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Clinica Chimica Acta 366 (2006) 185 – 189

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Elevated serum levels of proinflammatory cytokines and biomarkers of matrix remodeling in never-treated patients with familial hypercholesterolemia

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Received 14 September 2005; received in revised form 30 September 2005; accepted 30 September 2005
Available online 8 November 2005

Abstract

Background: Familial hypercholesterolemia (FH) is a common inherited disorder of lipoprotein metabolism, whose origin involves mutations in the gene coding for the low-density lipoprotein receptor protein. Although FH is monogenic, wide variation occurs in the onset and severity of atherosclerosis in these patients.
Methods: Since data on levels of inflammatory proteins and/or active factors in FH patients who have never received lipid-lowering treatment are lacking, serum levels of MMP-3, active MMP-9 and TIMP-1 as well as pro-inflammatory cytokines (TNF-α, IL-18) were determined in never-treated homozygous FH Moroccan patients ($n=4$) and compared to those of heterozygous FH subjects ($n=7$) and of healthy control subjects ($n=5$).
Results: When compared to controls, homozygous FH patients exhibited levels of active MMP-9 and TIMP-1 ($p<0.05$), and of both high sensitive-CRP and IL-18 which were significantly elevated ($p<0.05$ and $p<0.01$, respectively). In heterozygous FH patients, intermediate values between FH homozygotes and healthy controls were observed for these markers, with the exception of MMP-9 activity whose levels were significantly elevated ($p<0.05$). Multivariate analysis revealed a positive correlation between apolipoprotein B, TIMP-1 and IL-18 levels, and between hs-CRP and IL-18 ($p<0.01$).
Conclusions: Although the sample size of this FH group was limited, our data suggest that nontreated homozygous FH patients, and to a lesser degree heterozygous FH patients, exhibit not only a markedly proinflammatory vascular state but also pronounced extracellular matrix remodeling, as reflected by elevated circulating levels of inflammatory cytokines and MMPs.

Keywords: Atherosclerosis; Inflammation; Metalloproteinase; Familial hypercholesterolemia; Matrix remodeling

1. Introduction

Familial hypercholesterolemia (FH) is a common autosomal dominantly inherited disorder of lipid/cholesterol metabolism whose origins involve either mutations in the low-density lipoprotein (LDL) receptor gene or in the gene encoding apolipoprotein B or the in PCSK9 gene [1–4]. As a result of the disruption of LDL receptor function, patients

Abbreviations: FH, familial hypercholesterolemia; MMP, matrix metalloproteinase; TIMP, tissue inhibitor metalloproteinase; TNF-α, tumor necrosis factor; IL, interleukine; Hs-CRP, high sensitive C-reactive protein; CAD, coronary artery disease.

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doi:10.1016/j.cca.2005.09.027

with FH display markedly elevated circulating levels of LDL cholesterol from birth, ranging from 7 to 15 mmol/l in heterozygous patients to up to move than 25 mmol/l in the homozygous state. As high levels of LDL-cholesterol are a major risk factor for cardiovascular disease (CAD), FH subjects exhibit a significantly increased risk of premature CAD, due to the formation of atherosclerotic lesions in childhood [5,6].

Atherosclerosis is recognized as a chronic inflammatory disease of the arterial wall in which an inflammatory response is a key event that leads to the formation of atheromatous lesions [7]. Fragilisation of the fibrous cap of atherosclerotic plaques is intimately related to the presence of an inflammatory state which plays a crucial role in plaque rupture, the major pathophysiological event underlying acute coronary syndromes [8–10]. Expression of a spectrum of inflammatory factors, such as C-reactive protein (CRP) and proinflammatory cytokines including TNF-α and IL-18, constitutes a strong link between inflammation and atherogenesis in coronary artery disease [11]. Proinflammatory cytokines are responsible for vascular endothelial dysfunction and activation, leading for example to the overexpression of endothelial adhesion molecules [12], to enhanced uptake of oxidized LDL through increased expression of scavenger receptors in monocyte-derived macrophages [13], to plaque fragilisation [14] and to induction of cell proliferation in the arterial wall [15]. The matrix metalloproteinases (MMPs) and their inhibitors, the tissue inhibitors of metalloproteinases (TIMPs) constitute an additional group of biomarkers and of actors in the inflammatory process, and play a central role in subendothelial vascular extracellular matrix remodeling [16–18]. Experimental data have established that MMPs and TIMPs are involved in the development of atherosclerotic lesions and their fragilisation [16–19]; moreover, we recently observed that asymptomatic hyperlipidemic subjects at high cardiovascular risk display elevated circulating levels of MMP-3, MMP-9 and TIMP-1 [20].

There is a paucity of information on the inflammatory state in untreated FH subjects, and more specifically on circulating levels of cytokines, MMPs and TIMPs [21,22]. However, although the cause of FH is monogenic, wide variation exists in the onset and severity of atherosclerotic disease in these patients [23]. We hypothesized that comprehensive evaluation of the inflammatory state of never-treated FH patients might provide not only further insight into variation in disease severity but might also improve cardiovascular risk assessment. We therefore determined serum levels of several inflammatory markers, i.e. high sensitive CRP (hs-CRP), IL-18 and tumor necrosis factor (TNF)-α, and of matrix remodeling factors, i.e. MMP-3, MMP-9 and TIMP-1, in homozygous FH patients who had never received either lipid-lowering or anti-inflammatory treatment. We equally evaluated the potential relationships between these inflammatory molecules and the plasma lipid profile.

2. Materials and methods

2.1. Patients

Three groups of Moroccan subjects were studied: 4 homozygous FH subjects (M/F ratio: 3/1; age: 16.2±8.0 years, mean±SD); 7 heterozygous FH subjects (M/F ratio: 5/2; age: 42.5±22.6 years), and 5 healthy control subjects (M/F ratio: 3/2; age: 24.2±7.2 years). The diagnosis of FH was established using Simon Broome's criteria [24] and confirmed by the presence of LDL receptor gene mutation. The control subjects and FH patients are issued from 3 unrelated families with distinct LDL receptor gene mutations. All patients included in the study had never been treated for hypercholesterolemia. Exclusion criteria for both groups were: hypothyroidism, renal deficiency, hepatic disease, diabetes mellitus, arthritis, cancer and treatment with anti-inflammatory drugs. All patients gave their informed consent prior to inclusion in the study. The mean body mass indices were 22.7±7.4, 23.4±5.9 and 22.1±7.2 kg/m^2 in homozygous, heterozygous and control groups, respectively. CAD had been documented by ECG and cardiac scan in 1 homozygous and 1 heterozygous FH patient. Control individuals had no past history or evidence of CAD, or hypertension.

2.2. Analysis of circulating lipids, lipoproteins and inflammatory markers

Venous blood samples were collected by venipuncture after a 12-h overnight fast. Serum total cholesterol (TC) and triglyceride levels (TG) were measured by routine enzymatic methods (Boehringer Mannheim, FRG). Serum HDL cholesterol (HDL-C) was enzymatically determined by separating HDL from plasma by precipitation of the (LDL+VLDL) fraction with a phosphotungstic acid–magnesium chloride solution (Boehringer Mannheim, FRG). Serum LDL-C was calculated according to the Friedewald formula [25]. Apolipoproteins AI and B, lipoprotein (a) [Lp(a)], and hs-CRP levels were measured using immunonephelometric methods on a BNII analyzer (Dade-Behring, France) using the manufacturer's reagents. Detection limit for hs-CRP was 0.2 mg/l; intra- and interassay CV were <5% [20].

Serum levels of MMP-3, active MMP-9 and TIMP-1 proteins were determined using commercially available enzyme-linked immunoassays (R&D Systems, France). Intra-assay CVs were <7%; between assay CVs were <9%. Sensitivities were 0.009, 0.08, and 0.005 ng protein/ml for MMP-3, TIMP-1 and active MMP-9, respectively.

Serum TNF-α and IL-18 concentrations were measured using commercially available enzyme-linked immunoassays (Bender Med Systems GmbH and R&D Systems, France, respectively). Intra-assay CVs were <10%; between assay CVs were <15% (<10% for IL-18). Detection limits were 5.8 and 12.5 pg protein/ml for TNF-α and IL-18, respectively.