Influence of serum amyloid A on the decrease of high density lipoprotein-cholesterol in active sarcoidosis

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Abstract

Objective: We have previously observed low levels of high density lipoprotein (HDL) cholesterol in active sarcoidosis. The aim of this study was to analyze the role of serum amyloid A (SAA) on this lipid disorder. Methods: Eighty five untreated sarcoid patients, 40 with active disease and 45 with inactive disease, were recruited. Sarcoidosis activity was evaluated by means of clinical, chest X-ray, gallium-67 scan, serum angiotensin converting enzyme (peptidyl-dipeptidase A) values, and pulmonary function tests. Analysis of lipoprotein metabolism included: serum cholesterol, low density lipoprotein (LDL)-cholesterol, HDL-cholesterol, HDL2-cholesterol, HDL3-cholesterol, apolipoprotein A-I (apo A-I), apolipoprotein B (apo B), and triglyceride concentrations. Serum amyloid A protein and lecithin-cholesterol acyltransferase (LCAT) activity were measured. Results: In active sarcoidosis we found significantly reduced levels of HDL-cholesterol (1.17 ± 0.36 vs. 1.44 ± 0.39 mmol/l, P = 0.002), HDL2-cholesterol (0.78 ± 0.23 vs. 1.02 ± 0.21 mmol/l, P < 0.0001), and apo A-I (1.36 ± 0.29 vs. 1.61 ± 0.27 g/l, P < 0.0001) and significantly increased levels of triglyceride (1.51 ± 0.64 vs. 1.03 ± 0.46 mmol/l, P < 0.0001), and apo B (1.14 ± 0.25 vs. 0.99 ± 0.27 g/l, P = 0.012) versus inactive sarcoidosis. Serum amyloid A concentrations were significantly increased in the patients with active disease (155.45 ± 154.01 mg/ml) compared to the inactive sarcoid patients (89.70 ± 65.36 mg/ml) (P = 0.011). There were no significant differences in cholesterol, LDL-cholesterol, HDL2-cholesterol or LCAT values between groups. Multivariate logistic regression analysis showed that HDL-cholesterol (regression coefficient b = −1.96; S.E. = 0.87; P = 0.02) and SAA (regression coefficient b = 0.01; S.E. = 0.004; P = 0.01) were the two variables independently associated with disease activity. Moreover, a significant negative correlation was observed between SAA levels and both HDL-cholesterol (r = −0.39; P = 0.01) and apo A-I (r = −0.35; P = 0.03) levels, in the active sarcoid group. Conversely, no correlation was found in the inactive sarcoid group. Conclusion: The low HDL-cholesterol and apo A-I concentrations seen in active sarcoid patients are associated with a significant increase of SAA levels. We suggest that the displacement of apo A-I by SAA on HDL accounts for the lower level of HDL-cholesterol seen in active sarcoidosis. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Sarcoidosis; Cholesterol; Activity; Serum amyloid A

1. Introduction

Sarcoidosis is a multisystemic granulomatous disease of unknown etiology that is characterized by the proliferation of monocyte-macrophages and the formation of noncaseating epithelioid granulomas that most often affect the lymph nodes, lungs, eyes, skin, liver and spleen [1]. The activated reticuloendothelial system could alter serum lipoprotein concentrations, as occurs with thermolysin-like metalloendopeptidase and angiotensin converting enzyme [2]. Some authors have already described a relationship between diseases involving monocyte-macrophages and hypcholesterolemia in patients with myeloproliferative disorders [3], Gaucher type I disease [4] and rheumatoid arthritis [5,6]. Kindman et al. [7] first published on the subject of lipid metabolism in sarcoid patients and found reduced serum values of cholesterol and HDL-cholesterol, though there was no comparison between clinically...
active and inactive disease. We have already reported that the decrease in HDL-cholesterol that is observed in patients with sarcoidosis is limited to those with active disease [8]. However, the underlying mechanism of this phenomena has not yet been directly studied. Serum amyloid A protein is an acute-phase reactant of hepatic synthesis that may be transiently associated with HDL during acute inflammation and may potentially serve as a transient cholesterol-binding protein [9]. Serum amyloid A may act either displacing apo A-I, which may result in increased catabolism of HDL, or inhibiting LCAT activity, which leads to low levels of esterified serum cholesterol [10,11]. To our knowledge, there is only one description in the medical literature about SAA in sarcoid patients that found elevated SAA levels, though there was neither comparison between active and inactive disease nor study of lipid metabolism [12].

The aim of the present study was to analyze the role of SAA protein in the decrease of HDL-cholesterol observed in patients with active sarcoidosis.

2. Patients and methods

2.1. Patients

The diagnosis of sarcoidosis was made according to the following criteria: (1) a compatible clinical and radiological picture; (2) histological demonstration of noncaseating granulomas in one or more tissues with negative stains and cultures for mycobacteria and fungi, or a positive Kveim test; and (3) exclusion of other granulomatous diseases. A total of 85 consecutive outpatients (ten men and 75 women) from a sarcoid clinic were included in the study. None of the patients was acutely ill, nor was receiving any steroids, B-blocking agents, thiazide diuretics, hormone replacement therapy or oral contraceptives at the time of the study. None of the patients was obese nor was on a hypolipidemic diet or losing weight. Alcohol intake in sarcoid patients was minimal. Patients were excluded if they had diabetes, hyperlipidemia or coronary artery disease, or if they had liver or kidney impairment or showed clinical signs of neoplastic disease or thyroid dysfunction, after undergoing a careful physical examination. As our patients were not elderly and patients with moderate or severe hyperlipidemia were excluded from the study, TSH was not routinary measured. All patients were matched for age, sex, and body mass index (Table 1) and met the clinical criteria just listed.

2.2. Evaluation of sarcoidosis activity

The parameters used for sarcoidosis activity in this study were based on those reported by James and Jones Williams [13], and are described in Table 2. Minimal residual fibrotic radiological changes not associated with other findings were not considered as sign of active disease. In patients with doubtful or minor clinical, radiological or functional derangements, abnormalities of the serum angiotensin-converting enzyme and gallium scan helped to establish the presence of activity [14,15]. According to this definition, sarcoidosis patients were classified into two different groups: 40 with active disease and 45 with inactive disease.

3. Serum samples and analytic procedure

Venous blood for lipoprotein analysis was drawn after a 12-h overnight fast, into blood-collection tubes without anticoagulant. After clotting, each specimen was centrifuged at 1200 × g for 20 min, and stored at 4°C until analysis. High-density lipoprotein cholesterol and HDL2-cholesterol were obtained from serum by selective immunoprecipitation with polyethilenglycol solutions of different concentration and pH (IMMUNO AG, Vienna, Austria). Cholesterol and triglycerides were determined by the enzymatic-colorimetric methods CHOD-PAP and GPO-PAP, respectively, commercialized by Boehringer Mannheim. The serum concentration of HDL2-cholesterol was calculated as the difference between the concentrations of HDL-cholesterol and HDL3-cholesterol. The serum concentration of LDL-cholesterol was calculated by using the Friedewald equation. Apolipoprotein A-I and apo B were measured by immunological turbidity test
(Boehringer Mannheim, Germany). Serum amyloid A was quantified from frozen samples by a solid phase sandwich enzyme-linked immuno-sorbent assay (ELISA) with a monoclonal antibody specific for SAA (Cytoscreen™, Biosource international, CA). The intra and inter-assay coefficient of variation were 4.9 and 7.8%, respectively. The analytical method used for peptidyl-dipeptidase A (serum angiotensin-converting enzyme) was a spectrometric method (Sigma Diagnostics).

To determine LCAT activity, the initial rate of cholesterol sterification was measured in human plasma. Briefly, to obtain plasma, blood was collected into tubes that contained heparin, cooled immediately in ice water and centrifuged at low speed with a refrigerated centrifuge. Each sample was separated in two parts, one of them processed immediately and the other was incubated at 37°C for 40 min with continuous shaking. Both parts were performed as follows (part I immediately and part II after 40 min). One milliliter of LCAT reagent (Wak-Chemie, Medical GMBH, Germany) was added to 50 μl of plasma, mixed, incubated for 25 min at room temperature and their absorbances measured spectrophotometrically at 546 nm. The difference of both measured absorbances (OD part I-OD part II) was extrapolated into a calibration curve made with cholesterol standard (Wak-Chemie, Medical GMBH, Germany) at the same time. Results were expressed as nmol of cholesterol/h per ml plasma and was the mean of sextuplicate.

3.1. Statistical analysis

All calculations were performed using the SPSS-PC 6.0 software package. These programs were simple data description and comparison of two groups by t tests and the Mann–Whitney U-test for variables that were not normally distributed. These tests were used to assess the univariate statistical association of demographic, clinical and laboratory variables with disease activity. Logistic regression was used to assess the multivariate statistical associations of these variables between active sarcoidosis and inactive sarcoidosis groups. A stepwise method was used for the logistic regression, including the following variables in the model: age, sex, body mass index, HDL-cholesterol, LDL-cholesterol, triglyceride, LCAT and SAA values. The correlations were calculated using the Pearson correlation coefficient. Results are presented as mean ± S.D.: P < 0.05 were considered statistically significant.

4. Results

Table 3 shows the concentrations of serum cholesterol, triglyceride, LDL-cholesterol, HDL-cholesterol and its subfractions HDL2-cholesterol and HDL3-cholesterol, and apo A-I and apo B in the patients with untreated active and inactive sarcoidosis. Mean HDL-cholesterol serum concentration was 1.17 ± 0.36 mmol/l in the active sarcoid patients as compared with 1.44 ± 0.39 mmol/l in the inactive disease group (P = 0.002).

Apo A-I concentrations were significantly reduced in the patients with active disease (1.36 ± 0.27 g/l) compared to the inactive disease group (1.61 ± 0.27 g/l) (P < 0.0001). HDL2-cholesterol concentrations were significantly decreased in patients with active disease as compared with values in the inactive disease group (P < 0.0001). Active sarcoid patients had higher levels of triglyceride and apo B concentrations than did inactive subjects (P < 0.0001 and P = 0.012, respectively).

Serum concentrations of cholesterol, LDL-cholesterol, and HDL2-cholesterol were similar in both groups.

Table 4 shows the SAA concentrations and LCAT values. Serum amyloid A concentrations were significantly increased in the patients with active disease (155.45 ± 154.01 mg/ml) compared to the inactive sarcoid patients (89.70 ± 65.36 mg/ml) (P = 0.011). The Mann–Whitney U-test confirmed the significance of the SAA values (P = 0.004). There were no significant differences in LCAT values between groups.

Multivariate logistic regression analysis showed that disease activity was independently associated with HDL-cholesterol values (regression coefficient b = −1.96; S.E. = 0.87; P = 0.02) and SAA levels (regression coefficient b = 0.01; S.E. = 0.004; P = 0.01). No

<table>
<thead>
<tr>
<th>Active sarcoidosis (n = 40, x ± S.D.)</th>
<th>Inactive sarcoidosis (n = 45, x ± S.D.)</th>
<th>P</th>
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<tbody>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>5.41 ± 0.99</td>
<td>5.29 ± 1.13</td>
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<tr>
<td>Triglyceride (mmol/l)</td>
<td>1.51 ± 0.64</td>
<td>1.03 ± 0.46</td>
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<tr>
<td>LDL-cholesterol (mmol/l)</td>
<td>3.53 ± 0.85</td>
<td>3.42 ± 1.12</td>
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<tr>
<td>HDL-cholesterol (mmol/l)</td>
<td>1.17 ± 0.36</td>
<td>1.44 ± 0.39</td>
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<tr>
<td>HDL2-cholesterol (mmol/l)</td>
<td>0.37 ± 0.18</td>
<td>0.42 ± 0.21</td>
</tr>
<tr>
<td>HDL3-cholesterol (mmol/l)</td>
<td>0.78 ± 0.23</td>
<td>1.02 ± 0.21</td>
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<tr>
<td>Apolipoprotein A-I (g/l)</td>
<td>1.36 ± 0.29</td>
<td>1.61 ± 0.27</td>
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<tr>
<td>Apolipoprotein B (g/l)</td>
<td>1.14 ± 0.25</td>
<td>0.99 ± 0.27</td>
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other variables were associated with the activity of sarcoidosis.

HDL-cholesterol levels were negatively correlated with serum angiotensin-converting enzyme levels \((r = -0.38; P = 0.01)\) and SAA levels \((r = -0.39; P = 0.01)\) in patients with active sarcoidosis (Fig. 1), whereas no correlation between these variables was found in the inactive sarcoid group \((r = 0.06; P = 0.65\) and \(r = -0.04; P = 0.75,\) respectively). Moreover, the concentrations of the main protein fraction of HDL, the apo A-I, showed also a significant negative correlation with SAA levels in the active sarcoid group \((r = -0.35; P = 0.03)\), but no correlation was found in the inactive disease group \((r = -0.02; P = 0.87)\).

5. Discussion

This study reinforces the relationship between sarcoidosis activity and lipoprotein metabolism. The present data coupled with our previous results confirming that the decrease in HDL-cholesterol serum concentrations is significantly associated with disease activity [8]. HDL-cholesterol was again the only lipid value independently associated with disease activity in the multivariate analysis. We also found, in keeping with our previous report, a significant negative correlation between serum angiotensin-converting enzyme levels, a well-known marker of inflammatory activity in sarcoidosis [16], and HDL-cholesterol levels in the active sarcoid group.

This pattern of depressed HDL-cholesterol and apo A-I has already been described in patients with a variety of systemic autoimmune diseases [5,17–20]. For instance, Lahita et al. [17] showed a pattern of dyslipoproteinemia similar to our untreated active sarcoid patients in a group of patients with active systemic lupus erythematosus and demonstrated an association between anticardiolipin antibodies and low levels of HDL-cholesterol and apo A-I in patients who were not taking corticosteroids. Svenson et al. [21] suggested that it was the degree of inflammatory activity that determined the lipoprotein alterations observed in their patients with untreated chronic inflammatory arthritides. Other authors have demonstrated a significant inverse correlation between apo A-I levels and several inflammatory parameters in a group of patients with active juvenile chronic arthritis [22].

The precise mechanism of the reduced serum HDL-cholesterol concentrations in sarcoid patients remains

<table>
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<th>Table 4</th>
<th>Serum amyloid A and lecithin-cholesterol acyltransferase values</th>
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<tr>
<td></td>
<td>Active sarcoidosis ((n = 40, \bar{x} \pm \text{S.D.}))</td>
</tr>
<tr>
<td>Serum amyloid A (mg/ml)</td>
<td>155.45 \pm 154.01</td>
</tr>
<tr>
<td>Lecithin-cholesterol acyltransferase (nmol/ml per h)</td>
<td>215.23 \pm 122.13</td>
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\(a\) By Mann–Whitney \(U\)-test.

Fig. 1. Correlation between the serum concentrations of HDL-cholesterol and serum amyloid A in patients with active sarcoidosis.
unknown. The factors that account for the decline in HDL-cholesterol and apo A-I have not been directly studied. The results of the current study show a significant increase of SAA levels in sarcoid patients classified as having inflammatory activity as compared with those having inactive disease. Although SAA levels in inactive sarcoid patients were higher than those described in healthy controls from other studies, we believe the magnitude of this increase is not high enough to interact with HDL metabolism. Elevated SAA levels were independently associated with disease activity. The presence of raised SAA levels has already been described in sarcoid patients, though a comparison between subgroups of patients with active and inactive disease could not be performed because of the small sample size [12]. Serum amyloid A is an acute phase protein produced in response to cytokines synthesized by activated monocyte-macrophages [23,24].

This is the first report of the correlations among apo A-I, HDL-cholesterol and SAA levels in sarcoidosis. In our active sarcoid patients, we have demonstrated a significant inverse correlation between HDL-cholesterol and SAA levels. Moreover, a significant negative correlation was observed as well between apo A-I and SAA levels in the active sarcoid group. Alterations in serum lipids and their relationship to acute phase proteins have already been reported in other diseases. Memon et al. [25] found a significant negative correlation between SAA and HDL-cholesterol levels in lepromatous leprosy patients, and instead, there was no statistically significant correlation between C-reactive protein and HDL-cholesterol levels in lepromatous leprosy patients. We suggest that the displacement of apo A-I from the lipoprotein surface, with the free apoprotein likely to be rapidly removed from the plasma, may act with HDL metabolism. Elevated SAA levels were independently associated with disease activity. The presence of raised SAA levels has already been described in sarcoid patients, though a comparison between subgroups of patients with active and inactive disease could not be performed because of the small sample size [12]. Serum amyloid A is an acute phase protein produced in response to cytokines synthesized by activated monocyte-macrophages [23,24].

Several studies have shown that, during the acute phase response, SAA is released from the liver and circulates in plasma incorporated into HDL particles, mainly of HDL3 density, with a concomitant decline in plasma HDL concentration [27–29]. Serum amyloid A displaces apo A-I from the HDL [30,31]. Van-Lenten et al. [32] reported a 73% decrease of apo A-I levels, as SAA levels in acute phase HDL increased, concomitantly. Alternatively, SAA may act inhibiting LCAT activity that leads to low levels of esterified serum cholesterol and quantitative and qualitative changes in HDL2 and HDL3 particles [11]. In our patients there were no significant differences in LCAT values between groups, measured as the initial rate of cholesterol esterification with an exogen substrate. It may be possible that using lipoproteins of patients as substrate, an influence of SAA in LCAT activity would have been revealed.

The marked increase in plasma SAA level and its association with HDL (HDL–SAA) displaces apo A-I from the lipoprotein surface, with the free apoprotein likely to be rapidly removed from the plasma. Previous investigations have demonstrated that macrophages are capable of binding and endocytosing HDL–SAA [33]. Our findings emphasize that SAA plays a role in cholesterol metabolism during the course of inflammation and are in accordance with the suggested mechanism of SAA displacing apo A-I, which may result in increased catabolism of SAA-containing HDL particles.

Low HDL-cholesterol levels are a risk factor for atherosclerosis. Epidemiological studies have established a negative correlation between coronary artery disease (CAD) and levels of HDL. Other studies have observed that inflammatory responses may have an effect in the atherosclerotic process and some have suggested a potential pathophysiological link between SAA protein and the development of CAD [29,34–36]. Despite the fact that in patients with active sarcoidosis the HDL-cholesterol concentrations were low with elevated SAA levels, there have so far been no reports that indicate an increasing occurrence of atherosclerotic disease in patients with sarcoidosis.

In summary, the decrease of HDL-cholesterol seen in patients with active sarcoidosis is independently associated with a significant increase of SAA levels in these patients. We suggest that the displacement of apo A-I from the HDL particles by SAA accounts for the decline of HDL-cholesterol seen in patients with active sarcoidosis. Further studies are needed to investigate the composition of HDL particles from subjects with active and inactive sarcoidosis that could demonstrate that the rapid clearance of these particles from plasma is the final mechanism that lowers HDL-cholesterol.

References


