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Activin A and follistatin in patients with nonalcoholic fatty liver disease

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ABSTRACT

Objective. There are limited data on the role of activin A and its binding protein, follistatin, in nonalcoholic fatty liver disease (NAFLD). The main aim was the evaluation of serum activin A and follistatin levels in patients with biopsy-proven NAFLD vs. controls.

Methods. This was a case–control study. Fifteen patients with nonalcoholic simple steatosis (SS), 16 with steatohepatitis (NASH), and 52 (24 lean and 28 obese) controls were recruited. Activin A and follistatin were measured using ELISA.

Results. Activin A levels showed a trend towards progressive increase ($p = 0.010$) from the controls (lean: 356 ± 25, 95% CI 305–408; obese 360 ± 20, 95% CI 320–401 pg/ml) to SS (407 ± 28, 95% CI 347–466 pg/ml) and NASH patients (514 ± 70 95% CI 364–664 pg/ml); this association became non-significant after adjusting for adiposity. Follistatin was not different between groups (lean controls: 1.11 ± 0.08, 95% CI 0.95–1.28; obese controls: 1.00 ± 0.07, 95% CI 0.86–1.14; SS: 0.86 ± 0.07, 95% CI 0.70–1.02; NASH: 1.14 ± 0.09, 95% CI 0.90–1.37 ng/ml; $p = 0.13$). Within the NAFLD group of patients, follistatin was associated with NASH independently from activin A, gender and age, a relationship however likely reflecting the effect of adiposity.

Conclusions. Activin A is higher in patients with NASH than both lean and obese controls. Future clinical studies are needed to confirm and expand these findings, whereas mechanistic studies exploring underlying mechanisms are also warranted.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is a disease gaining increasing interest worldwide [1]. It ranges from simple nonalcoholic steatosis (SS) to nonalcoholic steatohepatitis (NASH), is characterized by steatosis, inflammation and fibrosis [2], and may lead to liver cirrhosis and hepatocellular carcinoma [3]. NAFLD shares common pathogenetic mechanisms with other components of the insulin resistance (IR) or metabolic syndrome [4], with adipokines playing a crucial role [5]. The prevalence of NAFLD increases in parallel with the epidemics of obesity and type 2 diabetes mellitus (T2DM) [6]. NASH diagnosis

Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; BMI, body mass index; FNDC, fibronectin type III domain containing; GGT, gamma-glutamyl transferase; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostatic model of assessment insulin resistance; IR, insulin resistance; NAFLD, nonalcoholic fatty liver disease; NAS, NAFLD Activity Score; NASH, nonalcoholic steatohepatitis; LDL-C, low-density lipoprotein cholesterol; RBP, retinol-binding protein; SS, simple steatosis; T2DM, type 2 diabetes mellitus; TGF, tumor growth factor.

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requires liver biopsy, an invasive method; therefore, developing noninvasive markers for NAFLD represents a field of extensive research, targeting to replace liver biopsy or identify candidates for liver biopsy [7]. Furthermore, despite the high prevalence of the disease, NAFLD treatment remains an unmet medical need [8].

Activin A is a member of the tumor growth factor (TGF)-β superfamily and is regarded as a multifunctional cytokine expressed in a wide range of tissues and cells, where it regulates cellular differentiation, homeostasis of cell number and tissue architecture, inflammation, cell proliferation and apoptosis [9]. In hepatocytes, a complex role has been attributed to activin A; it is reported to be beneficial against lipid accumulation, but it may promote hepatic inflammation and fibrosis [10]. It enhances the expression of collagen and TGF-β1, induces mitochondrial β-oxidation, downregulates fatty acid synthase activity, promotes decreased weight percentage of saturated fatty acids alters the composition of polyunsaturated fatty acids and promotes matrix metalloproteinase activity. Its expression is elevated in the fibrotic liver and it has been proposed to contribute to liver fibrosis through induction of matricellular proteins, including connective tissue growth factor, in hepatocytes and hepatic stellate cells. Moreover, it inhibits the proliferation and induces apoptosis of hepatocytes, contributing to the termination of liver regeneration; the proliferation of liver progenitor cells and their mediated liver regeneration are controlled by activin A [10,11].

Follistatin, the natural antagonist of activin A, is a widely expressed protein that binds and inactivates members of the TGF-β family, including activins [12]. It has been recently proposed that the liver is a major contributor to the circulating levels of follistatin in humans [13]. Deregulated expression of follistatin and activins has been implicated in hepatic diseases, including inflammation, fibrosis, liver failure and hepatocellular carcinoma [14].

In clinical terms, it has been proposed that activin antagonists, such as follistatin, may offer future therapeutic approaches in various conditions, including liver diseases [12]. The pathogenetic link between activin A, follistatin and NAFLD is of interest, since it may provide evidence for noninvasive assessment of the disease, but also the basis for future targeted treatment.

The primary aim of this study was the evaluation of serum activin A, follistatin levels and their ratio in patients with biopsy-proven NAFLD vs. lean and/or obese controls. Secondary aims were: a) the association of activin A and follistatin levels with IR, cardiometabolic risk factors, liver function tests, selected adipokines and irisin separately in patients and controls; b) the evaluation of serum activin A, follistatin levels and their ratio in specific histological lesions within NAFLD patients.

2. Patients and Methods

2.1. Patients

NAFLD patients were consecutively recruited on an outpatient basis at a single center (Second Medical Clinic, Aristotle University of Thessaloniki, Greece). Determination of eligibility was based on medical history, physical examination, liver function tests (serum aspartate transaminase [AST], alanine transaminase [ALT], gamma-glutamyl transferase [GGT], alkaline phosphatase) and liver ultrasound imaging performed during the screening visit.

Inclusion criteria for the NAFLD patients were: 1) age > 18 years; 2) bright liver on ultrasound imaging and abnormal liver function tests for at least 6 months before liver biopsy; and 3) patient's consent for liver biopsy. Individuals without NAFLD, living in the same region and being of similar gender and age to the patients, were recruited as controls from the same catchment area/study base at the Second Medical Clinic of Aristotle University of Thessaloniki and the Department of Endocrinology of 424 General Military Hospital, Thessaloniki, Greece. Inclusion criteria for the controls were: 1) age > 18 years; 2) no history of abnormal liver ultrasound imaging or abnormal liver function tests; 3) currently normal liver ultrasound imaging; and 4) currently normal liver function tests. The controls were subsequently divided into two groups: the obese control group, which included those of similar body mass index (BMI) and waist circumference to NAFLD patients, and the lean control group, in which those of lower BMI and waist circumference compared to NAFLD patients were included. Controls did not undergo a liver biopsy, due to obvious ethical considerations. Exclusion criteria for both NAFLD patients and controls aimed to exclude conditions resulting in secondary fatty liver disease (e.g. alcoholic, viral, autoimmune, drug-induced etc.) and were described in detail in our previous publication [15]. Cirrhotic patients (fibrosis stage 4) were also excluded.

The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the local ethics committees. All participants provided an informed consent.

2.2. Methods

Morning (8–9 am) fasting blood samples were collected 1–2 h prior to liver biopsy. Serum AST, ALT, GGT, triglycerides, urea, creatinine, uric acid, total cholesterol and high-density lipoprotein (HDL-C) were measured within 1 h after blood drawing, with standard methods using an automated analyzer (Olympus AU2700; Olympus, Germany). Sera were also immediately frozen, initially at −30 °C and later at −80 °C, for the measurement in one batch at the end of the study of the remaining parameters, which included: glucose (Roche Cobas c311, Roche Diagnostics, IN), insulin (immunoassay, Immulite 1000, Siemens Healthcare Diagnostics, NJ), adiponectin (radioimmunoassay, Millipore, MA), leptin (enzyme-linked immunosorbent assay [ELISA], Millipore, MA), irisin (Phoenix Pharmaceuticals, CA), retinol-binding protein (RBP)-4 (Biovendor Research and Diagnostic Products, NC) and chemerin (Biovendor Research and Diagnostic Products, NC), as previously described [15]. Activin A and follistatin were measured with ELISA: activin A (ELISA, R&D Systems, MN; sensitivity 3.67 pg/ml, intra-assay coefficient of variation [CV] 4.2%–4.4%, inter-assay CV 4.7%–7.9%); follistatin (ELISA, R&D Systems, MN; sensitivity 0.03 ng/ml; intra-assay CV 4.9%–7.5%; inter-assay CV 5.2%–7.3%).

Liver biopsy was performed under computed tomography-guidance by an experienced radiologist and interpreted by two experienced pathologists. NAFLD patients were subdivided into those with SS or NASH according to the criteria of NAFLD Activity Score (NAS) [16]. Steatosis grade, fibrosis stage, lobular and portal inflammation, and ballooning were dichotomized [16].

BMI was calculated by the formula: body weight (kg)/height^2 (m^2). IR was quantified by the homeostasis model of assessment—insulin