ASSESSMENT OF CARDIOVASCULAR RISK IN PATIENTS WITH TYPE 2 DIABETES MELLITUS AND NONALCOHOLIC STEATOHEPATITIS

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Abstract

The nonalcoholic steatohepatitis is the leading cause of hepatic morbidity and mortality, the patients being at very high risk of developing cirrhosis, liver failure and hepatocellular carcinoma. Its growing incidence and prevalence in patients with diabetes mellitus and metabolic syndrome indicates that these entities have at least one common pathogenic mechanism, where the main factors are visceral obesity and insulin resistance. Purpose: The purpose of this study was to explore the prevalence of nonalcoholic steatohepatitis in a group of patients with type 2 DM from the Timis County, as well as NASH relationships with other cardiovascular risk factors, such as obesity, dyslipidemia and arterial hypertension.

Material and method: The study included 310 patients with type 2 DM, 123 men (39.7%) and 187 women (60.3%) with mean age 59.6 ± 8.1 (39-79) years, treated within the Antidiabetes Center Timisoara between March-December 2012.

Results and discussions: The nonalcoholic steatohepatitis was diagnosed in 208 patients (71.5%), 90 men (78.9%) and 118 women (66.6%), its prevalence being higher in males. Analyzing the metabolic syndrome components, we found that all examined parameters were statistically significantly higher in patients with nonalcoholic steatohepatitis, showing an extremely high cardiovascular risk in this category of patients.

Conclusions: The therapeutic strategy in nonalcoholic steatohepatitis involves the decrease of visceral fat and the improvement of insulin resistance.

Keywords: Type 2 diabetes mellitus, Insulin resistance, Nonalcoholic steatohepatitis, Cardiovascular risk, Metabolic syndrome

Introduction

We are witnessing an epidemic of metabolic diseases, as demonstrated by the alarming increase in the number of cases with diabetes mellitus (DM).

At the end of 2012, over 371 million people with DM were recorded, of which 50% undiagnosed. In Europe, their number reached 55 million, of which 38.6% undiagnosed.

Type 2 DM is accompanied by a two-three times increase of the risk of cardiovascular disease (CVD), which is considered equivalent to a CVD. In fact, 50% of deaths in patients with DM are due to CVD and only in 15% of cases they are caused by its direct complications.

This is due to a presence of several cardiometabolic risk factors (CMR), such as: ▪ disorders of lipid metabolism, manifested by the increase of the small and dense particles of LDLc, serum TG, Apo B, and the decrease of HDLc, ▪ hypercoagulability, ▪ arterial hypertension (AHT), ▪ insulin resistance (IR) and ▪ inflammation.

The association with the nonalcoholic steatohepatitis (NASH) enhances this risk by increasing the release of proinflammatory cytokines and decreasing the adiponectin secretion.

NASH covers a wide spectrum of liver damage, from isolated macrovesicular steatosis to steatohepatitis associated by macrovesicular steatosis, lobular and perisinusiodal necroinflammmatory lesions, hepatocitary inflammation and Mallory bodies, to advanced fibrosis and cirrhosis, in the absence of a relevant alcohol consumption (<20 g/day). NASH is the leading cause of hepatic morbidity and mortality, the patients with NASH being in a very high risk to develop cirrhosis, liver failure and hepatocellular carcinoma (Brunt, 1999, Chitturi, 2001).

It is estimated that the prevalence of NASH in developed countries in Western Europe and the USA covers 20 to 30% of the population.

Although the exact cause is not fully known, the incidence and prevalence of NASH in patients with DM and metabolic syndrome (MS), shows that these entities have at least one common pathogenic mechanism, where visceral obesity and IR play the main role (Hsiao, 2007, Marchesini, 2002).

Type 2 DM is present in 21-45% of patients with NASH, and NASH is present in 49-86% of patients with type 2 DM. After Bugianesi E et al, NASH is associated with obesity in 60-95% of cases; with type 2 DM in 28-55% of cases and with dyslipidemia in 27-92% of

cases (Bugianesi, 2005). NASH is considered a hepatic manifestation of MS, reflected by the fact that 90% of patients with NASH meet more than one criterion for the diagnosis of MS, and 33% have three or more diagnostic criteria (Amarapurkar, 2004). Also, each additional criterion of MS enhances exponentially the risk of NASH.

Hyperinsulinemia, caused by the elevated insulin secretion by the pancreatic beta cells and reduced insulin degradation by the liver, is a compensatory phenomenon of IR. Hyperinsulinemia leads to increased fat mass and lipogenesis, being associated with increased concentration of free fatty acids (FFA) (Marceau, 1999, Marchesini, 2003).

Adipocytes represent an active endocrine organ that produces and secretes a number of cytokines (adipokines) that help regulate the metabolic processes. The most important proinflammatory cytokines secreted by adipocytes are tumor necrosis factor (TNF-alpha), resistin, leptin and plasminogen activator inhibitor (PAI), which contribute to the alterations leading to IR (Bray, 1992).

The concentration of adiponectin, produced exclusively by adipocytes, is low in patients with IR, type 2 DM and NASH. It is proven that adiponectin reduces IR by increasing FFA oxidation, decreasing triglycerides (TG) in the liver and muscle, and by inhibiting the liver glucose production.

NASH results from the TG accumulation in hepatocytes. FFA derived from food intake and released from the fat stores, become esterified into TG with the help of diacylglycerol acyltransferase enzyme (DGAT), the enzyme that catalyzes the final step of TG synthesis in the liver. Finally, TG are released from the liver as VLDL.

Leptin may play the role of an antisteatosis hormone, preventing the accumulation of FFA and fats in non-adipose tissues. In addition, leptin appears to be essential for the development of hepatic fibrosis, in response to chronic liver alterations induced by the transforming growth factor beta 1.

The oxidative stress is recognized as a mechanism responsible for the progression of liver damage. Oxidative stress includes oxygen free radicals and lipid peroxidation (Cortez-Pinto, 2001). The mitochondrial β-oxidation processes of FFA are deeply altered in patients with NASH, the consequence of this imbalance being the formation of oxygen free radicals, which are responsible for the increased synthesis of TNF-alpha, inducing lesions of necrosis, inflammation and hepatocyte fibrosis (Munteanu, 2008, Pessayre, 2001).

The increased TNF-alpha liver expression in NASH represents the connection between the development of IR and the hepatic steatosis. TNF-alpha holds a significant

mechanism in the development of peripheral IR in patients with obesity and type 2 DM (Koruk, 2004, Yadav, 2002).

Main Text
Objectives and aims

Starting from these premises, the principal aim of our study was to explore the prevalence of nonalcoholic steatohepatitis in a group of patients with type 2 DM treated within the Center of Diabetes from Timisoara, as well as NASH relationships with other cardiovascular risk factors (CVR): obesity, dyslipidemia, hypertension (AHT).

Materials and methods

The study included 310 patients with type 2 DM, 123 men (39.7%) and 187 women (60.3%) with mean age 59.6 ± 8.1 (39-79) years, treated within the Antidiabetes Center Timisoara between March-December 2012.

Data on alcohol consumption or use of medicines that might cause hepatic lesions were obtained by direct questioning of the patient, as well as confirmation of their family members.

Following parameters were assessed; sex, age, waist circumference (WC) as well as data concerning AHT presence. Body mass index (BMI) was calculated using the formula: body weight (kg) /height (m^2); the patients with BMI >30 kg/m^2 were considered obese.

Total cholesterol (TC), serum triglycerides (TG) and liver transaminases: ASAT and ALAT, viral markers, anti-hepatitis B antigen and anti-hepatitis C antibodies were measured in all patients.

Glycemic control was assessed by measuring HbA1c (by the latex-imunoagglutination inhibition assay, with the DCA analyzer and with the result displayed within 6-8 minutes).

The hepatic steatosis was measured by abdominal ultrasonography and classified as mild, moderate and severe. The fibrosis degree was established using the FibroScan, a device measuring the elasticity of the liver tissue by a mechanical wave that propagates through the liver, its speed being monitored by an ultrasound beam. This method has the advantage of being completely non-invasive, objective (minimum interobserver variability), reproducible and fast.

The result of the examination is actually the average of 10 successive assays validated by the device. On such basis, the degree of fibrosis is assessed from 0 to 4. Normal values must fall between 5.5 ±1.6 kPa. Age does not influence the values, but the values are higher in men (5.8 ± 1.5 kPa) than in women (5.2 ± 1.6 kPa). In obese individuals, the normal values are 6.3 ± 1.9 kPa for men and 5.4 ± 1,5 kPa for women.

The approximate correspondence between the degree of hepatic fibrosis and the results of FibroScan is as follows: ● F1< 7 kPa – can be mixed up with normal liver, ● F2>7.1 kPa, ●F3 > 9.5 kPa, ● F4 (cirrhosis) > 12,5 kPa. The predictive value is about 80% for fibrosis ≥ F2, 90% for fibrosis ≥ F3 and 95-97% for fibrosis = F4. In principle, the result is correct in approximately 95% of the tested patients, and the correlation between the FibroScan outcome and liver biopsy is about 90%.

The characteristics and biological variables were expressed by mean value ± standard deviation (SD).

Results

Of the 310 patients included in the study, hepatitis B virus was detected in 10 patients (3.2%); hepatitis C virus in 9 patients (2.9%) and one of the patients was infected with both viruses. After we excluded them, we analyzed the study group to ascertain NASH prevalence, as well as its relationship with other CVR factors.

Based on abdominal ultrasonography, the 291 investigated patients with type 2 DM were classified as follows: 83 patients (28.5%) without steatosis (S0), 61 patients (21%) with minimal or mild steatosis (S1), 79 patients (27.1 %) with moderate steatosis (S2) and 68 patients (23.4%) with severe steatosis. (S3), (Figure 1).
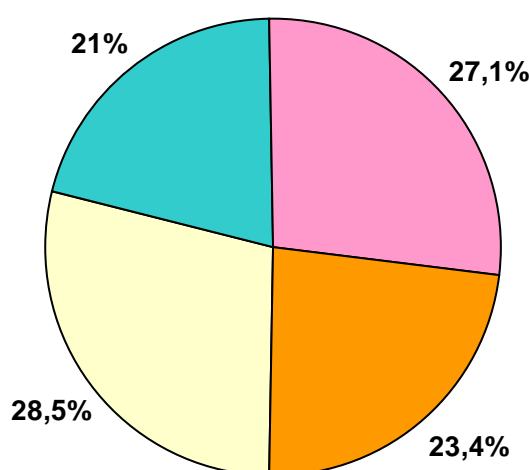

Figure 1. *Distribution of the group after the degree of steatosis*

NASH prevalence in the study group was 71.5%, being higher in men in women: 78.9%, respectively 66.6% (Figure 2).
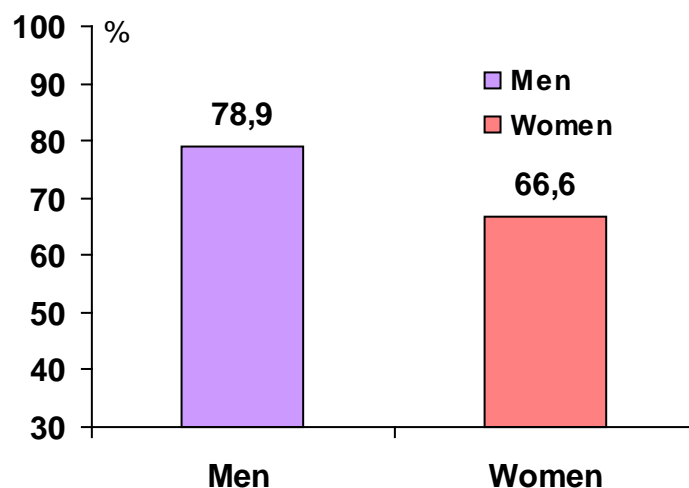
Figure 2. NASH prevalence, by gender

The main characteristics of the two patient groups (with NASH and without NASH) are shown in Table 1.

Table 1. Comparison between the two groups of patients, with and without NASH

Parameter	with NASH	without NASH	
No. (%)	208 (71.5%)	83 (28.5%)	
	Average ± SD	Average ± SD	p
Age (years)	60.3 ± 8.9	58.9 ± 6.8	0.19 (NS)
BMI (kg/m^2)	31.2 ± 5.4	28,4 ± 5	<0.0001 (ES)
WC (cm) men	109 ± 16.6	101.4 ± 15.8	<0.0001 (ES)
WC (cm) women	94.3 ± 11.5	84.5 ± 9.6	<0.0001 (ES)
DM (years)	9 ± 5.3	7 ± 4.6	0.0028 (FS)
HbA1c (%)	7.8 ± 1.8	7.3 ± 1.6	0.028 (S)
TC (mg/dl)	201.1 ± 41.2	178.6 ± 28.8	<0.0001 (ES)
TG (mg/dl)	209.3 ± 113.5	161.9 ± 75.9	0.0005 (ES)
HDLc (mg/dl)	39.4 ± 1.8	44.4 ± 2.1	<0.0001 (ES)
LDLc (mg/dl)	118 ± 39	100 ± 36	<0.0001 (ES)
ASAT (U/L)	30.8 ± 14.2	29.4 ± 25.8	0.555 (NS)
ALAT (U/L)	39.3 ± 26.9	31.9 ± 18.3	0.022 (S)
AHT no. (%) *	148 (71.1%)	38 (45.8%)	0.083 (NS)
SBP (mmHg)	159.8 ± 44.1	136.6 ± 32.8	<0.0001 (ES)

* Values are expressed as number and percentage

Statistically significant differences were noticed in all examined parameters, except age and ASAT value (Table 1).

Using the FibroScan we measured the degree of fibrosis in the patients included in the study, and they were classified as follows: 86 patients (29.6%) without fibrosis (F0); 118 patients (40.5%) with minimal or mild fibrosis (F1); 44 patients (15.1%) with moderate fibrosis (F2); 25 patients (8.6%) with severe fibrosis (F3) and 18 patients (6.2%) with cirrhosis (F4) (Figure 3).
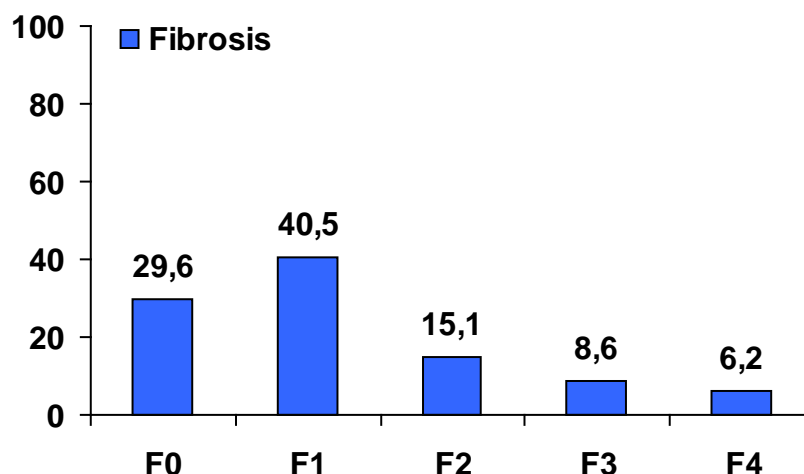
Figure 3. Distribution of study group, according to the degrees of fibrosis obtained with the FibroScan

Although there are slightly elevated liver transaminase levels, they may be normal even in the presence of severe liver damage. Note that elevated ALAT and ASAT were detected only in 39 patients (18.7%) of the ones with significant steatosis tested by abdominal ultrasound.

We analyzed the relationship between the mean values of ASAT and ALAT, and the degrees of steatosis and fibrosis within the study group (Table 2).

Table 2. Mean ASAT and ALAT values, depending on the degrees of steatosis and fibrosis at the study group.

Parameter	Steatosis				Fibrosis				
	0	1	2	3	0	1	2	3	4
ASAT (U/L)	24.5 ± 21.4	29.4 ± 24.2	32.6 ± 26.8	33.9 ± 28.4	24.6 ± 18.9	27.6 ± 20.8	30.2 ± 21.3	34.3 ± 22.6	33.8 ± 22.4
ALAT (U/L)	34.9 ± 18.3	35 ± 21.3	35.7 ± 24.1	36.8 ± 26.4	32.7 ± 19.7	34.8 ± 20.8	35 ± 21.1	36.8 ± 23.4	38.9 ± 24.6

We noticed that ASAT and ALAT values increase directly proportional to the increase of the degree of hepatic steatosis and fibrosis, but without statistically significant differences.

Analyzing the weight status of the patients with NASH, we found that 192 patients (92.3%) were overweight or obese (Figure 4).
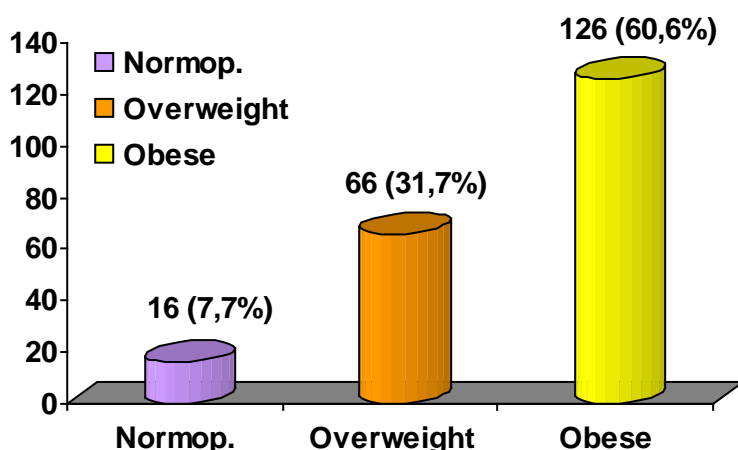
Figure 4. Weight status of the patients with NASH

AHT was found in 149 patients (71.6%) with NASH, with a statistically significant difference between the mean value of SAT in patients with NASH, as compared to those without NASH: 159.8 mmHg versus 136.6 mmHg (<0.0001 (ES). In patients with type 2 DM and NASH, the prevalence of dyslipidemia was 80.3%.

Given that all patients with NASH were diabetic and 92.3% of them were overweight or obese, the MS criteria were met in all 208 patients with NASH: 54 patients (26%) met 3 diagnostic criteria of MS; 84 patients (40.4%) 4 criteria and 70 patients (33.6%) 5 diagnosis criteria (Figure 5).
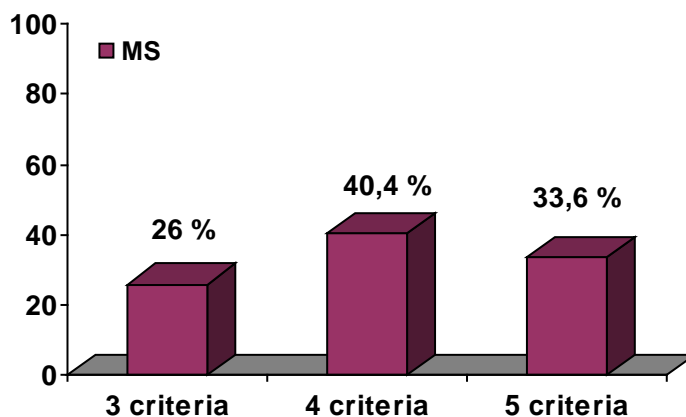

Figure 5. MS, depending on the number of diagnostic criteria in the study group

The main cause of death in patients with type 2 DM is CVD. The ADVANCE risk engine is a CVR calculator developed for people with DM. It estimates the probability of a major cardiovascular event in 4 years, including CV death, non-fatal myocardial infarction and non-fatal cerebral vascular accident (stroke).

To assess CVR, the ADVANCE risk engine records the following 10 factors: age at diagnosis, duration of DM, sex, arterial pressure (blood pressure), the presence of diabetic retinopathy and arterial fibrillation, urine albumin/creatinine ratio, non-HDLc, the existence of antihypertensive treatment and the HbA1c value.
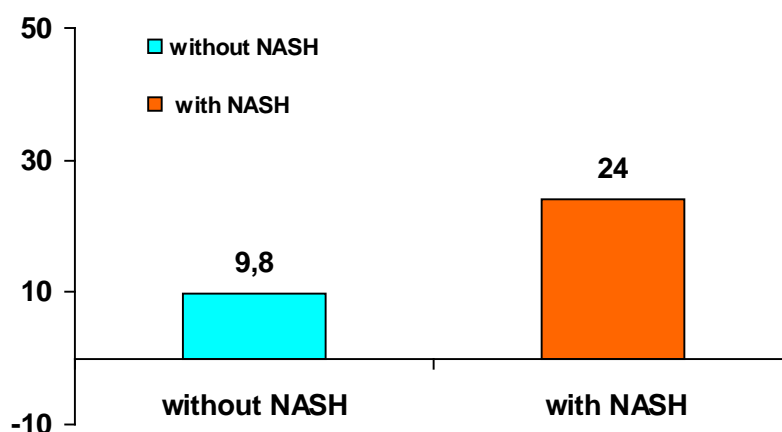

Figure 6. CVR in patients with type 2 DM and NASH, versus those without NASH, based on the ADVANCE risk engine

Based on the ADVANCE risk engine, we ascertained within the study group that the CVR in patients with type 2 DM and NASH is 2.5 higher than in those with type 2 DM without NASH: 24%, versus 9.8%. (Figure 6).

Discussions

NASH is nowadays considered as the hepatic manifestation of MS, caused by the same etiopathogenic mechanisms, namely IR, abdominal (visceral) obesity, high calorie/high fat foods and a sedentary lifestyle. NASH is considered to be an early manifestation form of IR and is predictive for the subsequent development of the other MS component parts.

Recent studies have shown that NASH is now the most important chronic liver disease leading to cirrhosis, hepatocellular carcinoma, and finally death. It seems that 20% of the patients with NASH develop cirrhosis, and 30-40% of those with cirrhosis require a liver transplant or die due to very severe complications (Pascale, 2010).

Chronic hyperinsulinemia causes the accumulation of TG in hepatocytes and, by definition, the accumulation of TG in hepatocytes is needed for the development of NASH.

The peripheral IR and the compensatory hyperinsulinemia lead to accumulation of fat in the liver. Normally, the insulin levels increase postprandial, stimulating glucose uptake in muscle and fat. The increase of postprandial insulinemia stops the release of FFA from the adipocytes (fat cells) and reduces the glucose produced by hepatocytes (Paloma, 2009).

Dyslipidemia, especially hypertriglyceridemia, stimulates the increase of VLDL-c and the decrease of HDL-c, accelerating the processes of atherosclerosis in patients with type 2 DM.

Several studies showed that NASH is 2-3 times more frequent in obese patients and in patients with type 2 DM, being present in almost all cases where these two diseases were associated.

Some recent data indicate a significant rise of NASH prevalence in children: from 2.6% a decade ago, to 5% at present in children with a normal weight; to 38% in the ones with obesity, and to 48 % in those with DM.

Ong et al. showed that the most common causes of death recorded in patients with NASH are due to cardiovascular and neoplastic diseases. For this reason, NASH is currently considered as the hepatic manifestation of MS, being an independent predictor of CV morbidity and mortality (Ong, 2008). Studies like Hoorn Study, the Framingham Heart Study and Valpolicella Heart Diabetes Study showed that there was a strong association between elevated serum transaminases and increased CVR.

Studies like the Hoorn Study, the Framingham Heart Study and Valpolicella Heart Diabetes Study showed the existence of a strong association between elevated serum transaminases and increased CVR (Goessling, 2008, Schindhelm, 2007, Targher, 2006).

Nowadays, NASH prevalence in patients with DM is estimated between 35-75%, even to 100% in patients with DM and obesity. NASH prevalence recorded in our study group reached 71.5%, being higher in men than in women.

At the FibroScan examination, almost 30% of the patients were diagnosed with fibrotic lesions, and 18 patients (6.2%) even showed cirrhotic lesions (F4 > 12.5 kPa).

MS elements were present in almost all patients included in the study: all had type 2 DM; 92.3% of them were overweight or obese; 71.6% were hypertensive and 80.3% were diagnosed with dyslipidemia.

Gianfranco Pagano et al. aimed to ascertain the presence of MS components in patients with NASH, as well as the correlation between liver lesion and peripheral hyperinsulinemia. Compared with the control group, the group with NASH recorded a higher value of insulin secretion and lower insulin sensitivity (Pagano, 2002). In addition, 9 patients (47%) showed at least 2 MS diagnostic criteria, according to the European Group for the Study of Insulin Resistance (EGIR) and World Health Organization (WHO) (Alberti, 1998, Balkau, 1999).

A study conducted in Taiwan included 16,486 patients and focused on establishing the correlation between the gravity of hepatic lesions and the presence of IR and MS. The patients were divided into 3 groups: without hepatic steatosis; with moderate steatosis and with severe steatosis. Prevalence of obesity, AHT, glycoregulation disorders and hypertriglyceridemia were statistically significant higher in the group of patients with severe steatosis. Also, BMI ≥ 30 kg/m^2, seems to be the most important factor in the progression from moderate to severe hepatic steatosis, in both sexes. The study shows that patients with severe hepatic lesions have an increased CVR and that they must be examined periodically to detect metabolic disorders. NASH is recognized as the most common liver disease in the U.S., with a prevalence of 10-24%; similar data were recorded in Europe and Japan. Many studies showed the direct correlation between NASH and MS components, as WC, serum TG and HDLc. Peripheral IR and compensatory hyperinsulinemia interfere with homeostatic mechanisms that lead to fat accumulation in the liver.

In conclusion, the central role of IR in the pathogenesis of NASH suggests that direct intervention in improving insulin sensitivity may be beneficial in slowing the progression or the reversal of this disease.

Conclusion

To prevent CVR, the therapeutic strategy in NASH has two goals: to reduce visceral fat and to improve IR. The only effective method is the lifestyle change (to reduce excess weight by 5-10% with a low fat diet and an appropriate and individualized physical exercise).

In the case of persons with DM, achieving and maintaining optimal glycemic control is the best way to prevent NASH.

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