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High-sensitivity C-reactive protein and liver enzymes in individuals with Metabolic Syndrome in Talca, Chile

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Abstract. Metabolic syndrome (MS) is a core set of disorders, including abdominal obesity, dyslipidemia, hypertension and hypertriglyceridemia that together predict the development of diabetes type 2 and cardiovascular disease. This study investigated the relationship between liver enzyme levels and high-sensitivity C-reactive protein (hs-CRP) in subjects with and without MS. Alanine-aminotransferase (ALAT), aspartate-aminotransferase (ASAT), γ-glutamyl transferase (GGT) and hs-CRP were measured in 510 subjects, aged 40 to 65 years old. Patients were selected from 1007 subjects from the Research Program for Cardiovascular Disease Risk Factors in Talca, Chile. Results showed that women with MS presented higher liver enzyme levels than those who did not have MS. This was not observed in male patients for the enzymes ALAT and ASAT. However, GGT and hs-PCR levels were higher in male and female patients with MS than in those without MS. In conclusion, it is important to search for the presence of MS when diagnosing fatty liver. Moreover, the presence of liver disease in patients with MS should be further investigated.

Introduction

Metabolic syndrome (MS) is a collection of disorders that includes abdominal obesity, dyslipidemia, hypertension and hypertriglyceridemia, which combined predict type 2 diabetes and cardiovascular disease (1). Authors previously proposed non-traditional components of MS, such as subclinical inflammation, microalbuminuria and more recently, the non-alcoholic fatty liver disease (NAFLD) which also predicts cardiometabolic risk (2,3).

NAFLD describes a clinical pathologic condition characterized by a significant deposit of fat in the liver parenchyma and spectrum disorders ranging from a simple steatosis to more severe forms that include a non-alcoholic steatohepatitis (NASH) which may progress to fibrosis and hepatic cirrhosis (4).

The histological characteristics of NAFLD are very similar to those described for the alcoholic liver disease (5). Thus, both are responsible for the metabolic imbalance that exceeds the ability of the liver to adapt to injury (6). This disease is often associated with obesity (7), diabetes mellitus type 2 (8,9), dyslipidemia (10) and hypertension (11). Each of these abnormalities carries a risk of cardiovascular disease and together defines the syndrome of insulin resistance (SIR) or MS (11). The syndrome of insulin resistance and oxidative stress play a crucial role in the pathogenesis of NAFLD and progression from impaired glucose tolerance to diabetes. Predictors of the progression to fibrosis in patients with NASH, such as age (>45 years), obesity (BMI >30 kg/m^2), cirrhosis (ASAT/ALAT >1) and diabetes are associated with an increase in insulin resistance (IR) (12,13). Even though it is believed that NASH is a multifactorial disease, IR appears to be the main factor responsible for the progression of a simple steatosis to steatohepatitis (14).

Obese and overweight individuals with NAFLD present with high levels of markers of liver disease such as aspartate-aminotrasferase (ASAT), alanine-aminotransferase (ALAT) and γ-glutamyl transferase (GGT) (15).

It should be considered that the adipocytes not only store energy but also respond to metabolic signals by releasing free fatty acids, secreting hormones and cytokines which exert a local (adipose tissue), central (nerve tissue) and peripheral effect (organs such as liver, muscles, pancreas) (16). Cytokines are substances released from adipose tissue, with a significant impact on the general homeostasis of the body, eating habits and insulin sensitivity (17). Besides high levels of free fatty acids being closely associated with IR, they appear to be cytotoxic and may lead to peroxisomal oxidation generating hydrogen peroxide as a source of oxidative stress (18-20).

The main adipocytokines are leptin, adiponectin and resistin, while other molecules that include tumor necrosis factor α (TNF-α), interleukin 6 (IL-6), plasminogen activator inhibitor 1 (PAI-1), complement proteins, proteins of the renin-angiotensin system also act as adipocytokines (21-23).

In contrast, studies showed that IL-6 and TNF-α are significant co-stimulators of C-reactive protein (CRP) in the

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Table I. Patient data.

Variable	Men			Women		
	Non-MS, n=75 Median (IQR)	MS, n=80 Median (IQR)	p-value[a]	Non-MS, n=171 Median (IQR)	MS, n=184 Median (IQR)	p-value[a]
Age	48.0 (13.0)	52.0 (12.0)	0.006	48.0 (10.0)	52.0 (10.0)	<0.001
Glycemia (mg/dl)	90.0 (11.0)	102.0 (31.0)	<0.001	87.0 (12.0)	99.0 (23.0)	<0.001
hs-PCR (mg/l)	1.1 (1.8)	2.1 (3.2)	<0.001	1.5 (2.9)	3.6 (5.0)	<0.001
ALAT (U/l)	7.8 (5.6)	8.5 (8.7)	0.344	5.4 (4.5)	6.9 (5.1)	<0.001
ASAT (U/l)	17.2 (7.5)	17.0 (7.9)	0.409	13.4 (5.3)	16.5 (8.4)	<0.001
GGT (U/l)	24.0 (23.0)	33.0 (35.0)	0.003	17.0 (14.0)	24.0 (28.0)	<0.001
Uric acid (mg/dl)	5.0 (1.2)	6.1 (1.6)	<0.001	3.9 (1.3)	4.4 (1.5)	<0.001
Cholesterol (mg/dl)	199.0 (47.0)	191.5 (51.0)	0.720	202.0 (50.0)	209.0 (54.0)	0.226
LDL-C (mg/dl)	120.0 (40.0)	111.0 (34.0)	0.099	116.5 (39.0)	122.0 (41.0)	0.250
Triglycerides (mg/dl)	128.0 (74.0)	185.0 (111)	<0.001	110.5 (59.0)	168.5 (80.0)	<0.001
Waist (cm)	93.0 (9.0)	103.0 (13.0)	<0.001	84.0 (13.0)	96.5 (16.0)	<0.001
HDL-C (mg/dl)	46.0 (17.0)	39.0 (11.0)	<0.001	59.0 (18.0)	46.0 (13.0)	<0.001
BMI kg/m^2	26.9 (3.8)	30.3 (5.1)	<0.001	26.3 (5.6)	31.8 (7.3)	<0.001
Pressure S. (mmHg)	122.0 (19.0)	143.0 (22.0)	<0.001	117.0 (19.0)	135.0 (23.0)	<0.001
Pressure D. (mmHg)	76.0 (13.0)	89.0 (14.0)	<0.001	73.0 (11.0)	84.0 (13.0)	<0.001

[a]Mann-Whitney U test. MS, metabolic syndrome; S., systolic; D., diastolic; IQR, interquartile range.

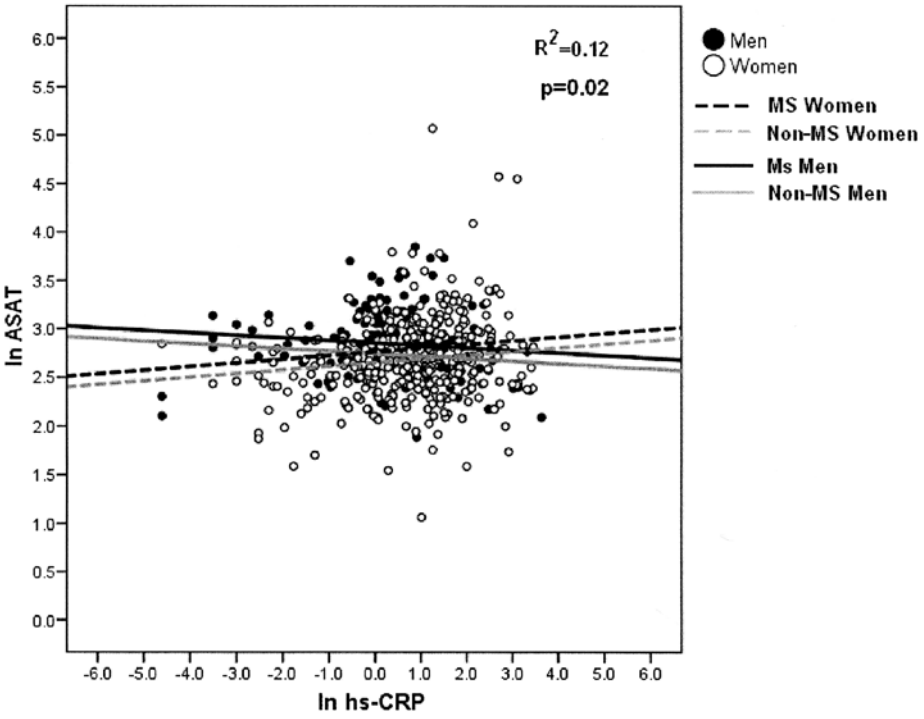

Figure 1. Scattering between In hs-CRP and In ASAT and the linear multivariate adjustment.

liver. Consequently, we proposed that due to its easy accessibility, the measurement of the high-sensitivity CRP (hs-CRP), may be used in conjunction with the measurement of liver enzymes as an adjunct to the diagnosis and evaluation of NAFLD and its relationship with MS (1).

Patients and methods

Patients. Patients with previous informed consent were selected from the Research Program for Cardiovascular Disease Risk Factors from the Universidad de Talca.

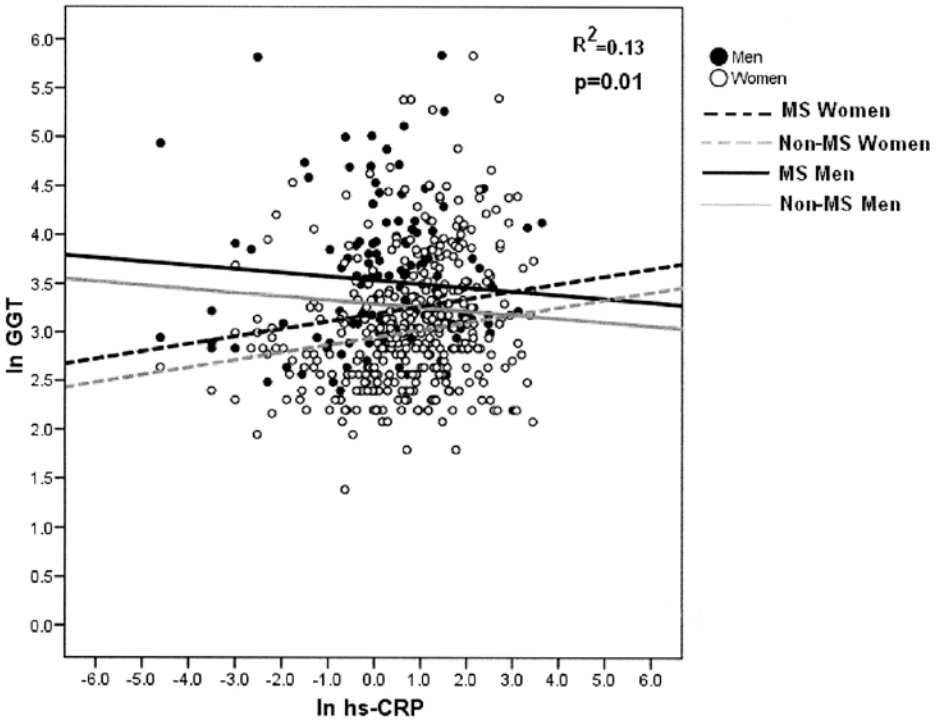

Figure 2. Scattering between ln hs-CRP and ln GGT and the linear multivariate adjustment.

Patients comprised a total of 510 adults between 40-65 years of age, and were classified into those who had MS (n=264) and those without MS (n=246), according to adult treatment panel III (ATP III) criteria (23). Blood pressure, waist index, height and weight were measured in all patients, and a blood sample after fasting was extracted for biochemical tests such as glucose, lipid profile, uricemia, liver enzymes and hs-PCR.

Methods. Blood pressure, height and weight were measured according to the World Health Organization recommendations (24,25). The venous blood samples from each patient were extracted after a 12-h fast. The biochemical characterization such as glucose, uric acid, lipid profile [total cholesterol, high density lipoprotein (HDL) cholesterol, triglycerides] was measured enzymatically. hs-PCR was conducted using the immunochemical method and the ASAT, ALAT and GGT enzymes were measured using the kinetic-spectrophotometric methodology. For all determinations, reagents from Roche Laboratories (Mannheim, Germany) were used with a Hitachi 711 auto analyzer. Human serum Biosystems (Barcelona, Spain) was used as a control.

Statistical analysis. Since measured variables were not normally distributed, median and interquartile ranges (IQR = 75th percentile-25th percentile) were used for description. The non-parametric Mann-Whitney U test was used to compare the variables of MS for men and women. To evaluate the effect of hs-CRP (log-transformed) in the liver enzymes as response variables, a multivariate regression analysis was used, controlled by MS, age and gender. A 0.05 level was considered to be significant.

Results

We studied 510 adults of whom 264, with an average age of 52.3±6.5 years, presented MS and a significantly higher BMI than adults without MS (31.2, 26.5, respectively) ($p<0.001$). The general characteristics of patients studied are shown in Table I, and it can be seen that there are significantly higher levels of liver enzymes in women with MS with respect to those without the syndrome, while in males these enzymes show no significant differences. In relation to hs-PCR, it is observed that both men and women with MS have significantly higher levels than those without MS ($p<0.001$, males and females, respectively).

Results of the multivariate linear regression on the ASAT and GGT enzymes showed that the enzyme levels are significantly higher among patients with MS than those who do not present it ($p<0.01$, $p<0.001$, respectively).

The multivariate analysis on the ASAT and GGT enzymes shows that there is a significant linear regression with hs-CRP, and the slope is modified by gender; positive in women and negative in men ($p<0.013$, $p<0.014$, respectively). There are also significant differences between MS and non-MS subjects ($p<0.001$, $p<0.0001$, respectively). The adjusted determination coefficient was 8.3 and 12.5% for each model (Figs. 1 and 2).

With regard to the linear relationship between the ALAT enzyme and hs-CRP, it can be seen that the slope increases significantly in patients with MS, while there is a negative relationship with non-MS subjects ($p=0.045$) (Fig. 3). Finally, there is a statistically significant difference between men and women. Men have higher ALAT levels than women ($p<0.0001$). The adjusted determination coefficient was 8.5% for the ALAT enzyme model (Fig. 3).

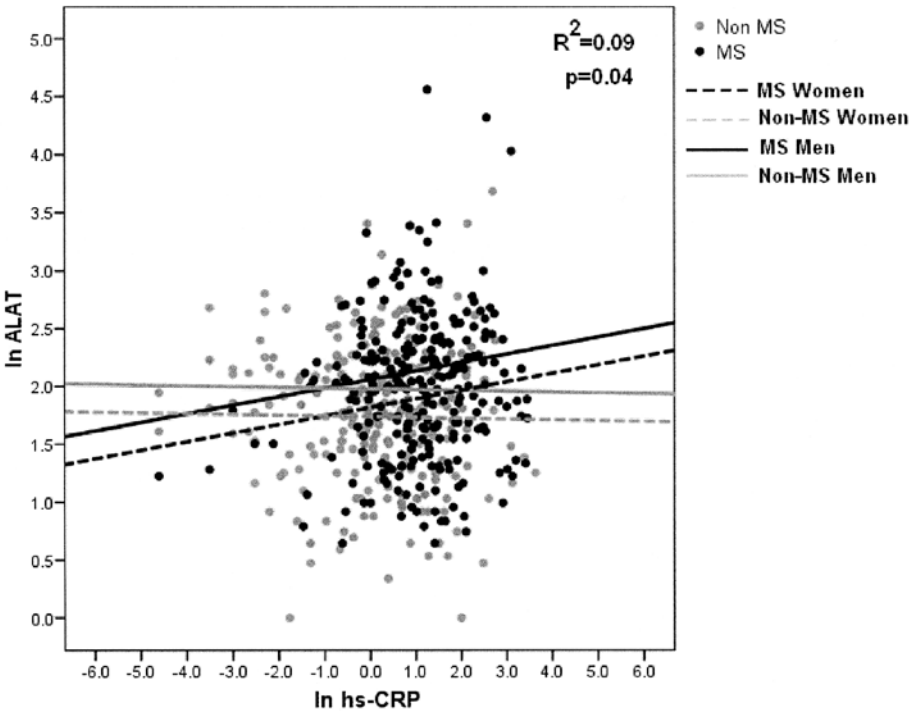

Figure 3. Scattering between ln hs-CRP and ln ALAT and the linear multivariate adjustment.

Increased levels of hs-CRP variably affect men and women, increasing ASAT and GGT enzyme levels in women and decreasing them in the case of men (p=0.02 and p=0.013, respectively) (Figs. 1 and 2).

With regard to the ALAT enzyme, a significant increase was observed in patients with MS, in accordance with the increase in the levels of hs-PCR (p=0.048) (Fig. 3). A statistically significant difference in the levels of this enzyme between men and women was also noted (p<0.001).

Discussion

The present study investigated the relationship of the levels of liver enzymes ALAT, ASAT and GGT in the presence of MS, and the levels of hs-PCR. The most relevant results were: a) we found higher levels of liver enzymes and hs-PCR in patients with MS compared to those without MS and b) from the multivariate analysis linear regression it was observed in women with and without MS that increased levels of hs-CRP induce an increased level of ASAT and GGT, exhibiting significantly higher levels of these enzymes in women with MS.

The finding of elevated liver enzymes in MS patients is somewhat expected as they have higher levels of obesity that determine, to a large extent, this alteration in liver enzymes. However, the pathogenic significance of these relations requires in-depth investigation. Several studies showed a relationship between high levels of ASAT, ALAT and GGT with impaired glucose tolerance and diabetes (26-29), although there is no clarity on the possible consequences of this interaction.

It was previously shown that adipocytokines act in hepatocytes and Küppfer cells initiating liver fibrosis. Peripheral IR in patients with NASH leads to an increase in the transport of fatty acids from adipose tissue to the liver. Thus, the fat metabolism routes are overloaded in the hepatocytes, which along with the increase of oxidative stress and/or mitochondrial dysfunction cause an increase in the adipocytokines, which begin a vicious cycle ending with the development of NASH (13,18,30).

Assuming that oxidative stress plays a critical role in the pathogenesis of NAFLD (3), as well as in the progression of the alteration of fasting glucose to diabetes, it is noteworthy that Nannipieri *et al* (29) found that GGT was an independent predictor of progression to glucose intolerance or diabetes. Similarly, we noted that the positive relationship between hs-PCR and levels of liver enzymes in patients with MS is important in identifying a subpopulation that possibly has more inflammatory and oxidative activity, which would have a higher risk of progressing to diabetes and/or towards NASH. Other studies that support this idea are those that have linked higher levels of GGT with the development of various diseases, including diabetes, Alzheimer's and atherosclerosis (15,31). The evidence supporting this hypothesis is based on previous studies indicating that GGT is an oxidative stress marker because of its importance in the transport of glutathione in cells (32).

Our study also showed significant gender differences (Table I and in Figs. 1 and 2). Although there are higher levels of hs-PCR in individuals with MS in relation to those without MS, it is interesting that the levels of women are superior to men with and without MS.

In light of the results that allow us to establish the relationships detailed above, we believe that future monitoring of these or other groups of patients with similar characteristics, with the aim of identifying groups most at risk, is significant.

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