The value of ultrasonography and computerized tomography in estimating the histopathological severity of nonalcoholic steatohepatitis

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Abstract

Background & Aims : **Liver biopsy is the gold standard for the diagnosis of non-alcoholic steatohepatitis (NASH), but is an invasive method. There is a need for non-invasive methods that can reflect the histopathological severity of NASH. The aim of this study was to compare the ultrasonography and computerized tomography findings with the histopathological severity in patients with NASH.**

Material and Methods : **Twenty-two consecutive patients with biopsy proven NASH and 20 age- and sex-matched healthy individuals were enrolled. Clinical and demographic data were collected at the time of liver biopsy. Histopathological grading and staging were made by an expert pathologist. Each patient underwent ultrasonography and computerized tomography.**

Results : **Liver ultrasonographic findings were not correlated with histopathological grade and stage (r : 0.134, *P* > 0.05 ; r : 0.130, *P* > 0.05). Mean liver densities obtained by computed tomography of NASH patients were lower than that of controls (*P* < 0.05) and liver/spleen density ratios were lower than that of controls (*P* < 0.05). These results were significantly correlated with histopathological grade (r : -0.716, *P* < 0.001 ; r : -0.663, *P* : 0.001), but not with the histopathologic stage (r : -0.416, *P* : 0.05 ; r : -0.356, *P* : 0.1).**

Conclusions : **Ultrasonography findings do not reflect histopathological severity in patients with NASH. Computed tomography attenuation of the liver is significantly correlated with histopathologic grade but not with histopathological stage.** (Acta gastroenterol. belg., **2005**, 68, **221-225**).

Key words : nonalcoholic steatohepatitis, histopathology, ultrasonography, computerized tomography.

Introduction

Nonalcoholic fatty liver disease (NAFLD) is a clinical and pathological condition that encompasses a broad spectrum ranging from simple steatosis to steatohepatitis (1). Nonalcoholic steatohepatitis (NASH) represents a subset of NAFLD and is characterized by the presence of steatosis along with inflammatory activity with or without fibrosis, indicating the possibility of progressive liver disease (2). The clinical importance of NASH arises mostly from this potential to progress to end-stage liver disease (3,4)

Because NASH is defined by characteristic findings on liver histology, diagnosis is established only by obtaining a liver biopsy (5). The use of liver biopsy for the diagnosis of NAFLD in routine clinical practice is however debated. Arguments against a liver biopsy include the limited ability to provide accurate prognostic information on an individual patient, even when liver biopsy is available ; the lack of an established effective therapy except weight loss for obese patients ; and the risks and costs associated with liver biopsy. On the other hand a liver biopsy remains the sole accurate method for the diagnosis of NASH (6). There is a need for a noninvasive method that can estimate the histopathological severity of NASH.

Several non-invasive imaging techniques, including ultrasonography (US), computed tomography (CT), and magnetic resonance imaging (MRI) can identify hepatic steatosis and have been advocated as noninvasive diagnostic tests for NASH (7). US is the most commonly used modality for the diagnosis of a fatty liver. Fatty infiltration of the liver produces a diffuse increase in echogenecity as compared with that of the kidneys on US, and a low density of hepatic parenchyma on CT (8). A CT scan was shown to be more specific for the diagnosis of fatty liver with comparable sensitivity but at a higher cost than US (5). These imaging techniques can show increased fat accumulation in the hepatic parenchyma (9) but relationship between US and CT findings with histopathological grade and stage is not well known.

The aim of this study was to investigate the relationship between histopathological severity of NASH with US and CT findings.

Materials and Methods

Patients

The study consisted of 22 consecutive patients with high alanine and aspartate aminotansferase (ALT, AST) levels who were diagnosed as NASH by histopathological examination and 20 age- and sex-matched healthy individuals as a control group, between May 2001 and November 2003. Liver biopsies were performed percutaneously according to Menghini method using 1.4 mm biopsy needle (Hepafix, B. Braun Melsungen AG, Germany). All samples were at least 15 mm in length.

The diagnosis of NASH was based on the following criteria :

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(i) the presence of steatosis (> 10%), lobular inflammation, and ballooning degeneration (with or without fibrosis) on liver biopsy ;
(ii) intake of less than 20 gr of ethanol per week, as confirmed by the physician and family members who were in close contact with the patient ; and
(iii) appropriate exclusion of other liver diseases such as alcoholic liver disease, viral hepatitis, autoimmune hepatitis, drug-induced liver disease, primary biliary cirrhosis, primary sclerosing cholangitis, biliary obstruction, and metabolic liver diseases. No patient had a history of jejunoileal by-pass.

Detailed histories of the patients and all drugs used by them were recorded. Presence of obesity, hyperlipidemia, and diabetes mellitus was investigated using well-known routine tests.

Informed consent was obtained from each patient and volunteer and the study protocol was approved by the institution's human research committee.

Pathology

Histopathological examination was carried out by an expert pathologist, who performed the grading and staging (10). The grade indicates the hepatic steatosis and the activity of necrosis and inflammation whereas stage reflects degree of fibrosis and architectural alterations.

Grading : Grading was made according to macrovesicular steatosis and necroinflammatory activity.

Grade 0 : No steatosis

Grade 1 : Steatosis up to 33% and mild necroinflammatory activity

Grade 2 : Steatosis between 33 and 66% with moderate necroinflammatory activity

Grade 3 : Steatosis over 66% and severe necroinflammatory activity.

Staging : Staging was made according to fibrosis.

Stage 1 : zone 3 perisinusoidal / pericellular fibrosis ; focal or diffuse ;

Stage 2 : Focal or diffuse periportal fibrosis with zone 3 perisinusoidal / pericellular fibrosis ;

Stage 3 : Focal and diffuse bridging necrosis with perisinusoidal / pericellular fibrosis and portal fibrosis ;

Stage 4 : Cirrhosis.

US and CT examination

US examination with a 3.5-5 MHz transducer. (Elegra ; Toshiba, Japan) aimed at obtaining the following images : sagital view of right lobe of the liver and right kidney, transverse view of the left lateral segment of the liver and spleen) The severity of echogenecity ; grade 1, slight, diffuse increase in fine echoes in liver parenchyma with normal visualization of diaphragm and intrahepatic vessel borders ; grade 2, moderate, diffuse increase in fine echoes with slightly impaired visualization of intrahepatic vessels and diaphragm ; grade 3, marked increase in fine echoes with poor or non visualization of the intrahepatic vessel borders, diaphragm, and posterior right lobe of the liver (11).

CT evaluation was performed with Hitachi W1000 using 120 Kvp, 100 mAs and slice thickness of 10 mm. Four slices were acquired in each patient on the basis of a scout view at the following levels : dome of the liver, immediately cephalad and caudal to the hepatic hilum, and close to the inferior margin of the right lobe. No contrast medium was used. The average of the four Housnfield values obtained for each slice was measured in a volume of interest (VOI, cm^2) ranging from 4-5 cm^2 for the dome and inferior margin to 15-20 cm^2 for the hilar region. The VOI was shaped in order to minimize the contribution of blood vessel. The ratio between the averaged liver density and the spleen density was also calculated (12).

Laboratory Analyses

Blood samples were collected from patients and control group after overnight fasting. AST, ALT, total protein, albumin, alkaline phosphatase, β-glutamyl transferase, Hbs-Ag, antiHCV, smooth muscle antibody, antimitochondrial antibody, serum cholesterol, triglyceride, fasting glucose levels and complete blood count were studied.

Patients with fasting serum glucose levels of more than 126 mg/dl in at least two separate samples were identified as having diabetes mellitus, and a finding of 140-200 mg/dl two hour after standard oral glucose loading was considered as impaired glucose tolerance test (12).

Statistical analysis

Student's *t* test was used to analyze to parametric variables, and Mann-Whitney U test was used for appropriate nonparametric variables. The relationships among the variables were analyzed using Spearman correlation test. All analyses were performed using the Statistical Package for the Social Sciences (SPSS) for Windows, version 10,0. *P* values of less than 0.05 were considered statistically significant.

Results

There was no significant difference between patient and control groups with respect to mean age and gender ($P > 0.05$ for each). Demographic, clinical, and laboratory data of the patients and controls are shown in Table 1.

Histopathologic features of the patients

Distribution of the patients according to histopathologic grade : seven patients (32%) had grade 1, 12 (54%) had grade 2, and three (14%) had grade 3 macrovesicular steatosis and inflammation.

Table 1. — **Demographic, clinical, and laboratory data in patients with nonalcoholic steatohepatitis and in a control group**

Parameters & Normal range	NASH (n = 22)	Controls (n = 20)	*P*
Age (years)	42 ± 9	43 ± 10	NS
Gender (Male/Female)	14/8	12/6	NS
AST (5-40 IU/L)	60.6 ±33.1	21.4 ± 17.4	< 0.001
ALT (10-28 IU/L)	120.4 ± 92.9	23.8 ± 14.5	< 0.001
ALP (38-145 IU/L)	109.8 ± 51.4	92.4 ± 58.2	NS
GGT (7-32 IU/L)	75.3 ± 84.9	37.3 ± 17.4	< 0.05
Total cholesterol (120-220 mg/dl)	200.1 ± 45.1	185.6 ± 42.7	NS
HDL cholesterol (35-60 mg/dl)	39.5 ± 9.8	40 ±10.4	NS
LDL cholesterol (< 130 mg/dl)	127.3 ± 38.1	101.7 ± 29.3	NS
Triglycerides (40-180 mg/dl)	224.9 ± 114.5	181.9 ± 48.7	NS
BMI ≥ 30	7 (31.8%)	–	
Hyperlipidemia	17 (77.3%)	–	
Diabetes mellitus	8 (36.4%)	–	
Abnormal OGTT	4 (18.2%)	–	
Mean liver density	35.03 ± 18.41	54.21 ± 2.50	< 0.001
Liver/spleen density ratio	0.702 ± 0.059	1.208 ± 0.082	< 0.001

Results are expressed as mean ± SD.

Abbreviations

NASH : Non-alcoholic stetaohepatitis ; **AST :** Aspartate amino transferase ; **ALT :** Alanine aminotransferase ; **ALP :** Alkaline phostatase ; **GGT :** Gamma glutamyl transferase ; **HDL :** High-density lipoprotein ; **LDL :** Low-density lipoprotein ; BMI : Body mass index ; **OGTT :** oral glucose tolerance test.

Table 2. — **The correlation between computed tomography and ultrasonography findings with histopathologic grade and stage in patients with nonalcoholic steatohepatitis**

	Histopathologic grade		Histopathologic stage	
	r :	*P* :	r :	*P* :
Liver Densities in CT	-0.716	<0.001	-0.416	>0.05
Liver/spleen density ratios	-0.663	<0.001	-0.356	>0.05
Sonographic grade	0.134	>0.05	0.130	>0.05

Distribution of the patients according to stage : four patients (18%) had stage 1, 14 (64%) had stage 2, and four (18%) had stage 3 fibrosis. Cirrhosis was not found in any of the patient.

In this study, ballooning degeneration was necessary to establish a diagnosis of NASH, and all patients had this. Mallory bodies were found in 11 (50%) patients.

Computed tomography and ultrasonography findings

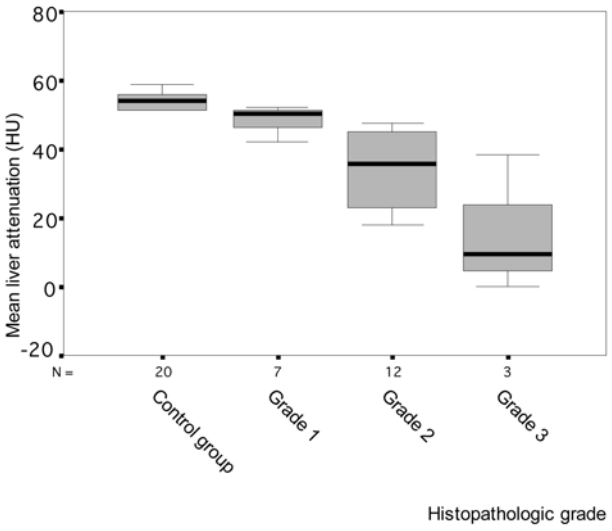
Mean liver densities of NASH patients were lower than that of controls ($P < 0.05$) and liver/spleen density ratios were lower than that of controls ($P < 0.05$).

A significant linear correlation between CT densities of liver and liver/spleen densities ratio with histopathologic grade were found (r : -0.716, $P < 0.001$; r : -0,663, P : 0.001). But there was no significant correlation between CT densities of liver and liver/spleen densities ratio with histopathologic stage (r : -0.416, P : 0.054 ; r :-0.356, P : 0.103). The sonographic grade of liver was not correlate with histopathologic grade and stage (r : 0.134, P : 0.553 ; r : 0.130, P :0.564). The correlation between computed tomography and ultrasonography findings with histopathologic grade and stage are shown in Table 2, Figure 1 and Figure 2.

Discussion

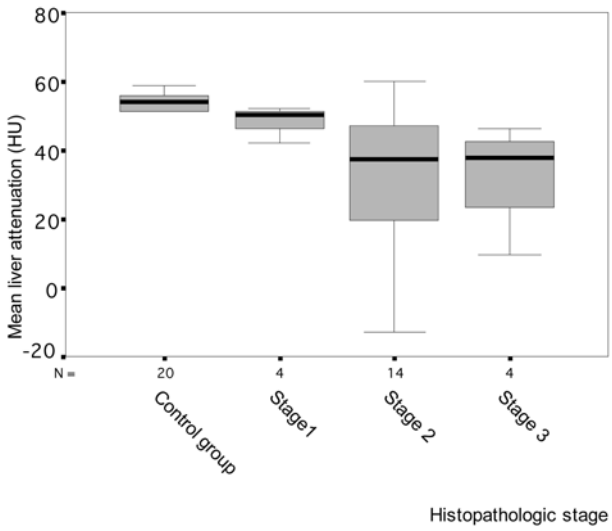
NAFLD is increasingly being recognized as an important and common condition, affecting approximately 20% of the general population (14). NASH, along with other forms of NAFLD, is a chronic liver disease that is attracting increasing significance (15). The diagnosis of NASH can be made with certainty only by examination of liver histology (1). The role of liver biopsy in diagnosing NASH thus remains controversial (16). Arguments can be made for and against the performance of liver biopsy. Most clinicians do not favour liver biopsy in routine practice. Nevertheless histological data is an essential component of clinical trials, and thus biopsy is often performed in tertiary centers with research interest in NAFLD (1). There are no accurate non-invasive methods for the diagnosis of NASH (6).

Non-invasive imaging studies are useful in determining the presence and amount of fatty infiltration of the liver. US is the most commonly used modality for the diagnosis of fatty liver (3). According to our results liver echogenicity is increased in all patients with NASH, but sonographic grade of liver disease is not correlated with histological grade and stage (r : 0.134, P : 0.553 ; r : 0.130, P : 0.564). Joseph *et al.* (17) reported sensitivity and specificity of US in 50 patients as 89% and 93% for



Abbreviations
HU : Hounsfield Unit

Figure 1. — The correlation between mean liver attenuation obtained by computed tomography with the histopathological grade.



Abbreviations
HU : Hounsfield Unit

Figure 2. — The correlation between mean liver attenuation obtained by computed tomography with the histopathological stage.

steatosis and 77% and 89% for fibrosis. In another study comparing liver biopsies and US findings in 165 patients, US had a sensitivity of 87.5% for detection of moderate steatosis. However, US was not considered as an appropriate method for detection of fibrosis (18). Population of the both studies was consisted of liver diseases with various etiologic causes including alcoholic, viral hepatitis B and C. There were only three cases (%1.8) of NASH in the latter study and none in the study of Joseph *et al.* In addition, only presence of steatosis in US and histopathologic findings was compared in both studies. However, in the present study, not only presence but also grade of steatosis in US and histpathological grade and stage compared (17,18).

Recently, using both 10-MHz as well as 3.5 MHz transducers, frequency-dependent attenuation of an ultrasound beam passed through the liver was shown to correlate well with its fat content (19). In the present study, absence of correlation between sonographic grade and histopathologic grade of liver may be explained by the histopathological grade definition composed not only of steatosis, but also of necroinflammatory activity of the liver. In studies in much more diseased populations with a high representation of alcoholic liver disease with advanced fibrosis, it is possible with ultrasound examination to detect fibrosis as an isolated finding or together with steatosis. The hyperechogenicity resulting from fibrosis is coarse and gives a dyshomogeneous brightness (17,18). In the present study the ratio of patients with advanced fibrosis was low (four patients had stage 3 and none of them stage 4) and it may cause of the absence of correlation with US grade and histological stage.

In the present study densities of liver determined with CT were significantly and inversely correlated with histopathologic grade ($r : -0.716$, $p < 0.001$), which indicates the activity of the steatohepatic lesion.

The normal CT attenuation values for the liver range from 50 to 75 Hounsfield units when a non-contrast enhanced scan is obtained. With increasing hepatic steatosis, the liver attenuation values decrease by about 1.6 Hounsfield units for every milligram of triglyceride deposited per gram of liver tissue (20). We chose not to administer intravenous contrast due to its associated morbidity and CT contrast has been shown to obscure the detection of fat (21). In most of these trials patients are selected mainly from steatosis alone, alcoholic hepatitis and steatosis associated conditions (22-26).

There was no significant correlation between CT attenuation of liver and histopathologic stage. ($R : -0.416$, $P : 0.054$). So CT attenuation does not estimate the stage of NASH ; however *P* value was at the margin of significance.

There are few reports that were using CT in NASH. Ricci *et al.* (12) and Cheng *et al.* (27) quantified the fat content in human and rats by calibrated CT. In these studies the test object that mimicking different degrees of fat content were used and, hepatic fat content were estimated by CT. Different techniques that can quantify the fatty infiltration of liver were reported (23,28). But they did not investigate the relation of CT with histopathologic stage.

Saadeh *et al.* (9) studied to evaluate the role of radiological modalities (US, CT, and MR) in establishing the diagnosis of NASH. According to this study only the severity of steatosis was reflected by these radiological

modalities. They used different grading and staging systems for histologic lesion. Brunt *et al.* (10) proposed a grading and staging system for NASH. To our best knowledge, this is the first study that using Brunt's scoring system for evaluating the CT.

In the present study liver/spleen density ratios like liver attenuation in CT were significantly and inversely correlated with histopathologic grade but not with stage (r : -0.663, *P* : 0.001 ; r :-0.356, *P* : 0.103). Liver/spleen density ratio has been reported to be correlated with the degree of fatty infiltration (29,30), and our results confirm this. Accordingly, it is a useful parameter for quantifying steatosis of liver but it is not true for histopathologic stage.

The absence of a second control group with NAFLD without NASH is limitation of our study. Perhaps it may due to selection criteria of patients for liver biopsy. The presence of fat in the liver can be diagnosed in many cases using various imaging modalities. The present study was primarily aimed to determine the relationship between US, CT and histopathologic severity. Necroinflammation and fibrosis besides steatosis are the main factors that effect the progression of the disease. Brunt's classification including steatosis and necroinflammation for grade evaluation initially may represent confounding variable, but it gives us histopathologic severity that clinically important.

The most common reported risk factors associated with NASH include obesity, type 2 diabetes mellitus, and hyperlipidemia (1,31). In the present study the percentages of obesity (BMI $\geq$ 30), hyperlipidemia, type 2 diabetes mellitus, and impaired glucose tolerance are 31.8%, 77.3%, 36.4%, 18.2%, respectively.

In conclusion, our results suggest that the CT attenuation of liver is strongly correlated with histopathologic grade in patients with NASH. Neither US nor CT can reflect the histopathologic stage.

References

1. Yu AS, Keeffe EB. Nonalcoholic fatty liver disease. Rev Gastroenterol Disord 2002 ; 2 : 11-9.
2. Feldstein AE, Canbay A, Angulo P *et al.* Hepatocyte apoptosis and fas expression are prominent features of human nonalcoholic steatohepatitis. Gastroenterology 2003 ; 125 : 437-43.
3. Alba LM, Lindor K. Review article : non-alcoholic fatty liver disease. Aliment Pharmacol Ther 2003 ; 17 : 977-86.
4. Henrion J. Surveillance for hepatocellular carcinoma... A Hotly debated issue ? Acta Gastroenterol Belg 2004 ; 67 : 255-264.
5. Neuschwander-Tetri BA. Fatty liver and nonalcoholic steatohepatitis. Clin Cornerstone 2001 ; 3 : 47-57.
6. Sanyal AJ. AGA technical review on nonalcoholic fatty liver disease. Gastroenterology 2002 ; 123 : 1705-25.
7. Diehl AM. Nonalcoholic steatohepatitis. Semin Liver Dis 1999 ; 19 (2) : 221-9.
8. Angulo P. Nonalcoholic fatty liver disease. N Engl J Med 2002 ; 346 (16) : 1221-31.
9. Saadeh S, Younossi ZM, Remer EM *et al.* The utility of radiological imaging in nonalcoholic fatty liver disease. Gastroenterology 2002 ; 123 : 745-750.
10. Brunt EM, Janney CG, Di Bisceglie AM, Neuschwander-Tetri BA, Bacon BR. Nonalcoholic steatohepatis : a proposal for grading and staging the histological lesions. Am J Gastroenterol 1999 ; 94 (9) : 2467-74.
11. Yajima Y, Ohta K, Narui T, Abe R, Suzuki H, Ohtsuki M. Ultrasonographical diagnosis of fatty liver : significance of the liver kidney contrast. Tohoku J Exp Med 1983 ; 139 : 43-50.
12. Ricci C, Longo R, Gioulis E *et al.* Noninvasive in vivo quantitative assessment of fat content in human liver. J Hepatol 1997 ; 27 : 108-13.
13. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 1997 ; 20 : 1183-97.
14. Joy D, Thava VR, Scott BB. Diagnosis of faty liver disease : is biopsy necessary ? Eur J Gastroenterol Hepatol 2003 ; 15 : 539-43.
15. Falck-Ytter Y, McCullough AJ, Younossi ZM, Marchesini G. Clinical features and natural history of nonalcoholic steatosis syndrome. Semin Liver Disease 2001 ; 21 : 17-26.
16. Sorbi D, McGill DB, Thistle JL *et al.* An assessment of the role of liver biopsies in asymptomatic patients with chronic liver test abnormalities [abstract]. Hepatology, 1999 ; 30 : 477A.
17. Joseph AEA, Saverymuttu SH, Al-Sam S, Cook MG, Maxwell JD. Comparision of liver histology with ultrasonography in assessing diffuse parenchymal liver disease. Clin Radiol 1991 ; 43 : 26-31.
18. Mathiesen UL, Franzén LE, İselius H *et al.* Increased liver echogenecity at ultrasound examination reflets degree of steatosis but not of fibrosis in asyptomatic patients with mild/moderate abnormalities of liver transaminases. Digest Liver Dis 2002 ; 34 : 516-22.
19. Fusamoto H, Suzuki K, Hayashi N *et al.* Obesity and liver disease : evaluation of fatty infiltration of the liver using ultrasonic attenuation. Nutr Sci Vitaminol 1991 ; 37 (Suppl) : S71-7.
20. Piekarski J, Goldberg HI, Royal SA, Axel L, Moss AA. Difference between liver and spleen CT numbers in the normal adult : its usefulness in predicting the presence of diffuse liver disease. Radiology 1980 ; 137 : 727-9.
21. Jacobs JE, Birnbaum BA, Shapiro MA *et al.* Diagnostic criteria for fatty infiltration of the liver on contrast-enhanced helical CT. AJR Am J Roentgenol. 1998 ; 171 : 659-64.
22. Bydder GM, Chapman RW, Harry D, Bassan L, Sherlock S, Kreel L. Computed tomography attenuation values in fatty liver. J Comput Tomogr 1981 ; 5 : 33-5.
23. Limanond P, Raman SS, Lassman C *et al.* Macrovesicular hepatic steatosis in living related liver donors : correlation between CT and histologic findings. Radiology 2004 ; 230 : 276-80.
24. Rockall AG, Sohaib SA, Evans D *et al.* Hepatic steatosis in Cushing's syndrome : a radiological assesment using computed tomography. Eur J Endocrinol 2003 ; 149 : 543-8.
25. Jiang J, Yang C, Fu K. Clinical characters and CT findings of steatohepatitis in highland area. Zhonghua Gan Zang Bing Za Zhi 2003 ; 11 : 84-5.
26. Wang B, Gao Z, Zou Q, Li L. Quantitative diagnosis of fatty liver with dual-energy CT. An experimental study in rabbits. Acta Radiol 2003 ; 44 : 92-7.
27. Cheng YF, Chen CL, Lai CY *et al.* Assesment of donor fatty livers for liver transplantation. Transplantation 2001 ; 71 : 1221-5.
28. Mendler MH, Bouillet P, Sidaner AL *et al.* Dual-energy CT in the diagnosis and quantification of fatty liver : limited clinical value in comparison to ultrasound scan and single-energy CT, with special reference to iron overload. J Hepatol 1998 ; 28 : 785-94.
29. Longo R, Pollesello P, Ricci C *et al.* Use of 1 H MRS in the quantitative in vivo determination of the fat content in human liver steatosis. JMRI 1995 ; 5 : 281-5.
30. Longo R, Ricci C, Masutti F, Vidimari R, Croce LS, Bercich L *et al.* Fatty infiltration of liver : quantification by 1H localized MR spectroscopy and comparision with CT. Invest Radiol 1993 ; 28 : 297-302.
31. James OFW NASH/NAFLD management. Acta gastroenterol belg 2002 ; 65 : 200-203.