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ORIGINAL ARTICLE

Shoulder adhesive capsulitis and hypercholesterolemia: role of APO A1 lipoprotein polymorphism on etiology and severity

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Abstract

Purpose Relationship between shoulder adhesive capsulitis (AC) and hypercholesterolemia is known. The connecting link might be represented by the correlation between HDL and transforming growth factor beta (TGF-β): normally, HDLs stimulate TGF-β expression; the latter is employed in the development of fibrous tissue. We assess whether the presence of the Apo-A1-G75A-polymorphism, which is correlated to an enhanced HDL function, could be a risk factor for the genesis and severity of AC.

Methods Peripheral blood samples of 27 patients [7M; 20F, mean age 54.81 (41–65)] with AC and hypercholesterolemia were submitted to polymerase chain reaction in order to evaluate the Apo-A1-G75A-polymorphism. Genome database was used as control. Two categories were obtained according to AC severity: type I (active forward flexion $\geq 100°$) and type II ($< 100°$). Data were submitted to statistics.

Results The prevalence of Apo-A1-G75A-polymorphism in the studied group and in the control group was 22.2% (10AG; 1AA; 16GG) and 19% (OR 1.22, IC 0.59–2.53, $p > 0.05$), respectively. Patients with type I and II capsulitis were 11 [flexion 148.0° (range 100°–165°)] and 16 [flexion 82.5° (range 50°–95°)], respectively. The prevalence of Apo-A1-G75A in type I was 18.1% (2AG; 9GG) and in type II was 56.3% (8GA; 1AA; 7GG), respectively (RR 1.87, IC 1.005–3.482, $p < 0.05$).

Conclusions Apo-A1-G75A-polymorphism is not necessary for the genesis, but it is a risk factor for severity of AC.

Level of Evidence III.

Keywords Adhesive capsulitis · Frozen shoulder · Frozen shoulder etiology · Hypercholesterolemia · Lipoproteins · Adhesive capsulitis severity

Introduction

Relationship between hypercholesterolemia and shoulder pathologies has been well elucidated. It has been observed that patients with rotator cuff tears were more likely to have hypercholesterolemia when compared with the control group [1, 2]. Beason et al. [3] hypothesized that rotator cuff healing would be inferior in rats receiving a high-cholesterol diet compared with those receiving standard chow; biomechanically they demonstrated that hypercholesterolemia may have a detrimental biomechanical effect on tendon healing in rat rotator cuff injury and repair model. In a similar experimental study, Chung et al. [4] observed that hypercholesterolemia had a deleterious effect on fatty infiltration and quality of cuff tendon-to-bone repair site.

In 1995, Bunker and Esler [5] observed that the fasting serum triglyceride and cholesterol levels were significantly elevated in the shoulder adhesive capsulitis. This paper has the merit of having observed the correlation between the two diseases; however, the mechanism by which hypercholesterolemia should cause capsulitis was not explained by the authors. The mentioned association was recently confirmed by Sung et al. [6]; unfortunately, once again, authors stated that further research is needed to evaluate if a non-optimal

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serum lipid level is a cause, a related factor or a result of primary shoulder adhesive capsulitis.

The fibrotic process that affects the rotator interval region is the predominant pathological element [7]. Several lines of evidence point to transforming growth factor beta (TGF beta) as a key cytokine whose sustained production underlies the development of tissue fibrosis [8]. Its expression is induced in both vitro and vivo by high-density lipoprotein (HDL) [9]. HDL is the smallest of the lipoprotein particles, and it is the densest because it contains the highest proportion of protein to lipids; its most abundant apolipoprotein is the Apo A1, whose structural stability is critical for the HDL function.

In the literature, many single nucleotide polymorphisms (SNPs) of APOA1 have been studied [10]; all of them are associated with a decreased function of HDL. Only the Apo A1 G75A polymorphism is correlated to an enhanced function of this lipoprotein.

The aim of our study was to assess whether the presence of this gene polymorphism could be a risk factor for the genesis and severity of adhesive capsulitis of the shoulder.

Materials and methods

A prospective observational study was performed. The studied group was composed of 186 patients with shoulder adhesive capsulitis. The diagnosis, performed by one of us (SG), was obtained after clinical examination (range of motion evaluation with a goniometer); coracoid pain test [11]; evaluation of the strength in external rotation with the arm adducted and physical examination of the posterosuperior (full can test; Patte test; external rotation lag sign; strength in external rotation) and anterior (lift-off test; Napoleon test; Bear-hug test) rotator cuff tendons; an X-ray (true AP and axillary X-ray views) and MRI of the involved shoulder.

Inclusion criteria were: reduction in the active and passive range of motion arose since less than 3 months.

Exclusion criteria for all attendees were: type I and II diabetes; patients younger than 40 years and older than 65 years; previous shoulder trauma and/or surgery; neck pain symptoms; rotator cuff tears including subscapularis tears; other ipsilateral upper limb pathologies (elbow; wrist and hand pathologies, neuropathies due to intrinsic or extrinsic factors); biceps and/or labral pathologies; shoulder instability; acromioclavicular arthritis; os acromiale; degenerative arthritis of the glenohumeral joint; autoimmune, rheumatologic and thyroid disease; Parkinson disease; workers' compensation claims. A total of 122 patients were excluded since they had one or more of the exclusion criteria. All 64 remained participants were submitted to peripheral blood examination in order to evaluate blood sugar, total cholesterol and thyroid hormones concentrations.

Peripheral blood samples of 30 patients with only hypercholesterolemia (total cholesterol levels > 200 mg/dl, LDL > 130 mg/dl, HDL > 60 mg/dl) [12] were collected using anticoagulant (EDTA) plexiglass tubes and stored at +4° and within 48 h submitted to polymerase chain reaction (PCR/IDL 03) in order to evaluate the gene APO A1 G75A(rs670) polymorphism (Fig. 1).

In order to evaluate shoulder adhesive capsulitis severity, we arbitrarily distinguished two categories: type I (patients with active forward flexion ≥ 100°) and type II (patients with active forward flexion < 100°).

The allele frequencies of the studied gene, obtained from a sample composed of 107 countrymen (extracted from the "1000 Genome" database), were considered as controls [13].

All participants signed an informed consent in accordance with Declaration of Helsinki; the study was approved by the Ethical Committee of our University.

Statistics

Calculation of sample size was done using G*Power 3.1.7 software (Heinrich-Heine-University, Dusseldorf, Germany). According a post hoc t test, assuming an α-value of 0.05 (sensitivity of 95%) and 29 patients group, we determined that the β-value is 0.19 (with a study power of 81%).

We used parametric tests after using the Kolmogorov–Smirnov test to verify that the variables were normally distributed. Unpaired sample *t* test and Chi-squared test were performed to evaluate differences between alleles frequencies in patients' group and control's sample, according to 1000 Genome database.

To evaluate risk of a shoulder's adhesive capsulitis' severe form in patients with a single nucleotide polymorphism, relative risk was calculated according to Altman.

Considering three genotypes (AA, AB, BB), alleles frequencies were calculated according to the functions $p = f(\mathrm{AA}) + \frac{1}{2}f(\mathrm{AB})$ and $q = f(\mathrm{BB}) + \frac{1}{2}f(\mathrm{AB})$ [14]. Because p and q are the frequencies of the only two alleles present at that locus, they must sum to 1. To check this condition, we performed the control function, $p + q = f(\mathrm{AA}) + f(\mathrm{AB}) + f(\mathrm{BB}) = 1$.

All statistical tests were 2-sided with a probability level of 0.05, and all results are expressed with 95% confidence interval. SPSS version 18 was used for calculations.

Results

The studied group consisted of 30 patients [9M; 21F, mean age 54.81 (41–65)]. The right and left shoulders were involved in 12 and 18 cases, respectively. No patient had bilateral disease. Right-handers were 24. All patients had

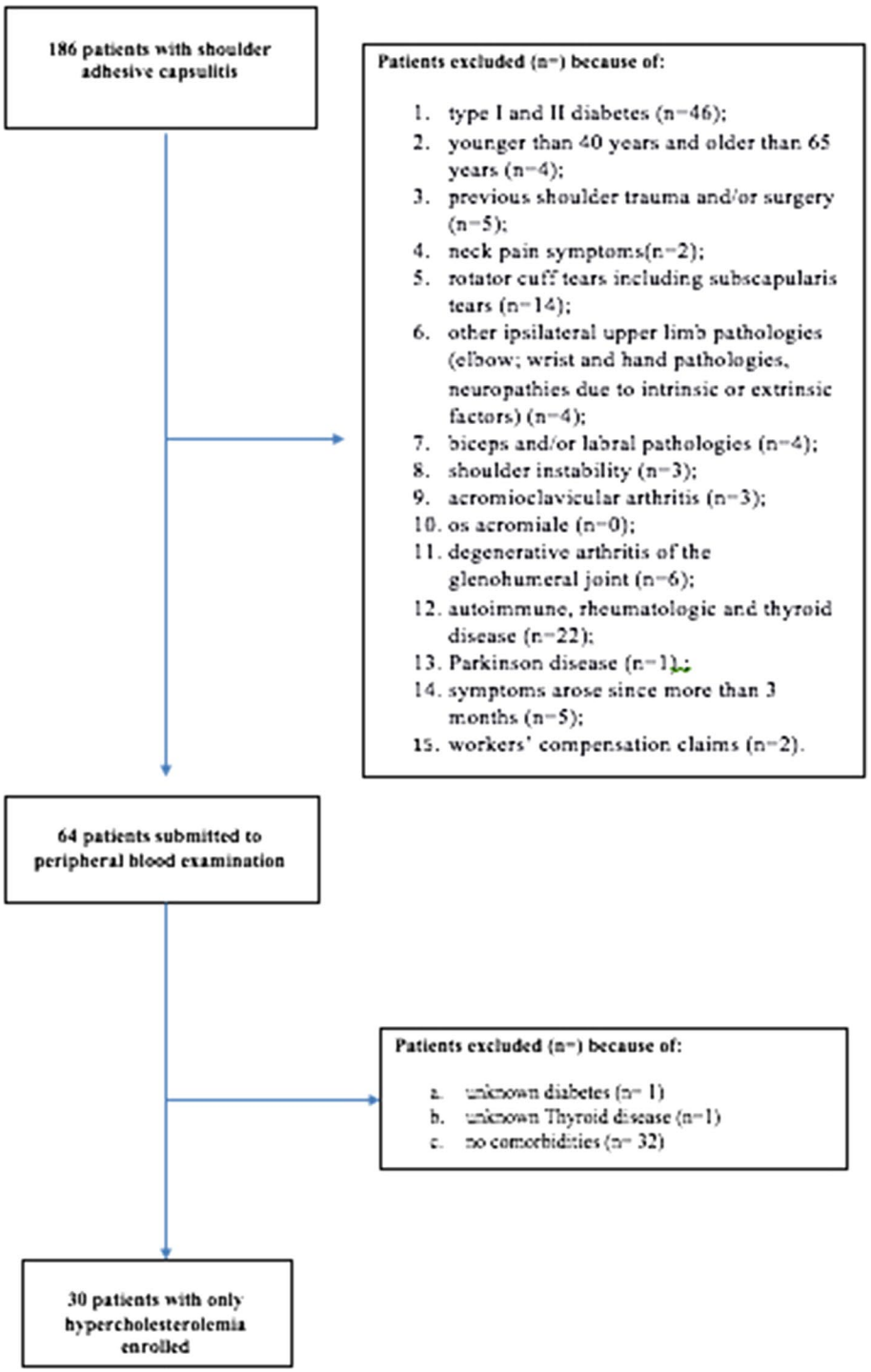

Fig. 1 Flowchart of participants through the trial

blood total cholesterol levels > 200 mg/dl (LDL > 130 mg/dl, HDL > 60 mg/dl).

The prevalence of allele A in position 75 in the gene Apo A1 in the studied group was 25.0% (13 AG genotype; 1 AA genotype; 16 GG genotype). The prevalence of the same allele in the control group was 19% (RR 1.23 IC 0.72–2.07, $p > 0.05$) (Fig. 2).

Patients with type I and II capsulitis were 11 [2M; 9F, mean age 55.55 (46–65); mean flexion 148.0° (100°–165°)] and 19 [7M; 12F mean age 54.27 (41–65); mean flexion 82.5° (50°–95°)], respectively. The prevalence Apo A1 75G > A in type I and II capsulitis was, respectively, 18.1% (2 AG genotype, 9 GG genotype) and 63.2% (11 GA genotype, 1 AA genotype and 7 GG genotype), respectively. A

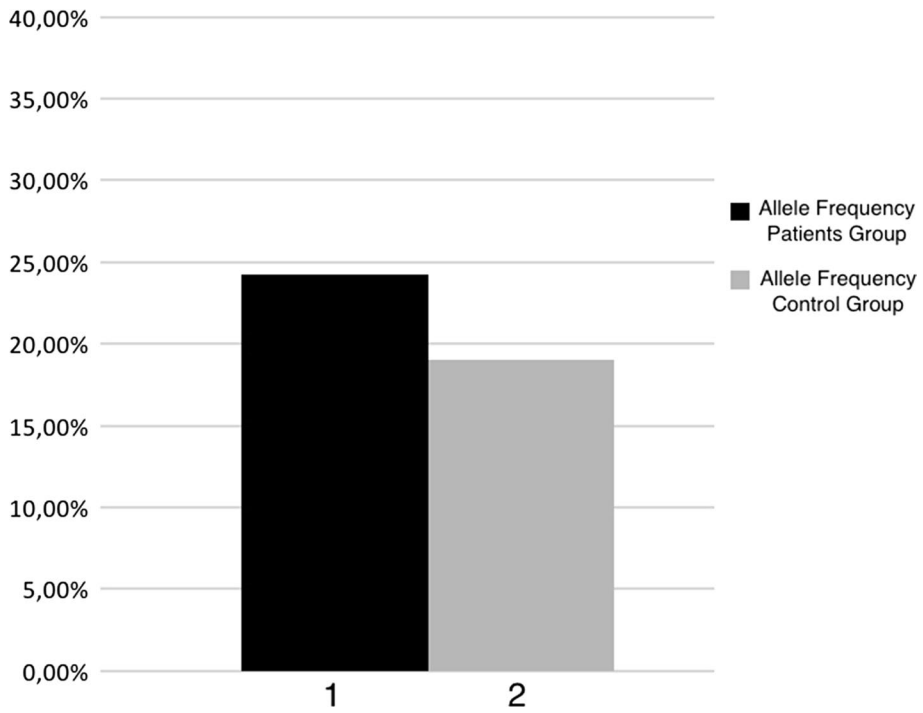

Fig. 2 Prevalence of allele A in position 75 in the gene Apo A1 in the two groups

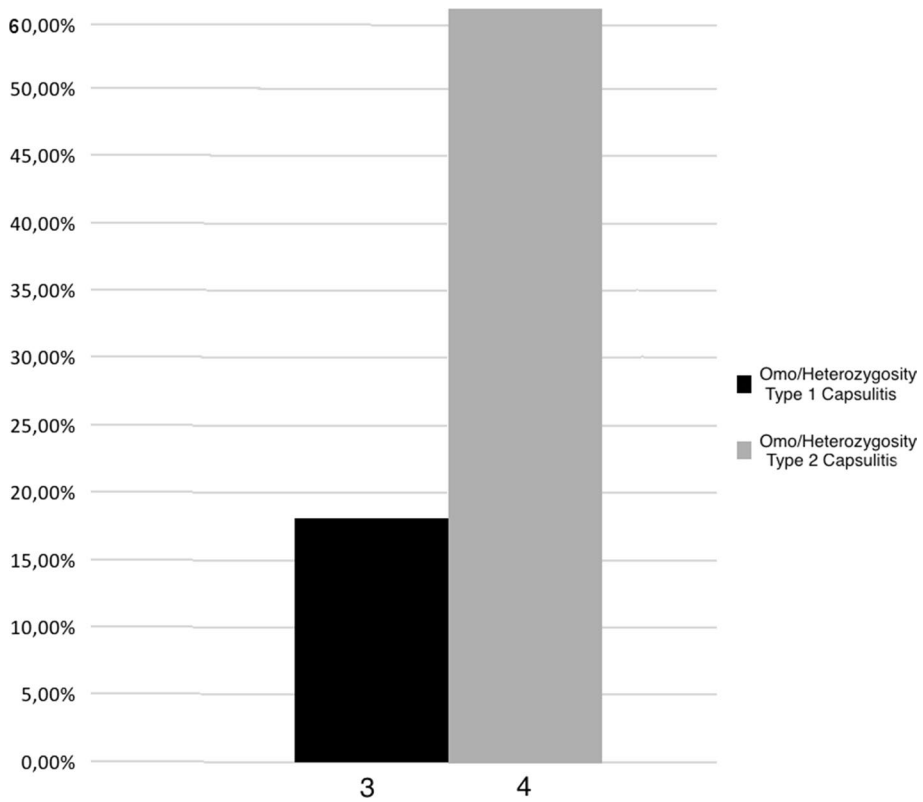

Fig. 3 Prevalence of allele A in position 75 in the gene Apo A1 in patients with type I and II adhesive capsulitis

significant difference between the two categories was found (RR 1.93, IC 1.06–3.53, $p < 0.05$) (Fig. 3).

Discussion

APO A1 is the major protein component of high-density lipoprotein (HDL) in plasma; HDL promotes cholesterol efflux from tissues to the liver for excretion. APO A1 is a cofactor for lecithin cholesterol acyltransferase (LCAT), an enzyme responsible for the formation of most plasma cholesteryl esters. The studied variant (G75A: substitution of a Guanosine with an Adenosine in position 75) is associated with higher serum levels of HDL and APO A1 [15]. HDL is known to be inversely associated with cardiovascular disease due to its anti-atherogenic functions [16]. However, during inflammation associated with various diseases as hypercholesterolemia, a cascade of reactions, known as the acute-phase response (APR), occurs [17]. Besides alterations in plasma proteins, the APR is associated with changes in lipoproteins [18]. Increasing evidence suggests that HDL is a critical part of the acute-phase response of the innate immune system [19]; in acute inflammation a reduction in levels of several plasma proteins involved in HDL-mediated reverse cholesterol transport has been showed [18]. Moreover, the composition of circulating HDL during the acute-phase response is altered. Analysis of the lipid composition shows that acute-phase HDL is depleted in cholesterol ester but enriched in free cholesterol, triglyceride and free fatty acid [20]. Because of these marked changes during APR, the acute-phase HDL behaves differently from normal HDL.

In our study group, the prevalence of the APO A1 G75A(rs670) polymorphism was 22%. It was similar to that registered in a control group extrapolated from an approved database; among data relative to different races, we extrapolated information relative to 107 countrymen founding that the prevalence for the studied polymorphism was 19%. According to this result, the presence of the APO A1 G75A(rs670) polymorphism is not a necessary factor for the development of shoulder adhesive capsulitis. This finding is in accordance with our expectations. In fact, shoulder adhesive capsulitis not only affects patients with hypercholesterolemia and altered HDL levels; it is frequent in patients with type I and II diabetes, with thyroid disease, but it also occurs in the absence of comorbidities highlighting its complex pathogenesis.

We found that the prevalence of the APO A1 G75A(rs670) polymorphism was significantly higher in patients with a more severe type of adhesive capsulitis with respect to those with less functional limitation. This finding suggests that APO A1 variant is a risk factor for the severity of shoulder adhesive capsulitis. Some studies demonstrated the role of Apo A1 in supporting and trigger the acute-phase response. Oliviero et al. [21], using nephelometric analysis to evaluate the concentrations of lipoproteins and Apo A1 in synovial fluid and serum, found that local inflammation facilitates the diffusion from the serum to the synovial fluid of such proteins by increasing the ratio synovial fluid/serum compared to subjects with no joint disease. They concluded that probably the reason lies in the vasodilatation of blood vessels already formed or in a process of neoangiogenesis. Considering the higher concentration of Apo A1 in the synovial fluid in inflammatory conditions, de Seny et al. [22] observed that the Apo A1 is able to induce the expression of IL-6, MMP-1 and MMP-3 by chondrocytes and fibroblast-like synoviocytes; through the analysis of many receptors and their antagonists they found that Apo A1 stimulates the TLR4 receptor, known receptor involved in the immune response. The presence of the APO A1 G75A(rs670) polymorphism causing an increase in HDL and Apo A1 levels leads to a greater inflammatory reaction and thus to a more severe clinical type of pathology.

This study has some limitations: the low sample size even the vast majority of the group was excluded in order to not create bias and to include only patients with high cholesterol and HDL levels; further clinical studies will be performed to evaluate if the presence of the APO A1 G75A polymorphism is a risk factor also for the prognosis of shoulder adhesive capsulitis in patient with hypercholesterolemia.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

Informed consent All patients signed an informed consent form in accordance with the Declaration of Helsinki.

Ethical approval According to our Country's law, this study did not require an ethics committee approval.

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