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The level of fatty acid-binding protein 4, a novel adipokine, is increased in rheumatoid arthritis and correlates with serum cholesterol levels

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ABSTRACT

Objective: To assess the expression of the novel adipokine Fatty Acid Binding Protein-4 (FABP4) in synovial tissues, serum and the synovial fluid of patients with rheumatoid arthritis (RA) and osteoarthritis (OA) and to study the relationships among FABP4, disease activity and metabolic status.
Methods: FABP4 levels were measured in the serum and synovial fluid of 40 patients with RA and 40 control patients with OA. The disease activity score (DAS28), C-reactive protein (CRP) levels and serum lipids were assessed in patients with RA. Immunohistochemical analysis and confocal microscopy were used to study the expression and cell-specific distribution of FABP4 in synovial tissues.
Results: The age, sex and body mass index (BMI) adjusted levels of FABP4 were significantly higher in the serum ($p = 0.001$) and synovial fluid ($p = 0.005$) of patients with RA when compared to OA patients. FABP4 levels were higher in females than in males and correlated positively with body mass index (BMI) in patients with RA. Independent of confounders, FABP4 levels correlated with total cholesterol and LDL cholesterol in patients with RA, but not in OA patients. FABP4 levels were not affected by disease activity. Furthermore, the increased expression of FABP4 that was otherwise restricted to synovial fibroblasts, macrophages and B-cells was noted in RA patients at levels higher than that observed in OA patients.
Conclusions: The observed elevation of FABP4 levels in RA patients and the positive correlation of the adipokine to cholesterol suggest that FABP4 may represent a potential link between RA and the increased risk of atherosclerotic changes.

1. Introduction

Rheumatoid arthritis (RA) is a systemic chronic inflammatory disorder associated with a persistent and destructive synovitis leading to cartilage and bone erosions. The underlying mechanisms of RA remain unknown; however, the inflammatory cells infiltrating the hyperplasic synovial membrane and aggressive synovial fibroblasts producing cytokines, proteolytic enzymes and other mediators represent key factors in the pathogenesis of RA. In the last decade, important advances in our understanding of the relationships between inflammation, the immune response and adipokines have been achieved [1]. Adipokines were originally found in adipose tissue and were linked with several metabolic disorders; however, recent data have demonstrated that adipokines are important mediators of the inflammatory and immune response in RA [1]. A number of studies have already demonstrated associations between several adipokines and the severity of RA [1–9].

Adipocyte Fatty Acid Binding Protein (AFABP, also known as aP2 or FABP4), a member of the cytoplasmic fatty acid binding protein multigene family (FABPs), has recently been described as a novel adipokine associated with insulin resistance, type 2 diabetes mellitus, and cardiovascular disease [9–12]. FABP4 is predominantly expressed in mature adipocytes, but recent publications have reported its presence in macrophages [13,14]. Similar to other adipokines, FABP4 has been found to influence both metabolic and inflammatory pathways. The decreased expression of FABP4 inhibits its most pro-inflammatory signals, including the production of cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS) and cytokines such as tumour necrosis factor-α (TNF-α), interleukin (IL)-1β, IL-6 and monocyte chemoattractant protein-1

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(MCP-1) [13]. Moreover, leukocyte recruitment and pulmonary cytokine production were down-regulated in a FABP4-knockout mouse model of allergic airway inflammation [15]. In contrast, Yang et al. demonstrated increased adipose tissue inflammation in FABP4-knockout obese mice [16].

Recently, a positive association between circulating FABP4 and pro-inflammatory factors, such as C-reactive protein (CRP), IL-6 and TNF receptor, has been demonstrated in patients with obesity, metabolic syndrome and type 2 diabetes mellitus [17–19]. We hypothesised that FABP4 levels might be higher and associated with the inflammatory response in patients with RA. Therefore, we assessed the FABP4 expression in synovial tissue, synovial fluid and serum from patients with RA and osteoarthritis (OA), and investigated the relationship between FABP4, disease activity and the lipid profile in RA patients.

2. Methods

2.1. Characteristics of patients

Forty patients with active RA (31 females and 9 males, mean age ± SD: 59.95 ± 14.06) who fulfilled the American College of Rheumatology criteria for the classification of RA [20] and forty patients with OA (20 females, 20 males, mean age ± SD: 62.55 ± 10.59) were enrolled in this study. RA patient disease activity was assessed according to the 28-Joint Count Disease Activity Score (DAS28) using the number of swollen and tender joints, CRP levels and patient responses using the global visual analogue scale (VAS). Body mass index (BMI) was calculated in all the studied patients as weight (kg)/height^2 (m^2). Table 1 lists the patient details and their disease characteristics. There were more females in the group of RA patients. As expected, patients with RA had higher CRP levels compared to patients with OA, while patients with OA had higher BMI and triglyceride serum levels compared to patients with RA (Table 1).

Table 1
Characteristics of patients with rheumatoid arthritis (RA) and osteoarthritis (OA).

Characteristics	RA (n = 40)	OA (n = 40)	p-Value
Gender (F/M)	31/9	20/20	0.011[a]
Age (years)	59.95 ± 14.06	62.55 ± 10.59	0.359[b]
CRP (mg/l)	24.22 ± 26.75	2.93 ± 2.96	<0.001[c]
BMI (kg/m^2)	25.97 ± 4.19	27.75 ± 3.22	0.039[b]
TC (mmol/l)	5.65 ± 1.20	5.72 ± 1.39	0.832[b]
LDL-C (mmol/l)	3.28 ± 1.01	3.38 ± 1.31	0.696[b]
HDL-C (mmol/l)	1.69 ± 0.50	1.55 ± 0.48	0.232[b]
TG (mmol/l)	1.31 ± 0.53	1.79 ± 0.85	0.006[c]
Drugs (DMARDs/GC)	35/24	3/2	–
Biological therapy	10*	–	–
SJC out of 28	6.08 ± 5.80	–	–
DAS28 score	4.25 ± 1.70	–	–
RF positivity, n (%)	22 (62.5%)	–	–
Anti-CCP positivity, n (%)	20 (50%)	–	–
S-FABP (ng/ml)	29.22 ± 17.00	19.61 ± 8.44	0.002[b]
SF-FABP (ng/ml)	23.96 ± 12.09	18.04 ± 6.20	0.008[b]

Anti-CCP, anti-cyclic citrullinated peptide antibody; BMI, body mass index; CRP, C-reactive protein; DAS28 score, disease activity score; DMARDs, disease-modifying antirheumatic drugs; F, female; GC, glucocorticoids; TC, total cholesterol; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; M, male; MTX, methotrexate; OA, osteoarthritis; RA, rheumatoid arthritis; RF, rheumatoid factor; SJC, swollen joints count; TG, triacylglycerol; S, serum; SF, synovial fluid.
The data are expressed as the mean (±SD).
* Out of 10 patients, 6 were treated with anti-TNF therapy, 1 with abatacept, 1 with rituximab and 1 with tocilizumab.
[a] Statistical test used: chi-square test.
[b] Statistical test used: independent samples t-test.
[c] Statistical test used: Mann–Whitney test.

2.2. Laboratory measurements

Fasting blood samples were collected from all the participants when they underwent therapeutic arthrocentesis or not more than 5 days post-arthrocentesis. Paired samples were centrifuged, and both the serum and synovial fluid were stored at −80 °C. Before analysis, the synovial fluids were incubated with Hylase Dessau for 30 min at 37 °C. The serum and synovial fluid concentrations of FABP4 were measured with a commercially available ELISA kit according to the manufacturer's protocol (BioVendor, Brno, Czech Republic). This assay has a detection limit of 0.1 ng/ml, with intra-assay and inter-assay coefficients of variability of 3.9% and 5.3%, respectively. Absorbances were measured using the Sunrise ELISA reader (Tecan, Salzburg, Austria) with 450 nm as the primary wavelength. CRP levels were determined via an immuno-turbidimetric technique using an Olympus biochemical analyser (model AU 400, Japan). The serum levels of anti-citrullinated protein/peptide autoantibodies (ACPA) and IgM rheumatoid factor (IgM-RF) were determined by an ELISA (Test Line s.r.o., Czech Republic). Serum lipids, including total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol and triglycerides (TG) were assessed (Beckman Coulter, Inc., Brea, CA, USA). Ethical approval was obtained and patients signed the informed consent prior enrolment.

2.3. Immunohistochemistry

Synovial samples for immunohistochemistry were obtained from patients with RA (n = 5) and OA (n = 7) at the time of arthroscopy or open joint surgery (1st Orthopaedic Clinic, 1st Faculty of Medicine, Prague, Czech Republic). Five-micrometres thick formaldehyde fixed paraffin sections were deparaffinised and rehydrated. Endogenous peroxidase activity was inhibited by the addition of 3% hydrogen peroxide in methanol for 30 min followed by 15 min of rinsing in tap water. Non-specific binding activity was avoided by pre-treatment of the sections with 1% normal bovine serum for 2 h. Immunohistochemical labelling was performed after antigen retrieval in 0.2 mol/l citrate buffer (pH 6.0) for 40 min at 91 °C. All the slides were incubated for 1 h at room temperature with polyclonal rabbit adipocyte Fatty Acid Binding Protein (FABP4) antibody (BioVendor, Brno, Czech Republic) diluted 1:400 in ChemMate antibody diluent (Dako, Cytomation, Glostrup, Denmark). The Envision kit (Dako, Cytomation, Glostrup, Denmark) was used to visualise sections incubated with the primary antibody. In addition, 3, 3-diaminobenzidine (Liquid DAB + Substrate, Dako Cytomation, Glostrup, Denmark) was used as a chromogene. The slides were counterstained with Mayer's haematoxylin. Isotype IgG (Dako, Cytomation) diluted 1:1000 was used as a negative control. All the sections were analysed semiquantitatively using a Nikon Eclipse E600 microscope operated by an experienced pathologist who was blind to the clinical data. The analysis included eight to ten different areas of the biopsies, and the intensity of FABP4 expression was scored on a four-point scale: 0 represented negative staining intensity, and scores of 1–3 represented weak, moderate and strong staining intensity, respectively.

2.4. Laser scanning confocal microscopy

For confocal microscopy, double immunolabeling was performed. The slides were incubated with the following primary anti-human antibodies: anti-human CD 68 clone PG-M1 (diluted 1.100), anti-human CD20 clone L26 (diluted 1:100), anti-human CD3 clone PC3 (diluted 1:100) or anti-human vimentin clone 3B4 (diluted 1:100) monoclonal antibodies (Dako, Cytomation, Denmark) and a primary polyclonal rabbit anti-human FABP4 antibody (BioVendor, Brno, Czech Republic). After a 1 h incubation, the slides