) or are due to an inherited monogenic or polygenic pattern, we sought to clarify the relationship between low HDL-C and inflammation by measuring the CRP

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concentrations in consecutive patients with nonselected hypoalphalipoproteinemia who attend our outpatient lipid clinic and by comparing them with the levels in control subjects with normal HDL-C concentrations. Case patients and control subjects were carefully matched for the presence of cardiovascular disease and associated risk factors. Because some existing infections are able to produce low-grade inflammation [9] and influence lipoprotein levels [10], we investigated whether the relationship between CRP and HDL-C could be mediated by concomitant chronic infections.

2. Methods and materials

2.1. Patients

This is a case-control study conducted in patients with dyslipidemia who attend the Atherosclerosis Clinic of the Carlos III Hospital (Madrid). All patients with hypoalphalipoproteinemia, defined as HDL-C below 40 mg/dL in 2 determinations at least 1 week apart, were recruited. Individuals with acute infection or inflammation, any chronic renal, hepatic, or inflammatory disease, diabetes, cancer, alcohol consumption of more than 30 g/d and those undergoing treatment with corticosteroids or lipid-lowering drugs were excluded from the study; women receiving hormonal replacement therapy were also excluded. Subjects were excluded if their triglyceride levels were higher than 300 mg/dL. Standard definitions were as recommended by the American College of Cardiology [11]. Control subjects were patients attending the same Atherosclerosis Clinic but with an HDL-C level higher than 45 mg/dL and with the same exclusion criteria. The 45-mg/dL cutoff served to ensure a clear distinction between case patients and control subjects. Control subjects were matched by gender, age (5-year groups), smoking habit, presence of cardiovascular disease, and weight (5-kg groups). The study was approved by the ethics committee of the hospital, and all participants were assured of anonymity of data when their agreement to participation in the study was being solicited.

2.2. Laboratory analysis

Lipid and lipoprotein levels were determined in fresh serum obtained from the participants after an overnight fast. Concentrations of cholesterol and triglycerides were measured by enzymatic methods (Boehringer Mannheim, GmbH, Mannheim, Germany). High-density lipoprotein cholesterol was determined after precipitation of apo-B–containing lipoproteins with phosphotungstic acid and $MgCl_2$. C-reactive protein concentrations were measured on serum stored at −80°C, which had been thawed previously on one occasion only. The assay was high-sensitivity immunonephelometry (Dade-Behring, Newark, NJ). Herpes simplex virus type 1 immunoglobulin (Ig) G antibodies were measured using an established enzyme-linked immunosorbent assay (SeroHSV1, Savyon Diagnostics Ltd, Ashdod, Israel). Cytomegalovirus IgG antibodies were measured using an enzyme-linked fluorescent assay technique (VIDAS CMV IgG, BioMérieux, Marcy l'Etoile, France). *Chlamydia* IgG antibodies were measured using a microimmunofluorescent antibody assay (*Chlamydia* MIF IgG, MRL Diagnostics, Cypress, CA), and *Helicobacter pylori* IgG antibodies were measured using a quantitative enzyme immunoassay (Pyloriset EIA-G III, Orion Diagnostica, Espoo, Finland). Test results were considered positive according to the cutoff points recommended by the manufacturers of the individual kits. All samples were run in duplicate, and the operator was blinded with regard to sample source.

2.3. Statistical analysis

Results are presented as mean±SD. Nonnormally distributed variables (CRP and triglyceride concentrations) were logarithmically transformed for the statistical analyses, but, for clarity, the nontransformed values are presented throughout the text and in the tables. Comparisons in nonmatched groups (infected vs noninfected subjects) were performed using the Student *t* test. For matched groups, comparisons were performed using the paired *t* test. The influence of triglycerides on CRP levels in case patients and control subjects was evaluated by analysis of covariance for repeated measures with the triglyceride concentration as a covariate. A stepwise regression analysis was performed to evaluate factors that could contribute to the variance in HDL-C. A 2-tailed *P* value of <.05 was accepted as indicative of statistical significance.

3. Results

A total of 172 subjects, 86 case patients (84% of whom were male) and 86 control subjects, was included in the study.

Table 1
Clinical characteristics of case patients and control subjects included in the study

	Case patients (n = 86)	Control subjects (n = 86)	*P*
Men (%)	84	84	NS
Age (y)	52 ± 12	52 ± 12	NS
BMI (kg/m^2)	26.5 ± 2.3	26.4 ± 2.3	NS
Current smokers (%)	33	33	NS
Alcohol consumption (g/wk)	53 ± 98	47 ± 67	NS
Cardiovascular disease (%)	19	19	NS
Hypertension (%)	31	28	NS
Systolic blood pressure (mm Hg)	139 ± 22	138 ± 20	NS
Diastolic blood pressure (mm Hg)	85 ± 14	83 ± 14	NS
Total cholesterol (mg/dL)	239 ± 40	249 ± 42	NS
Triglycerides (mg/dL)	151 ± 47	110 ± 45	<.0001
HDL-C (mg/dL)	34 ± 4	55 ± 9	<.0001
LDL-C (mg/dL)	175 ± 40	172 ± 42	NS
Total cholesterol/HDL-C	7.1 ± 1.5	4.6 ± 1.0	<.0001
Medications			
β-Blockers (%)	16	16	NS
Diuretics (%)	11	8	NS

NS indicates not significant.

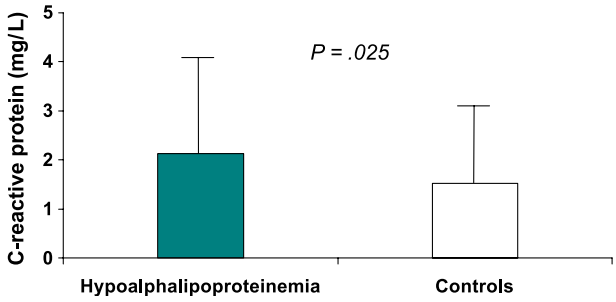

Fig. 1. C-reactive protein levels (mean ± SD) in subjects with hypoalphalipoproteinemia and those in the control group.

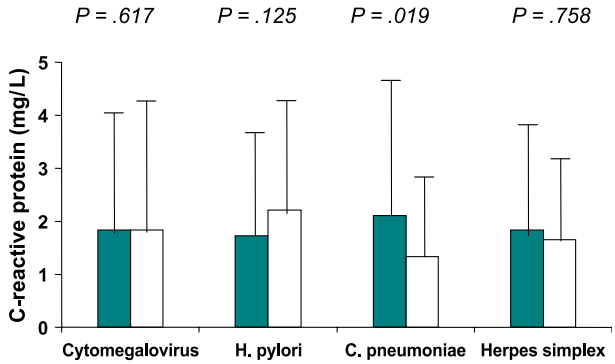

Fig. 2. C-reactive protein levels (mean ± SD) in subjects whose serologic findings were either positive (dark bars) or negative (white bars) for different chronic infections. Numbers in brackets relate to the positive or negative serologic findings for cytomegalovirus (154/18), *H pylori* (134/38), *C pneumoniae* (109/63), and herpes simplex virus 1 (162/10).

Clinical characteristics of the participants are presented in Table 1. Both groups were well balanced with respect to gender, age, body mass index (BMI), and smoking habit. No differences in LDL-C were found between the 2 groups. As expected, HDL-C concentration was lower and triglyceride concentrations were higher in case patients relative to control subjects. The concentrations of CRP were higher in subjects with low, compared to normal, HDL-C levels (2.13 ± 2.0 vs 1.52 ± 1.8 mg/L; $P = .025$; Fig. 1). C-reactive protein levels were higher in smokers ($P = .009$) and in those with a BMI ($P = .003$) and age ($P = .025$) above the median value for the study population. As subjects with hypoalphalipoproteinemia also had high triglyceride levels, we compared CRP levels in case patients and control subjects after controlling for triglycerides. C-reactive protein concentration continued to be elevated in case patients as compared with control subjects (2.13 ± 2.2 vs 1.53 ± 2.2 mg/L; $P = .034$). For the overall study population, CRP levels correlated with HDL-C concentrations ($r = -0.17$; $P = .02$) but not with triglyceride concentrations ($r = 0.10$; $P = .15$). In a stepwise regression analysis in which the covariates included age, gender, BMI, smoking status, alcohol consumption, plasma triglycerides, LDL-C, HDL-C, CRP levels, and β-blocker and diuretic treatments, the only variables that contributed significantly to the variance in HDL-C were triglyceride and CRP concentrations (15% and 3% of the variance, respectively).

The prevalence in the overall study population of seropositive *Chlamydia pneumoniae*, *H pylori*, cytomegalovirus, and herpes simplex type 1 was 63%, 78%, 89%, and 94%, respectively. No differences in the prevalence of these infections were found between subjects with low vs normal HDL-C concentrations (Table 2). Pathogen burden was defined as the sum of seropositivities against the 4 pathogens. All patients had at least one infection. An increased pathogen burden in the individuals with hypoalphalipoproteinemia was not observed. Similar results were obtained when separate analyses were performed to segregate the participants with respect to gender and age above and below the median and for subjects with or without CHD (results not shown).

Table 2
Percentage of seropositivity for different chronic infections in subjects with low HDL-C levels and a group matched for age and gender but with normal HDL-C concentrations

	Case patients (%)	Control subjects (%)	*P*
Herpes simplex virus 1	92.2	96.1	.344
C pneumoniae	63.7	58.8	.560
H pylori	76.5	80.4	.496
Cytomegalovirus	90.2	86.3	.523

C-reactive protein concentrations in subjects whose serologic findings were positive were compared with those whose serologic findings were negative for each specific infection (Fig. 2). Subjects who were seropositive for *C pneumoniae* had higher CRP levels (2.11 ± 2.14 vs 1.33 ± 1.43 mg/L; $P = .019$). The other infections did not reach statistical significance, albeit the number of noninfected individuals was too small to obtain categorical results (Fig. 2). However, pathogen burden did not relate significantly with CRP concentrations (data not shown).

4. Discussion

A number of primary and secondary prevention studies [12,13] have demonstrated an independent association between CRP and the incidence of CHD. Before incorporating CRP measurements into the CHD risk estimation, it is important to identify the factors that can influence its concentration in the circulation. In the present study, we found that nonselected individuals with hypoalphalipoproteinemia have an increased level of CRP, which, traditionally, has been seen as a marker of systemic inflammation. These data extend previous results obtained in subjects with primary hypoalphalipoproteinemia, a condition characterized by very low levels of HDL-C [4,5]. Our study has also demonstrated that chronic coincident infections, a possible cause of low HDL-C levels and increased CRP levels, do not mediate this relationship.

Epidemiological studies have demonstrated that CRP levels correlate with multiple risk factors for cardiovascular disease, including an inverse association with HDL-C [14-16]. This association is partly mediated by smoking,

obesity, insulin resistance, and diabetes—factors known to affect both CRP as well as HDL-C concentrations [15,17]. It is for this reason that we had excluded subjects with diabetes, and the case patients and control subjects were carefully matched for gender, age, BMI, smoking habit, and the presence of cardiovascular disease, thus avoiding the possible confounding effects of circumstances and conditions that could affect both parameters. However, because we had not measured waist circumference and glucose levels, we cannot rule out that part of our findings could be mediated by insulin resistance.

Different mechanisms that may explain our findings can be postulated. Elevated levels of CRP in persons with inherited forms of hypoalphalipoproteinemia [4] suggest that inflammation could be a consequence of the reduced HDL-C levels. This possibility is reinforced by the finding of very low levels of CRP in individuals with hyperalphalipoproteinemia [5]. Further support comes from a number of clinical and experimental studies demonstrating that HDL possesses anti-inflammatory activity [18,19]. However, it is also possible that inflammation could be the cause rather than the consequence of the reduced HDL-C levels. Experimentally induced inflammation in animals causes a decrease in HDL-C concentrations [20], and low HDL-C levels have been reported in various inflammatory diseases as well as a variety of infections in human beings [16,21-27]. Although chronic infections could partially explain a decrease in HDL-C observed in population studies, their involvement in the pathogenesis of hypoalphalipoproteinemia has yet to be documented. It is for this reason that we evaluated the effect of the prevalence of some chronic infections, previously related with the presence of atherosclerosis [28,29], coronary events [30], and lipid abnormalities [16,21-27], in subjects with and without low concentrations of HDL-C. Our results did not show any increased prevalence of any particular infection or an increased infection burden in case patients as compared with control subjects. This indicates that although these agents could explain a minor degree of variation in HDL-C concentration in the overall population, they probably do not have any significant effect on the pathogenesis of hypoalphalipoproteinemia as a distinct entity.

In our study, subjects whose serologic findings were positive for *C pneumoniae* had elevated levels of CRP. Elevated levels of inflammatory markers have been described previously in this and other chronic infections, and some authors have postulated that this could be the link between infections and cardiovascular disease [29-31].

Although the prevalence of these infections in our study sample was relatively high, they did not differ significantly from that reported for this age group within general populations in other European countries [28]. It is possible that this high prevalence precludes finding significant differences between individuals with normo- and hypoalphalipoproteinemia. Our sample size had a power calculation of 80% for detecting a difference of 18.5% (in absolute terms) in the prevalence of *C pneumoniae* infection between case patients and control subjects, with an estimated bilateral error of 0.05 for a percentage of discordant pairs of 46%. To have detected a difference between the 2 groups below 5%, it would have been necessary to have recruited 1456 pairs of study subjects.

We conclude that nonselected subjects with low HDL-C are in a proinflammatory state. Although chronic infections can have a moderate influence on HDL-C levels in the general population, their role in the pathogenesis of hypoalphalipoproteinemia is probably negligible.

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