Reference number to be mentioned by correspondence : AG/OM200828

Serum adiponectin levels in different types of non alcoholic liver disease. Correlation with steatosis, necroinflammation and fibrosis

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Abstract

Background and study aims : **In recent studies adiponectin has been implicated in the pathogenesis of non alcoholic liver disease (NAFLD), a common chronic liver disease with a broad spectrum of histopathologic findings. The aim of this study was to investigate the correlation between serum adiponectin levels and steatosis, necroinflammation and fibrosis in different types of NAFLD patients.**

Patients and methods : **Forty three patients with elevated liver enzymes and biopsy proven non alcoholic fatty liver disease and 38 patients with clinically diagnosed NAFLD and permanently normal liver enzymes were prospectively enrolled in the study. Patients with biopsy proven NAFLD were divided into two groups : non alcoholic steatohepatitis (NASH) : 25 patients and simple steatosis : 18 patients. Serum adiponectin levels were measured with an ELISA immunoassay, and BMI, fasting serum glucose, total and HDL cholesterol, fasting triglyceride levels and insulin resistance were determined.**

Results : **Groups did not differ in age, sex, BMI, waist circumference and HOMA – IR. Only patients with confirmed NASH had lower serum adiponectin levels in comparison to NAFLD patients with both abnormal (6.6 ± 4.7 μg/mL vs 10.8 ± 5.6 μg/mL, p = 0.01) as well as normal liver enzymes (6.6 ± 4.7 μg/mL vs 9.2 ± 4.8 μg/mL, p = 0.01). For the whole NAFLD group with elevated liver enzymes no correlation was found between serum adiponectin levels and the degree of liver steatosis or fibrosis stage. Also no correlation was found between adiponectin levels and BMI, ALT, AST, γ GT or HOMA-IR.**

Conclusions : **Patients with established NASH have lower serum adiponectin levels than NAFLD patients with normal or abnormal liver enzymes. Adiponectin was not associated with the severity of hepatic fibrosis.** (Acta gastroenterol. belg., **2008**, 71, **000-000**).

Introduction

Non alcoholic fatty liver disease (NAFLD) is a common chronic liver disease with a broad spectrum of histopathologic findings, extending from simple steatosis to non alcoholic steatohepatitis (NASH) (1). While simple steatosis is a non progressive disorder non alcoholic steatohepatitis is a progressive disease which may result in liver cirrhosis and occasionally hepatocellular carcinoma (2-4). It is estimated that 20-30% of the total population of Western countries have fatty liver disease, 2-3% have NASH (5,6) and the incidence has recently increased (7).

Adipokines, adipose tissue derived proteins, including leptin, tumor necrosis factor α (TNF-α), resistin and adiponectin (8,9) have an active role in energy homeostasis and they have been linked to many aspects of the metabolic syndrome in many recent human and animal studies (10-13). Increasing evidence suggests that adiponectin plays an important role in the pathophysiology of the metabolic syndrome. Adiponectin levels correlate inversely with body mass index (BMI), plasma triglyceride levels and fasting insulin concentration (14).

In previous studies lower serum adiponectin levels have been observed in patients with NAFLD in comparison to age and BMI matched control groups (15-17). In these studies low adiponectin levels were reported for the whole group of patients with NAFLD and increased aminotransferases. However the microscopic lesions in these patients vary from simple steatosis to established non alcoholic steatohepatitis irrespective of ALT levels. Some studies have shown that a percentage of patients with normal ALT levels have NASH (18-19) but the number of patients in these studies is small. In addition it is not widely accepted to perform systematically liver biopsies in this cohort of patients.

The aim of this study was to explore the serum adiponectin levels in a population of NAFLD patients, to investigate differences in serum adiponectin levels among patients with different types of NAFLD (NAFLD with normal aminotransferases, simple steatosis with elevated liver enzymes, established steatohepatitis) and to investigate the correlation between serum adiponectin levels and hepatic inflammation and fibrosis in these patients.

Patients/Materials – Methods

Patients with elevated liver enzymes for more than six months and biopsy proven non alcoholic liver disease (NAFLD), and patients with clinically diagnosed (bright liver on ultrasound) and permanently normal liver enzymes were enrolled in the study. The population of this study was derived from the hepatology outpatient clinic of a tertiary hospital. Exclusion criteria were :

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Submission date :
Revised version :
Acceptance date :

alcohol consumption (> 30 gr/day for males, > 20 gr/day for females), infection by chronic hepatitis B or/and chronic hepatitis C, autoimmune hepatitis, primary biliary cirrhosis, patients with metabolic or genetic diseases such as Wilson disease, haemochromatosis, alpha-1-antithrypsin insufficiency, Wolman disease, patients with hepatocellular carcinoma or other malignancies. Other exclusion criteria were adrenal or pituitary disease, consumption of drugs that induce steatosis such as corticosteroids, calcium channel blockers, amiodarone, tamoxifen, antiviral agents (zidovudine, didanosine), or hormones such as estrogens, patients who underwent gastrointestinal by pass or excision and patients who receive total parenteral nutrition.

Clinical examination and liver ultrasound was performed on each patient. Anthropometric data including body mass index (BMI) and waist circumference were obtained. Patients with elevated liver enzymes underwent liver biopsy. A transcutaneous liver biopsy was performed with a 16-gauge needle by two experienced gastroenterologists. All patients gave informed written consent before a liver biopsy was performed. The specimen was evaluated by an experienced hepatopathologist according to the NAFLD Activity Score (NAS) and Fibrosis Staging by Kleiner *et al.* (20). For the evaluation of steatosis we considered as grade 0 : < 5% of affected hepatocytes, grade 1 : 5-33%, grade 2 : 33-66% and grade 3 : > 66% of hepatocytes. For the evaluation of NASH we took into account the total score for steatosis (0-3), lobular inflammation (0-3), and hepatocellular ballooning (0-2). Scores of 0-2 are considered not diagnostic of NASH, 3-4 are borderline and 5-8 are positive for NASH. Based on the histopathologic criteria patients with biopsy proven NAFLD were divided into two groups : patients with non alcoholic steatohepatitis (NASH) $\geq$ 5 criteria and patients with simple steatosis $\leq$ 2 criteria. We excluded patients who were borderline in order to have a better correlation between serum adiponectin levels and inflammation. Fibrosis was estimated as perisinusoidal, portal, periportal fibrosis, bridging fibrosis and cirrhosis. The stages of fibrosis were divided in two groups : mild fibrosis : stages 0-2 and advanced : stages 3-4.

On the morning of liver biopsy we collected venous blood samples after an overnight fast to determine the levels of glucose, insulin, C-peptide, total and HDL cholesterol, triglyceride, high density lipoproteins (HDL), aspartate aminotransferase (AST), alanine aminotranferase (ALT), gamma-glutamyltranspeptidase (GGT), albumin, bilirubin, alkaline phoshate (ALP), international normalized ratio (INR) and adiponectin.

Serum adiponectin levels were measured with Radioimmunoassay (Human adiponectin RIA KIT ; LINCO RESEARCH St, Charles, MO USA). Insulin resistance index was determined using homeostasis model of assessment for insulin resistance HOMA – IR = fasting insulin level (Mu/L) X early morning fasting blood glucose level (mg/dl) X 0,00551/22,5 (21).

Serum adiponectin levels were correlated to BMI, serum glucose, cholesterol, triglyceride levels and other parameters. Data were analyzed using the SPSS statistical package (SPSS, version 10.0, USA). Continuous variables were expressed as mean ± standard deviation (SD). Student's *t*-test for independent samples was used to assess the differences between NAFLD patients with normal and elevated transaminases. Comparisons among groups A, B and C were performed using the one-way ANOVA, followed by Bonferroni's post hoc test when variances across groups were equal or by Dunnette's T3 post hoc test when variances were not equal. Variance equality was tested by Levene statistical analysis. Categorical variables were expressed as percentages and differences between groups were tested for significance by using the Chi-square test. Multiple regression analysis was performed to assess independent predictors of serum adiponectin levels. In all cases, a *P*-value of less than 0.05 was considered as significant.

Results

Patient's characteristics

From January 2004 to December 2007 forty nine consecutive patients with elevated liver enzymes and biopsy proven non alcoholic fatty liver disease (NAFLD), and 38 patients with clinically diagnosed NAFLD (bright liver on ultrasound) and permanently normal liver enzymes were enrolled in the study. These patients were referred in our hospital due to abnormal liver function tests, or due to abnormal ultrasound. Based on the histopathologic criteria patients with biopsy proven NAFLD were divided into two groups : 25 patients with non alcoholic steatohepatitis ($\geq$ 5 criteria) (group A) and 18 patients with simple steatosis ($\leq$ 2 criteria) (group B). Six patients were borderline (3-4 criteria) and they were excluded from our study. The group C is the group of patients with normal enzymes and clinically diagnosed NAFLD.

The three groups had similar anthropometric and biochemical data. Age, sex, BMI and waist circumference were similar in all groups. Glucose levels, insulin, C-peptide HOMA – IR, cholesterol, triglycerides and HDL did not differ between the three groups. The liver synthetic function (albumin, prothrombin time) was normal in all patients (Table 1).

Serum adiponectin levels and correlations

Serum adiponectin levels were 8.3 ± 5.3 μg/mL in the group of patients with elevated liver enzymes and 9.2 ± 4.8 μg/mL in the group of patients with normal enzymes. There was not statistical difference between these two groups. When we divided the group of abnormal transaminases according to the presence of necroinflammation in the liver specimen to NASH (group A) and simple steatosis (group B) the levels of adiponectin were : 6.6 ± 4.7 μg/mL in group A and 10.7 ± 4.8 μg/mL

Table 1. — **Patient's characteristics**

	Biopsy proven NAFLD Increased aminotransferases		NAFLD Normal aminotransferases
	NASH (Score ≥ 5) (group A)	Steatosis (Score ≤ 2) (group B)	(group C)
Male/female	13/12	9/9	20/18
Age	45.7 ± 15.6	49.2 ± 12.6	48.4 ± 16.4
BMI	29.5 ± 3.8	29,9 ± 3.8	28.6 ± 2.9
Waist circumference (cm)	99.0 ± 10.5	99.0 ± 11.5	96.6 ± 8.7
Glycose (mg/dL)	105.2 ± 18.5	116.2 ± 39.4	106.1 ± 34.5
Insulin (μU/mL)	13.1 ± 12.8	12.9 ± 8.4	15.5 ± 7.3
C-peptide (ng/mL)	2.3 ± 1.5	2.2 ± 1.8	4.6 ± 3.5
HOMA-IR	3.6 ± 3.5	4.7 ± 3.5	4.3 ± 2.8
Cholesterol (mg/dL)	221.0 ± 62.3	230.6 ± 54.7	218.6 ± 58.6
Triglyceride (mg/dL)	136.2 ± 54.6	114.5 ± 41.6	126.8 ± 68.9
HDL (mg/dL)	46.2 ± 12.8	49.4 ± 9.7	51.6 ± 12.1
SGOT (U/L)	38.2 ± 13.8 *	44.0 ± 21.9**	20.5 ± 4.8
SGPT (U/L)	65.0 ± 24.8*	61.6 ± 37.3**	25.2 ± 11.9
Ggt (U/L)	68.0 ± 64.7*	84.0 ± 52.4**	28.1 ± 16.9
Albumin	4.4 ± 0.8	4.4 ± 0.7	4.6 ± 0.3
bilirubin (mg/dL)	0.51 ± 0.14	0.67 ± 0.36	0.62 ± 0.2
ALP (U/L)	88.3 ± 32.6	86.1 ± 26.2	71.1 ± 22.3
INR	1.0 ± 0.0	1.0 ± 0.0	0.9 ± 1.1

* $p < 0.05$ (NASH vs Group with Normal Aminotransferases)
** $p < 0.05$ (Simple Steatosis vs Group with Normal Aminotransferases).

in group B ($p < 0.05$). Only patients with confirmed NASH had lower serum adiponectin levels in comparison to NAFLD patients with both normal and abnormal transaminases (Table 2).

Significant difference between patients with established NASH and simple steatosis was observed only for adiponectin serum levels ($p = 0.011$). All other clinical and biochemical factors showed no statistically significant difference between the two groups, both with elevated transaminases.

For the whole NAFLD group with elevated and normal liver enzymes no correlation was found between adiponectin levels and age, waist circumference, BMI, fasting glucose, HOMA-IR and γ-GT. A negative correlation of adiponectin with ALT (r -237, $p = 0.038$) and AST (r -235, $p = 0.039$) was observed.

Also for the group of patients with elevated liver enzymes and biopsy proven NAFLD no correlation was found between serum adiponectin levels and the degree of liver steatosis (r : 0.07, $p = 0.69$) or fibrosis stage (r :-0.12, $p = 0.47$). Serum adiponectin levels were 7.7 ± 4.9 μg/mL in the group of patients with 0-1 hepatic steatosis in comparison to 9.2 ± 5.6 μg/mL in patients with 2-3 liver steatosis. Also patients with advanced fibrosis did not present lower serum adiponectin levels compared to patients with no or mild fibrosis (8.0 ± 6.1 μg/mL vs 8.9 ± 4.7 μg/mL, $p = 0.67$) (Table 2).

Discussion

Adiponectin is an adipocytokine secreted by adipocytes with antidiabetic, antilipogenic and antiatherogenic actions. Serum adiponectin circulates as low and high molecular multimers while two adiponectin receptors have been cloned (22). These receptors are found in many organs but they are predominantly expressed in muscle cells and liver (23). Adiponectin levels correlate negatively with insulin resistance and an altered lipid pattern. Previous studies have shown that adiponectin reduces body fat and improves hepatic and peripheral insulin sensitivity (24-26). Also reduced systemic adiponectin levels increase the risk of cardiovascular disease in both diabetic and non diabetic patients (27).

More and more data indicate that adiponectin plays an important role in the pathophysiology of NASH (9,28). Adiponectin seems to confer protective effects against alcoholic and non alcoholic liver disease but the underlying mechanisms are not yet clear (13,29).

The main finding of this study is the difference of serum adiponectin levels between the different groups of patients with NAFLD. Serum adiponectin levels were lower only in patients with histologically established non-alcoholic steatohepatitis. We did not find any difference between NAFLD patients with normal liver enzymes and NAFLD patients with abnormal liver

Table 2. — **Serum adiponectin levels and histological findings**

Histological Findings	Number of patients	Serum adiponectin levels μg/mL
NASH	25	6.6 ± 4.7
Simple steatosis	18	10.7 ± 4.9*
Normal aminotransferases	38	9.2 ± 4.8 **
Steatosis 0-1	18	7.7 ± 4.9
Steatosis 2-3	25	9.2 ± 5.6
Fibrosis 0-2	29	8.9 ± 4.7
Fibrosis 3-4	14	8.0 ± 6.1

* $p < 0.05$ (NASH vs Simple Steatosis)
** $p < 0.05$ (NASH vs Group with Normal Aminotransferases).

enzymes but without established NASH. The low levels of adiponectin in the NASH group of patients may reflect lack of hepatoprotection in these patients.

Our study had a limitation. In recent studies it has been shown that normal ALT levels do not exclude the presence of necroinflammation in the liver (18-19). But in these studies the majority of patients with normal ALT levels have less advanced liver disease comparing to patients with elevated ALT levels and NASH was independently predicted by ALT. As it is unreasonable to perform liver biopsies in every patient with normal ALT levels we accepted that the cohort of patients with normal ALT levels had minimal or no necroinflammation.

In experimental studies a reduction in the fat deposition in liver of ob/ob mice has been observed after treatment with recombinant adiponectin (13). Also a reduction in the activity of two key enzymes involved in fatty acid synthesis, including acetyl-CoA carboxylase and fatty acid synthase, was observed in this study. This may lead to a conclusion that adiponectin may prevent liver from excessive triglyceride accumulation and subsequent injury. This may be true to some degree in the general population as in previous studies serum adiponectin was lower in patients with NAFLD than in controls (15-17). But in our study with only NAFLD patients, adiponectin levels did not correlate with the degree of steatosis. In other studies patients with NASH and patients with simple steatosis were evaluated as one group, the group of NAFLD compared with normal population while the subgroups of patients differed in BMI, lipid profile or glucose levels and HOMA-IR. In our study the three subgroups were all NAFLD patients and they were similar in anthropometric and biochemical parameters. The only difference was the presence or absence of necroinflammation in liver specimen. This difference may be a result of hypoadiponectinaemia in the subgroup with steatohepatitis.

Adiponectin seems to protect liver from injurious effects of fat accumulation. It has been shown that is has several anti-inflammatory properties including modulation of endothelial inflammatory response by inhibiting nuclear factor Kb activation and blocking TNF-a release (30). In previous studies it has been demonstrated that adiponectin suppresses macrophage function, inducts anti-inflammatory cytokines in human leucocytes and modulates lymphopoiesis (31-32).

Adiponectin may act by counteracting detrimental effects of TNF-a on hepatocytes which is increased in patients with NASH. Decreased levels of adiponectin may be important in the shift from simple steatosis to NASH. In animal models exogenous administration of adiponectin decreases the plasma concentration and hepatic expression of TNF-a and supresses hepatic inflammation (13). In a previous study systemic infusion of anti-TNF-a antibodies in seven patients with severe alcoholic steatohepatitis resulted in a long term decrease of circulating adiponectin levels (33). This reduction suggests that anti-TNF-a elimination besides other mechanisms might be a possible adiponectin's action.

In previous studies thiazolidinediones, peroxisome proliferator–activated receptor γ (PPARγ) agonists, increase plasma adiponectin levels, ameliorate insulin resistance and improve glucose and lipid metabolism in type 2 diabetes (34). Pioglitazone, a thiazolidinedione derivative, has been found in two recent studies to reduce hepatic necroinflamation in animal models (35) and in patients with NASH (36-37). Moreover discontinuation of pioglitazone treatment was associated with subsequent decrease in adiponectin levels, elevation in serum alanine aminotransferase levels and worsening of hepatic inflammation (38). These findings reinforce the hypothesis of the anti-inflammatory effects of adiponectin.

The correlation between adiponectin and fibrosis is controversial. In a previous experimental study Kamada *et al.* showed that administration of adiponectin attenuated carbon tetrachloride induced liver fibrosis in adiponectin knockout mice (39). Savvidou *et al.* found that patients with NASH assosiated cirrhosis had lower adiponectin levels than patients with NASH without cirrhosis (40). Musso et al demostrated a negative correlation between adiponectin and fibrosis in patients with NASH. In our study adiponectin levels was not correlated with fibrosis (17). Similar results were found in the studies of Hui *et al.* and of Aygun *et al.* but in these studies there was comparison between patients with NAFLD and normal controls (16,41). In our study all the groups had fatty liver disease.

It is well known that advanced fibrosis and cirrhosis are found more often in older patients. The fact that in our study adiponectin levels were not correlated with the severity of fibrosis may be due to the younger age of our patients and the smaller percentage of patients with cirrhosis in comparison to other studies. Unlike fatty infiltration and necroinflammation, which can be altered over a short period of time, fibrosis progression may occur over a much longer period usually over decades in NAFLD patients. For this reason it is important to follow up these patients for a long period of time to exctract safe conclusions about the role of adiponectin in the progression of fibrosis.

Until now the gold standard for the diagnosis of non alcoholic steatohepatitis is liver biopsy but in a recent study Shimada *et al.* demonstrated that evaluation of adiponectin levels in combination with HOMA-IR and serum type collagen 7S level can indicate patients with non alcoholic steatohepatitis (42). These data can demonstrate that adiponectin may be used as a non invasive test for the diagnosis of NASH.

In conclusion our study demonstrates that only patients with established NASH have lower serum adiponectin levels than NAFLD patients with normal or abnormal liver enzymes. Adiponectin seems to protect the liver from inflammation caused by fat and it is not associated with hepatic fibrosis. Further studies are needed to determine the role of adiponectin on inflammation and liver fibrosis.

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