

## HEPATIC STEATOSIS IN PATIENTS WITH HEPATITIS C

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### Abstract

**Aims:** The aims of this study was to indentify independent predictive factors of liver steatosis in patients with hepatitis C and to examine the correlations between ultrasound-diagnosed HS and anthropometric, clinical, biochemical and sonographic measurements. **Materials and methods:** We included 173 patients with CHC. HS has a high prevalence in diabetes mellitus patients, and we considered patients with CHC and diabetes as a separate subgroup for analysis: 113 patients (54 men/59 women) with hepatitis C – group A and 60 patients (28 men/32 women) with hepatitis C and T2DM – group B. We performed anthropometric measurements (weight, height, BMI (body mass index), as well as laboratory analysis and ultrasonography. All patients signed an informed consent before inclusion in the study. **Results:** In all patients with CHC, in univariate analysis triglycerides, hypertension, MetS, HOMA-IR >2, T2DM, AST/ALT ratio >1, and BMI >30kg/m<sup>2</sup> was associated with HS. Logistic regression analysis revealed five factors independently related with ultrasound-diagnosed HS: the triglyceride levels, hypertension, the BMI, AST/ALT ratio, and T2DM. **Conclusions:** This study revealed a high prevalence of ultrasound-diagnosed HS in patients CHC and CHC and T2DM. Identification of risk factor for HS has therapeutic importance, because targeted therapies might prevent the progression of HS to fibrosis and cirrhosis.

**keywords:** hepatitis C, hepatic steatosis, HOMA-IR, body mass index

### Introduction

Hepatic steatosis (HS) is a common condition [1,2] and is an independent risk factor for disease progression in patients with HCV (hepatitis C virus) infection [3]. Various mechanisms are involved in the development

of hepatic steatosis. HCV core protein induces production of reactive oxygen species and lipid peroxidation [4]. HCV core protein also regulates secretion of very low-density lipoprotein, triglycerides, and apolipoprotein B through regulation of fatty acid synthase, microsomal triglyceride transport protein,

peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$ ), and sterol regulatory element binding protein-1c [5,6,7,8]. Thus, HCV itself is directly involved in the development of hepatic steatosis. In addition, insulin is an anabolic hormone and promotes hepatic lipogenesis through activation of hydroxymethylglutaryl-CoA reductase and acetyl-CoA carboxylase [9]. In addition, insulin inhibits lipolysis through regulation of phosphodiesterase type 3B [10]. In HCV core gene transgenic mice, the development of insulin resistance precedes the development of hepatic steatosis, suggesting that insulin resistance may induce hepatic steatosis [11,12]. However, hepatic steatosis could also cause insulin resistance [13,14], and therefore, the initial step in HCV-related metabolic disorders remains unclear in patients with HCV infection.

The aims of this study was to identify independent predictive factors of liver steatosis in patients with hepatitis C and to examine the correlations between ultrasound-diagnosed HS and anthropometric, clinical, biochemical and sonographic measurements.

We used the term hepatic steatosis (HS) and not non-alcoholic fatty liver disease because we used only ultrasonographic criteria. Hepatic ultrasound is a sensitive procedure for detecting liver fat (sensitivity 91-100, specificity 93-100) [15].

### **Material and methods**

The study was conducted during January 2008 - December 2009 at "Prof. N. Paulescu" National Institute of Diabetes, Nutrition and Metabolic Diseases, "Dr. Carol Davila" Clinical Central Military Emergency Hospital, and Dr. I. Cantacuzino Clinical Hospital -

Bucharest, Romania. We included 173 patients with CHC. HS has a high prevalence in diabetes mellitus patients, and we considered patients with CHC and diabetes as a separate subgroup for analysis: 113 patients (54 men/59 women) with hepatitis C – group A and 60 patients (28 men/32 women) with hepatitis C and T2DM – group B. We performed anthropometric measurements (weight, height, BMI (body mass index), as well as laboratory analysis and ultrasonography. All patients signed an informed consent before inclusion in the study.

### **Anthropometric parameters**

Height was measured using a standard stadiometer and the nearest one centimeter was recorded. Weight was measured with subjects wearing only light clothing; the nearest 0.5 kilogram was recorded. Waist circumference (WC) was measured at the end of a normal expiration, midway between the inferior margin of the ribs and superior border of the iliac crest in a horizontal plane, and was rounded to the nearest 0.1 cm, according to the previously published literature [16]. Body mass index (BMI; kg/m<sup>2</sup>) was calculated according to the Quetelet equation (weight in kilograms divided by height in square meter) [17]. BP was measured on the right arm with subject having relaxed for at least 5 minutes, via a standard sphygmomanometer of appropriate cuff size and was repeated with 10 minutes interval. The average of these two measurements was recorded for systolic and diastolic BP. We used IDF criteria for MetS: central obesity (waist circumference over 94cm in men, and 80cm in women or BMI over 30kg/m<sup>2</sup>) plus any two of the following

factors: 1. triglycerides  $\geq 1.695$ mmol/l (150mg/dl) or treatment; 2. lower high density lipoprotein-cholesterol  $< 40$ mg/dl in men or  $< 50$ mg/dl in women or treatment; 3. blood pressure  $\geq 130/85$ mmHg or medication; 4. fasting blood glucose  $\geq 5.6$ mmol/l (100mg/dl) or medication for diabetes [18].

### **Laboratory methods**

Venous blood samples were collected in the morning following a 12-hours fast; the laboratory tests included serum lipids (cholesterol, triglyceride, TG; HDL-cholesterol – HDL-C), glucose profile (fasting plasma glucose, FPG; glycated hemoglobin, HbA1c; fasting plasma insulin, FPI; peptid C), liver profile (alaninaminotransferases, ALT; aspartataminotransferases, AST; gamma-glutamyltranspeptidase, GGT; alkaline phosphatase, ALP; bilirubin, albumin), blood count for all patients.

IR (insulin resistance) was determined using Homeostasis Model Assessment (HOMA-IR) (fasting insulin level (mUI/l) $\times$  fasting glucose level (mg/dl)/405 [19]; a HOMA-IR index value of more than 2.0 was considered as criteria of insulin resistance.

### **Imaging studies**

The differentiation between steatosis and steatohepatitis can be made only by a histological approach [20]. The most common and less invasive imaging technique used for NAFLD diagnosis is ultrasonography.

First, examination of all visible liver parenchyma was performed with a 3-5 MHz transducer. Liver parenchyma was examined with sagittal as well as longitudinal guidance of the probe and completed by lateral and intercostals views. The presence of steatosis

was recognized as a marked increase in hepatic echogenicity “bright liver”, poor penetration of the posterior segment of the right lobe of the liver, and poor or no visualization of the hepatic vessels “blurring of the vascular margins”. The increased hepatic echogenicity is diffuse and easy to appreciate by comparison with the lower echogenicity of the kidney or spleen.

The hepato-renal contrast is an ultrasound index for quantification the liver steatosis [21,22]. Hepatomegaly was defined as a liver size above 155mm measured as a subcostal diameter in the midclavicular line by using the 3-5 MHz probe.

### **Statistical analyses**

Data were analyzed using the SPSS package for Windows, version 19.0, SPSS Inc., Chicago, Illinois, USA. As a first step, the two groups (CHC and CHC with T2DM) were analysed separately.

Patients characteristics are given as mean $\pm$ SD for continuous variables. One-way analysis of variance and Chi square and Fisher exact tests analyses were used to compare means and proportions, respectively were used to test differences in proportions. No multiplicity adjustments were used as no intermediate analysis was performed.

Pearson's and Spearman's correlation coefficient was calculated to measure the relationship between variables. Receiver operating characteristics curves were constructed for each test in order to evaluate the sensitivity, specificity, positive and negative predictive values, and the area under the receiver operating characteristic curve (AUROC). Significance was defined at the 0.05 level of confidence.

## Results

### Characteristics of the study population

Baseline characteristics of the 173 subjects included in the study are summarized in **Table 1** according to whether they had CHC (n=113) or CHC and T2DM (n=60), respectively. Average age did not differ significantly between patients with and without T2DM (53.6±7.8 years vs. 53.0±7.8 years, p=0.655). In CHC and T2DM patients,

the prevalence of ultrasound-diagnosed HS was 73.3% (n=44), higher in female (56.8%, n=25); in patients only with hepatitis C the prevalence of HS was 52.2%, higher in men (52.5%, n=31). This difference did not reach statistical significance (p=0.19 for group A and p=0.27 for group B). There were no significant differences with regard to the amount of alcohol consumption and the percentage of smokers between groups.

**Table 1. Characteristic of patients**

	CHC n=113	CHC+T2DM n=60	p value
Sex (F/M)	59/54	32/28	0.508
Environment (U/R)	70/43	48/12	0.011
Smoking	33.6% (n=38)	40% (n=24)	0.083
Metabolic waist circumference	72.6% (n=82)	80% (n=48)	0.187
BMI ≥30 kg/m <sup>2</sup>	32.7% (n=37)	33.3% (n=20)	0.534
HypoHDL-C	63.7% (n=72)	75% (n=15)	0.089
HyperTG	53.1% (n=60)	55% (33)	0.469
Hypertension	50.4% (n=57)	75% (n=45)	0.001
MetS	51.3% (n=58)	93.3% (n=56)	0.0001

Abbreviations: F, female; M, male; U, urban; R, rural; BMI, body mass index; HDL-C, high density lipoprotein – cholesterol; TG, triglycerides; MetS, metabolic syndrome.

### Prevalence of features of the metabolic syndrome in patients with hepatitis C and ultrasound-diagnosed HS

In patients with CHC (group A) prevalence of MetS was 51.3% (n=58); 34 patients had hepatic steatosis and MetS. The mean BMI of the whole study cohort (28.1±4.6 kg/m<sup>2</sup>) indicated a tendency to be slightly overweight as it was observed in the patients with T2DM but also in HCV populations [23]. Noteworthy, BMI as well as WC, HC and WHR were significantly higher in patients with ultrasound-diagnosed HS with CHC and MetS (**Table 2**).

Furthermore, HDL-C levels were significantly lower in the MetS patients with

HS (36.3±7.2mg/dl vs. 47.6±12.6mg/dl, p<0.0001) and triglyceride levels were higher in MetS patients (177.5±40.1mg/dl vs. 111.9±39mg/dl, p<0.0001), while serum levels of total cholesterol did not significantly differ. In contrast, systolic and diastolic blood pressure, as well as fasting glucose levels, insulin level and HOMA-IR did not differ significantly between both groups with hepatitis C (**Table 2**).

Remarkably, the rates of reported hypertension (88.6%, n=38) were significantly higher (as defined by history, review of medical files and/or use of hypertensive medication).

**Table 2. Characteristic of patients with CHC and hepatic steatosis**

		Mean	Std. Deviation	p value
Waist circumference (cm)	MetS-HS	86.52	15.00	0.0001
	MetS+HS	100.62	13.64	
BMI (kg/m <sup>2</sup> )	MetS-HS	25.85	4.12	0.0001
	MetS+HS	30.74	5.03	
WHR	MetS-HS	0.92	0.08	0.0001
	MetS+HS	1.02	0.11	
FPI (uU/ml)	MetS-HS	13.1	5.8	0.230
	MetS+HS	14.9	5.9	
Peptid C	MetS-HS	3.1	1.6	0.377
	MetS+HS	2.8	1.3	
FPG (mg/dl)	MetS-HS	90.5	17.7	0.276
	MetS+HS	95.6	17.2	
HOMA-IR	MetS-HS	3.0	1.8	0.217
	MetS+HS	3.7	1.9	
HbA1c (%)	MetS-HS	5.8	0.5	0.222
	MetS+HS	6.1	1.2	
Cholesterol (mg/dl)	MetS-HS	198	36.6	0.985
	MetS+HS	197.9	38.0	
Triglycerides (mg/dl)	MetS-HS	111.9	39.0	0.0001
	MetS+HS	177.5	40.1	
HDL-C (mg/dl)	MetS-HS	47.6	12.6	0.0001
	MetS+HS	36.3	7.2	
AST (UI/L)	MetS-HS	47.4	27.6	0.053
	MetS+HS	62.9	31.0	
ALT (UI/L)	MetS-HS	57.6	31.5	0.170
	MetS+HS	69.9	34.7	
GGT (U/L)	MetS-HS	54.8	37.7	0.014
	MetS+HS	89.2	59.5	
Albumin (g/dl)	MetS-HS	4.4	0.4	0.008
	MetS+HS	4.1	0.5	

In group A patients (CHC) hepatic serum parameters of ultrasound-diagnosed HS patients shown that mean AST and GGT serum levels were slightly higher in HS group as compared to patients without HS signs (**Table 2**). In contrast, serum ALT levels were higher but these differences did not reach statistical significance. The AST/ALT ratio was higher ( $0.89\pm 0.2$  vs.  $0.87\pm 0.2$ ,  $p=0.02$ ) in the HS group.

Constructing a ROC curve revealed that the best discriminating cut-off value of 56.8 U/l for AST would yield sensitivity and specificity rates of 46.6% and 28.6%, respectively, for the identification of HS (AUC=0.603, data not shown). The best discriminating cut-off value for the GGT of 30.5 would yield similar sensitivity and specificity rates (84.5% and 65.7%, respectively; AUC=0.585; data not shown). Further, mean serum levels of total bilirubin,

alkaline phosphatase, albumin were similar in both groups.

### Prevalence of features of the metabolic syndrome in patients with hepatitis C and T2DM and ultrasound-diagnosed HS

In patients with CHC and T2DM (group B) prevalence of MetS was 93.3% (n=56); 42 patients had hepatic steatosis and MetS. WC and BMI was higher in patients with HS and MetS but not WHR. FPG, FPI, cholesterol, triglycerides, HOMA-IR were higher in patients with MetS (all p over 0.05), probably because of the small number of patients

without MetS. In patients with diabetes and hepatitis C, hypertension was present in 97,1% (n=34).

### Multivariate analysis of risk factors for HS

In all patients with CHC, in univariate analysis triglycerides, hypertension, MetS, HOMA-IR >2, T2DM, AST/ALT ratio >1, and BMI >30kg/m<sup>2</sup> was associated with HS. Logistic regression analysis revealed five factors independently related with ultrasound-diagnosed HS: the triglyceride levels, hypertension, the BMI, AST/ALT ratio, and T2DM (Table 3).

**Table 3. Multivariate analysis of risk factors for ultrasound-diagnosed HS**

Variables	Adjusted Odds Ratio	95 % CI	P value
Gender	0.67	0.3-1.4	0.65
TG>150 mg/dl	1.85	1.27-2.7	0.005
Hypertension	1.7	1.19-2.4	0.022
MetS	1.62	1.147-2.308	0.06
HOMA-IR	0.75	0.38-2.39	0.178
T2DM	1.69	1.105-2.605	0.013
AST/ALT ratio	0.39	0.18-0.82	0.006
BMI > 30 (kg/m <sup>2</sup> )	1.42	1.02-2.18	0.032

Abbreviations: HS, hepatic steatosis; CI, confidence interval; TG, triglycerides; MetS, metabolic syndrome; HOMA-IR, Homeostasis Model of Assessment - Insulin Resistance; T2DM, type 2 diabetes mellitus; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI = body mass index.

In women, using binary logistic regression, independent risk factor for HS were diabetes (OR=2.89, 95%CI 1.49-5.86, p=0.004), BMI over 31.8 kg/m<sup>2</sup> (OR=2.5, 95%CI 1.1-5.67, p=0.008), hypertension (OR=2.45, 95%CI 1.44-4.15, p=0.0001), hypertriglyceridemia (OR=2.21, 95%CI 1.28-3.82, p=0.0002), WHR >0.87 (OR=2.21, 95%CI 1.44-3.37, p=0.002), HOMA-IR (OR=2.21, 95%CI 1.44-3.37, p=0.001), GGT >21.1U/L (OR=2.04, 95%CI 1.32-3.1, p=0.014), AST > 48 U/L (OR=1.67, 95%CI 1.01-2.08, p=0.0034), ALT >44 U/L (OR=1.6, 95%CI 0.99-2.57, p=0.045).

In men, independent risk factor for HS were diabetes (OR=2.2, 95%CI 1.4-3.38, p=0.047) and AST/ALT > 1 (OR=3.54, 95%CI 1.29-9.67, p=0.047).

### Discussions

The prevalence of ultrasound-diagnosed HS was 59.5% (57.3% in patients with CHC and 77.1% in CHC and T2DM patients); in our study prevalence was similar to that observed in previous ultrasound-based studies in the CHC populations [24,25]; in general population from Western countries, the Near-East and Japan prevalence of HS is between

20% to 30% [26,27]. Most likely, this high prevalence can be explained or may have been expected, respectively, based on the higher prevalence of (components of) the metabolic syndrome in our patients.

There is conflicting evidence on gender as a risk factor for HS. While earlier studies indicated a higher HS prevalence in women [28], studies both in Caucasian [29] and Asian populations [30] found male gender to be an independent HS risk factor. In line with a recent study [31] we did not observe a significant difference with regard to the gender proportion in patients with ultrasound-diagnosed HS.

In our study, smoking is not a risk factor for SH, as observed in other studies [32]. It may be speculated that smoking is an independent risk factor for hepatic inflammation and fibrosis as observed in patients with chronic HCV [33] infection. Hypoxia was identified as a potential underlying mechanism of progression of hepatic diseases and in the light of a recent study, linking the oxygen-sensitive transcription factor hypoxia-inducible factor HIF-1 to hepatic lipid metabolism [34], hypoxia may also be involved in the observed association between nicotine and hepatic steatosis.

Insulin resistance plays a central role in HS pathogenesis. Insulin resistance is the main feature of metabolic syndrome, which embodies obesity, hypertension, diabetes, and dyslipidemia [35]. HS is considered to be the hepatic component of MetS [36,37,38]. Although overweight and obesity are present in the majority of patients with HS, steatohepatitis can also occur in subjects with normal body weight [39]. A direct correlation

between the degree of obesity and NASH development has been observed [40]; however, not all obese patients will have HS; in our study 28.35% of patients with normal weight have HS. HS evidenced by ultrasound is clearly more pronounced in cases of insulin resistance. Some authors suggest that hyperinsulinemia of NAFLD is the result of the decreased insulin extraction by the liver [41].

According to Kotronen et al. [42], 90% of individuals with NAFLD have at least one risk factor for MS, and 33% have all the features of MetS. In our study 65.9% of patients have MetS and 10.4% have all five components. The presence of multiple metabolic disorders such as diabetes mellitus, obesity, dyslipidemia and hypertension is associated with a potentially progressive, severe liver disease [43].

In line with this, diabetes and hypertension as well as BMI and waist-hip-ratio as surrogates for obesity were significantly higher in the group of ultrasound-diagnosed HS.

In the entire group, there were five factors independently related with ultrasound-diagnosed HS: the triglyceride levels, hypertension, the BMI, AST/ALT ratio, and T2DM. We observed a difference between men and women regarding risk factors. In women, independent risk factor for SH were diabetes, BMI over  $31.8\text{kg/m}^2$ , hypertension, hypertriglyceridemia,  $\text{WHR}>0.87$ , HOMA-IR,  $\text{GGT}>21.1\text{U/L}$ ,  $\text{AST}>48\text{U/L}$ ,  $\text{ALT}>44\text{U/L}$ . In men, independent risk factor for HS were diabetes and  $\text{AST/ALT}>1$ .

It is widely accepted that serum levels of transaminases are neither specific nor sensitive enough to screen for HS [44,45], and

this is confirmed in the present study. AST, ALT and GGT have the sensitivity and specificity not satisfactory to identify HS.

## Conclusions

Hepatic steatosis is commonly seen in patients with chronic hepatitis C due to host metabolic factors such as obesity, hyperlipidemia, metabolic syndrome and type 2 diabetes mellitus in which insulin resistance plays an important role. This study revealed a high prevalence of ultrasound-diagnosed HS in patients CHC and CHC and T2DM.

## REFERENCES

1. Negro F, Sanyal AJ. Hepatitis C virus, steatosis and lipid abnormalities: clinical and pathogenic data. *Liver Int* **29** Suppl 2: 26-37, 2009.
2. Ong JP, Younossi ZM, Speer C, Olano A, Gramlich T, Boparai N. Chronic hepatitis C and superimposed nonalcoholic fatty liver disease. *Liver* **21**: 266-271, 2001.
3. Leandro G, Mangia A, Hui J, Fabris P, Rubbia-Brandt L, Colloredo G, Adinolfi LE, Asselah T, Jonsson JR, Smedile A, et al. Relationship between steatosis, inflammation, and fibrosis in chronic hepatitis C: a meta-analysis of individual patient data. *Gastroenterology* **130**: 1636-1642, 2006.
4. Korenaga M, Wang T, Li Y, Showalter LA, Chan T, Sun J, Weinman SA. Hepatitis C virus core protein inhibits mitochondrial electron transport and increases reactive oxygen species (ROS) production. *J Biol Chem* **280**: 37481-37488, 2005.
5. Negro F. Mechanisms and significance of liver steatosis in hepatitis C virus infection. *World J Gastroenterol* **12**: 6756-6765, 2006.
6. McPherson S, Jonsson JR, Barrie HD, O'Rourke P, Clouston AD, Powell EE. Investigation of the role of SREBP-1c in the pathogenesis of HCV-related steatosis. *J Hepatol* **49**: 1046-1054, 2008.
7. Negro F. Peroxisome proliferator-activated receptors and hepatitis C virus-induced insulin resistance. *PPAR Res* **2009**: 483485, 2009.
8. Perlemuter G, Sabile A, Letteron P, Vona G, Topilco A, Chrétien Y, Koike K, Pessayre D, Chapman J, Barba G, et al. Hepatitis C virus core protein inhibits microsomal triglyceride transfer protein activity and very low density lipoprotein secretion: a model of viral-related steatosis. *FASEB J* **16**: 185-194, 2002.
9. Geelen MJ, Harris RA, Beynen AC, McCune SA. Short-term hormonal control of hepatic lipogenesis. *Diabetes* **29**: 1006-1022, 1980.
10. Kawaguchi T, Yamagishi S, Sata M. Branched-chain amino acids and pigment epithelium-derived factor: novel therapeutic agents for hepatitis c virus-associated insulin resistance. *Curr Med Chem* **16**: 4843-4857, 2009.
11. Shintani Y, Fujie H, Miyoshi H, Tsutsumi T, Tsukamoto K, Kimura S, Moriya K, Koike K. Hepatitis C virus infection and diabetes: direct involvement of the virus in the development of insulin resistance. *Gastroenterology* **126**: 840-848, 2004.
12. Koike K. Hepatitis C as a metabolic disease: Implication for the pathogenesis of NASH. *Hepatol Res* **33**: 145-150, 2005.

- 13. Kawaguchi T, Osatomi K, Yamashita H, Kabashima T, Uyeda K.** Mechanism for fatty acid "sparing" effect on glucose-induced transcription: regulation of carbohydrate-responsive element-binding protein by AMP-activated protein kinase. *J Biol Chem* **277**: 3829-3835, 2002.
- 14. Kawaguchi T, Takenoshita M, Kabashima T, Uyeda K.** Glucose and cAMP regulate the L-type pyruvate kinase gene by phosphorylation/dephosphorylation of the carbohydrate response element binding protein. *Proc Natl Acad Sci USA* **98**: 13710-13715, 2001.
- 15. Lewis JR, Mohanty SR.** Nonalcoholic fatty liver disease: a review and update. *Dig Dis Sci* **55**: 560-578, 2010.
- 16. Alberti KG, Zimmet P, Shaw J.** Metabolic syndrome--a new world-wide definition. A Consensus Statement from the International Diabetes Federation. *Diabet Med* **23**(5): 469-480, 2006.
- 17. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D.** A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. *Ann Intern Med* **130**(6): 461-470, 1999.
- 18. International Diabetes Federation.** The IDF Worldwide Definition of the Metabolic Syndrome [online], 2005.
- 19. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC.** Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* **28**: 412-419, 1985.
- 20. Oh MK, Winn J, Poordad F.** Review article: diagnosis and treatment of non-alcoholic fatty liver disease. *Aliment Pharmacol Ther* **28**: 503-522, 2008.
- 21. Osawa H, Mori Y.** Sonographic diagnosis of fatty liver using a histogram technique that compares liver and renal cortical echo amplitudes. *J Clin Ultrasound* **24**: 25-29, 1996.
- 22. Webb M, Hanny Yeshua H, Zelber-Sagie S, Santo M, Barazovski E, Katz R, Halpern Z, Oren R.** A practical index for ultrasonographic quantification of liver steatosis. The 58th Annual Meeting of the American Association for the Study of Liver Diseases. Boston, MA. Totowa, NJ: *The Humana Press*; 2007.
- 23. Rusu E, Dragomir A, Radulian G, Jinga M, Cheta D.** Obesity in diabetic patients with hepatitis C. *International Journal of Diabetes in Developing Countries* **28** (suppl 1): 20, 2008.
- 24. Hourigan LF, Macdonald GA, Purdie D et al.** Fibrosis in chronic hepatitis C correlates significantly with body mass index and steatosis. *Hepatology* **29**: 1215-9, 1999.
- 25. Monto A, Alonzo J, Watson JJ, Grunfeld C, Wright TL.** Steatosis in chronic hepatitis C: relative contributions of obesity, diabetes mellitus, and alcohol. *Hepatology* **36**: 729-36, 2002.
- 26. Bellentani S, Bedogni G, Miglioli L, Tiribelli C.** The epidemiology of fatty liver. *Eur J Gastroenterol Hepatol* **16**: 1087-1093, 2004.
- 27. Bellentani S, Saccoccio G, Masutti F, et al.** Prevalence of and risk factors for hepatic steatosis in Northern Italy. *Ann Intern Med* **132**: 112-117, 2000.
- 28. Falck-Ytter Y, Younossi ZM, Marchesini G, McCullough AJ.** Clinical features and natural history of nonalcoholic steatosis syndromes. *Semin Liver Dis* **21**: 17-26, 2001.
- 29. Browning JD, Szczepaniak LS, Dobbins R, Nuremberg P, Horton JD, Cohen JC, Grundy SM, Hobbs HH.** Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. *Hepatology* **40**: 1387-1395, 2004.
- 30. Fan JG, Zhu J, Li XJ, Chen L, Li L, Dai F, Li F, Chen SY.** Prevalence of and risk factors for fatty liver in a general population of Shanghai, China. *J Hepatol* **43**: 508-514, 2005.
- 31. Bedogni G, Miglioli L, Masutti F, Tiribelli C, Marchesini G, Bellentani S.** Prevalence of and risk factors for nonalcoholic fatty liver disease: the Dionysos nutrition and liver study. *Hepatology* **42**: 44-52, 2005.
- 32. Targher G, Bertolini L, Rodella S, Lippi G, Franchini M, Zoppini G, Muggeo M, Day CP.** NASH predicts plasma inflammatory biomarkers independently of visceral fat in men. *Obesity (Silver Spring)* **16**: 1394-1399, 2008.

- 33. Hezode C, Lonjon I, Roudot-Thoraval F, Mavier JP, Pawlotsky JM, Zafrani ES, Dhumeaux D.** Impact of smoking on histological liver lesions in chronic hepatitis C. *Gut* **52**: 126-129, 2003.
- 34. Rankin EB, Rha J, Selak MA, Unger TL, Keith B, Liu Q, Haase VH.** Hypoxia-inducible factor 2 regulates hepatic lipid metabolism. *Mol Cell Bio* **29**: 4527-4538, 2009.
- 35. Marchesini G, Brizi M, Morselli-Labate AM, Bianchi G, Bugianesi E, McCullough AJ, Forlani G, Melchionda N.** Association of nonalcoholic fatty liver disease with insulin resistance. *Am J Med* **107**: 450-455, 1999.
- 36. Younossi ZM.** Review article: current management of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis. *Aliment Pharmacol Ther* **28**: 2-12, 2008.
- 37. Tarantino G.** Should nonalcoholic fatty liver disease be regarded as a hepatic illness only? *World J Gastroenterol* **13**: 4669-4672, 2007.
- 38. Tarantino G, Conca P, Pasanisi F, Ariello M, Mastrolia M, Arena A, Tarantino M, Scopacasa F, Vecchione R.** Could inflammatory markers help diagnose nonalcoholic steatohepatitis? *Eur J Gastroenterol Hepatol* **21**: 504-511, 2009.
- 39. Sanyal AJ.** AGA technical review on nonalcoholic fatty liver disease. *Gastroenterology* **123**: 1705-1725, 2002.
- 40. Edmison J, McCullough AJ.** Pathogenesis of non-alcoholic steatohepatitis: human data. *Clin Liver Dis* **11**: 75-104, 2007.
- 41. Goto T, Onuma T, Takebe K, Kral JG.** The influence of fatty liver on insulin clearance and insulin resistance in non-diabetic Japanese subjects. *Int J Obes Relat Metab Disord* **19**: 841-845, 1995.
- 42. Kotronen A, Westerbacka J, Bergholm R, Pietiläinen KH, Yki-Järvinen H.** Liver fat in the metabolic syndrome. *J Clin Endocrinol Metab* **92**: 3490-3497, 2007.
- 43. Marchesini G, Bugianesi E, Forlani G, Cerrelli F, Lenzi M, Manini R, Natale S, Vanni E, Villanova N, Melchionda N, et al.** Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome. *Hepatology* **37**: 917-923, 2003.
- 44. Neuschwander-Tetri BA, Caldwell SH.** Nonalcoholic steatohepatitis: summary of an AASLD Single Topic Conference. *Hepatology* **37**: 1202-1219, 2003.
- 45. Fracanzani AL, Valenti L, Bugianesi E, Andreoletti M, Colli A, Vanni E, Bertelli C, Fatta E, Bignamini D, Marchesini G, Fargion S.** Risk of severe liver disease in nonalcoholic fatty liver disease with normal aminotransferase levels: a role for insulin resistance and diabetes. *Hepatology* **48**: 792-798, 2008.

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