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Article

NON-ALCOHOLIC FATTY LIVER DISEASE: RELATION WITH THE CARDIOVASCULAR RISK

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ABSTRACT

The purpose of this research study is to assess the presence of metabolic cardiovascular risk factors (lipid dismetabolismul, diabetes mellitus type 2), anthropometric (obesity) and smoking in subjects with and without steatosis (non-viral and non-alcoholic) and the influence of steatosis on cardiovascular risk. Subjects were examined by abdominal ultrasonography under á jeun conditions of at least 5 hours. Abdominal ultrasounding is the most commonly used method for detecting steatosis, is widespread and reproducible. Non-alcoholic fatty liver viewed on ultrasounds shows a bright echostructure (comparable to the kidneys) with a posterior attenuation of the signal and a blurred vascular hepatic drawing. In the light of these recommendations, assessment of non-HDL cholesterol levels contributes to a more accurate stratification of cardiovascular risk than the calculation of LDL cholesterol. The target level of non-HDL-cholesterol is with 30 mg/dL higherr than the corresponding target level of LDL - cholesterol.

Keywords: cardiovascula risk, liver dissease

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1. INTRODUCTION

Non-alcoholic hepatic steatosis (NASH) or non-alcoholic fatty liver (non alcoholic fatty liver disease - NAFLD) is an advanced stage of non-alcoholic fatty liver, more specifically, to extra fat is added an inflammatory component, which increases the risk of cardiovascular disease and of cardiovascular and overall mortality [1].

In the last years, the disease has reached epidemic proportions and it is considered the most common cause of chronic liver disease in Western countries. Approximately 20-30% of the adults in western countries suffer from non-alcoholic steato-hepatitis. The prevalence increases to 70-90% among people who are obese or have diabetes [2]. Recently, a growth of healthcare costs for patients with NAFLD by 26% was estimated and this does not exclude the possibility that it will become the leading cause of liver transplant in 2020, without considering the complications associated with NAFLD related to diabetes and the cardiovascular disease.

Close correlations between non-alcoholic fatty liver, abdominal obesity and insulin resistance are making difficult an indication of the exact cause of determinant factors of cardiovascular risk in patients with NAFLD. The liver is a target to system disorders as well as it is a source of pro-atherogenic molecules that enhance arterial damage [3].

NAFLD can be regarded as a hepatic manifestation of the metabolic syndrome defined by central obesity, hypertension, hypertriglyceridemia, low HDL cholesterol, and hyperglycemia [4].

The purpose of this research study is to assess the presence of metabolic cardiovascular risk factors (lipid dismetabolismul, diabetes mellitus type 2), anthropometric (obesity) and smoking in subjects with and without steatosis (non-viral and non-alcoholic) and the influence of steatosis on cardiovascular risk.

2. METHODS

The present study included 50 hypertensive consecutive patients (34 women, 16 men) with no documented atherothrombotic cardiovascular disease, who were asking for family doctor periodic control. Blood pressure was measured according to standard recommendations, and high pressure was defined as the registered values over 140/90 mmHg or pre-existing blood pressure - lowering medication [5]. Weight status was assessed by calculating body mass index (BMI) and waist circumference measurements. Overweight and obesity were defined by a BMI $\geq$ 25 kg/m^2 and $\geq$ 30 kg/m^2 [6]. Lipid metabolic parameters (total cholesterol - TC, triglycerides - TG, high density lipoprotein - HDL) and glucose were evaluated á jeun using a Reflotron device. The level of low-density lipoprotein - LDL was calculated using Friedewald's formula (the calculation cannot be carried out for triglyceride levels over 400 mg / dL) [7] :

$$LDL\ [mg/dL] = CT\ [mg/dL] – HDL\ [mg/dL] – TG\ [mg/dL]/5$$

Non-HDL cholesterol was estimated by the difference between total cholesterol level and serum HDL-cholesterol level [8]. Patients who were currently or have attended in the last year to normolipemiant treatments were excluded. The presence of type 2 diabetes was taken from patient records by medical letters previously issued by the diabetes doctors.

For each subject we estimated the risk of fatal cardiovascular event in the next 10 years using the SCORE system, the version online [9]. To achieve the purposed goal, we hypothesized that all subjects enrolled had a measured systolic blood pressure of 140 mmHg. Subjects with SCORE risk <1% had a low absolute cardiovascular risk, the risk score between 1 and 5% (inclusive) had moderate cardiovascular risk, subjects with SCORE risk between 5 and 10% (inclusive) recorded a high cardiovascular risk, and those with a SCORE risk over 10% had very high cardiovascular risk [6].

Subjects were examined by abdominal ultrasonography under á jeun conditions of at least 5 hours. Abdominal ultrasounding is the most commonly used method for detecting steatosis, is widespread and reproducible. Non-alcoholic fatty liver viewed on ultrasounds shows a bright echostructure (comparable to the kidneys) with a posterior attenuation of the signal and a blurred vascular hepatic drawing. These characteristics have a diagnostic sensitivity to non-alcoholic liver steatosis of 82 to 94% and a specificity of more than 82%. The discrimination power of the ultrasound method is much lower than senior imaging methods (computer tomography, magnetic resonance imaging) in focal lesions of hepatic steatosis [10]. The gold standard in hepatosteatosis diagnosis is liver biopsy, the absence of this being one of the limitations of the study. Enrolled patients completed a questionnaire on lifestyle: those who reported drinking were excluded. Smokers were considered those who said they smoked at least 10 cigarettes daily. Continuous variables were expressed as mean ± standard deviation. Comparisons were performed using unpaired Student's T test. To calculate the likelihood, logistic regression was used. P values < 0.05 were considered significant. Statistical analyzes were performed using Epi Info 6 software (version 6.04d, CDC - USA , WHO Geneva - Switzerland).

3. Results and Discussions

3.1. Results

There were included 50 consecutive hypertensive patients (34 women, 16 men) with no documented atherothrombotic cardiovascular disease, who were asking for family doctor regularly control. Basic characteristics of the patient group are shown in Table 1.

Table 1: Basic characteristics in the study group BMI-body mass index, TC-total serum cholesterol levels; TG-the level of triglycerides, HDL-blood levels of high density lipoprotein; LDL - low density lipoprotein serum level

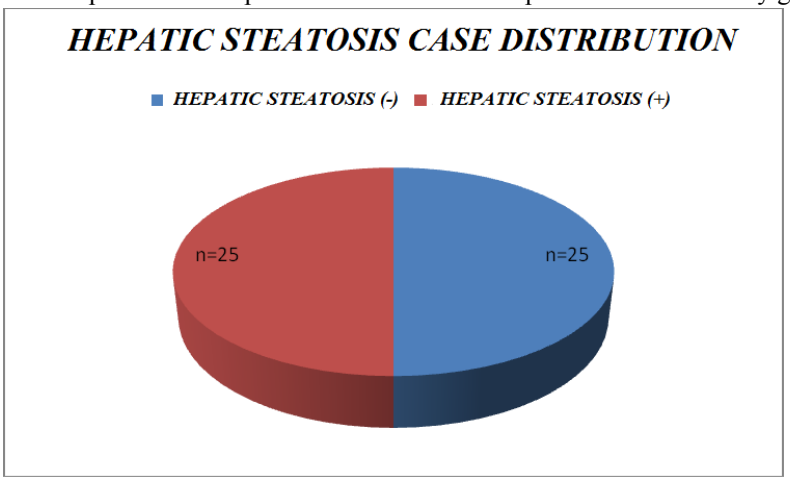
Parameter	Mean value ± standard deviation
Age, years	60,46 ± 11,79
BMI, kg/m^2	31,17 ± 6,81
TC, mg/Dl	202,54 ± 50,71
TG, mg/Dl	152,44 ± 69,48
HDL, mg/dL	47,34 ± 12,49
LDL, mg/dl	125,72 ± 47,54
Non-HDL, mg/Dl	154,85 ± 49,95
TG/HDL	3,62 ± 2,29
Blood glucose level, á jeun, mg/dL	105,28 ± 33,66

Within this group of hypertensive patients with documented atherothrombotic cardiovascular disease reported to the condition of hypertension, the presence of dislipidemic metabolism is observed with slightly ascended fractions of the non-protective lipoprotein (LDL) . Mild obesity (grade I), as defined by the body mass index, appears to be a characteristic to the sample of the patients enrolled in the study. Also carbohydrate perturbed metabolism is objectified by á jeun blood glucose levels (105.28 ± 33.66 mg/dL). Smoking is an unhealthy habit met in 42 % of the subjects.

The absolute SCORE cardiovascular risk was assessed, using the Heart Score online project [9]. To report the SCORE risk to the condition of non-alcoholic fatty liver without taking into account the variation of hemodynamic status, SCORE risk calculation was performed considering for calculation in the equation the value of 140 mmHg for systolic blood pressure. It can be noted that the sample of patients enrolled in the study are exposed to a moderate level of absolute cardiovascular risk (4.18 % ± 2.97 %).

Abdominal ultrasonography examination revealed the presence hepatosteatosis in half of the patients taken into the study (Figure 1).

Figure 1: Hepatic steatosis presence in non-alcoholic patients from the study group



Elevated serum total cholesterol levels (≥ 190 mg/dL) are not predictive for the non-alcoholic steatosis condition: OR = 2.225 (95% CI, 0.7105 to 7.227, p = NS).

Correlation between total serum cholesterol level and the diagnosis of hepatic steatosis is shown in Table 2.

Table 2: Concordance between CT levels – hepatic steatosis in the study group

	Steat hep (+)	Steat hep (-)
CT>=190	16	11
CT<190	9	14
	25	25

Serum triglyceride levels above 150 mg/dL are an important and statistically significant relationship factor for the possibility of non-alcoholic fatty liver existence: OR =

15.41 (95% CI, 3.971 to 71.98, p = 0.000015). Correlation between serum triglyceride levels and diagnosis of hepatic steatosis is shown in Table 3.

Table 3: Concordance between TG levels and hepatic steatosis in the study group

	St hep (+)	St hep (-)
TG>=150	19	4
TG<150	6	21
	25	25

Values above 100 mg/dL in serum LDL-cholesterol also cannot be placed in direct relation to the possibility of non-alcoholic hepatic steatosis: OR = 2.079 (95% CI, 0.6109 to 7.495, p = NS). Correlation between serum LDL cholesterol and hepatic steatosis diagnosis is shown in Table 4.

Table 4: Correlation between LDL levels and hepatic steatosis in the study group

	St hep (+)	St hep (-)
LDL>=100	19	15
LDl<100	6	10
	25	25

Non-HDL cholesterol is significantly related to the possibility of non-alcoholic fatty liver: OR = 2.727 (95% CI, 0.8431 to 9.326, p = 0.0047). Correlation between non-HDL-cholesterol levels and the diagnosis of hepatic steatosis is shown in Table 5.

Table 5: Concordance between non-HDL and steatosis in the study group

	St hep (+)	St hep (-)
NonHDL>=130	18	12
NonHDL<130	7	13
	25	25

A value of the ratio between serum triglyceride levels and low HDL-cholesterol value above 3 is a high and significantly predictive factor of fatty liver: OR = 4.422 (95% CI, 1.352 to 15.57, p = 0.0065). Concordance between the triglycerides / HDL-cholesterol ratio and hepatic steatosis diagnosis is shown in Table 6.

Contrary to expectations given by the results received from the relationship between lipid parameters and the chances of diagnosing non-alcoholic fatty liver, diabetes mellitus type 2 is not related to ultrasound diagnosis of fatty liver: OR = 2.213 (95% Cl, 0.6149 to 8.633, p = NS). Correlation between the presence of type 2 diabetes and hepatic steatosis diagnosis is shown in Table 7.

Table 6: Concordance between TG / HDL ratio and hepatic steatosis in the study group

	St hep (+)	St hep (-)
TG/HDL>=3	18	9
TG/HDL<3	7	16
	25	25

Table 7: Concordance between type 2 diabetes and hepatic steatosis in the study group

	St hep (+)	St hep (-)
DZ (+)	9	5
DZ (-)	16	20
	25	25

Smoking also cannot be related to ultrasound diagnosis of hepatic steatosis: OR = 2.263 (95% CI, 0.7132 to 7.482, p = NS). Concordance between the condition of smoking and diagnosis of hepatic steatosis is shown in Table 8.

Table 8: Concordance smoking - steatosis in the study group

	St hep (+)	St hep (-)
Fumat	13	8
NonFumat	12	17
	25	25

A value of the SCORE absolute cardiovascular risk over 5% is not statistically significant with the possibility of correlation with ultrasound diagnosis of hepatic steatosis: OR = 0.4095 (95% CI, 0.1142 to 1.377, p = 0.07). Concordance between cardiovascular risk score and the diagnosis of hepatic steatosis is shown in Table 9.

Table 9: Concordance between the SCORE risk and hepatic steatosis in the study group

	St hep (+)	St hep (-)
SCORE>=5%	6	11
SCORE<5%	19	14
	25	25

3.2. Discussion

Non-alcoholic fatty liver is the most common cause of chronic liver disease in the general population living with "modern" habits, slowly progressing from simple fatty infiltration of liver (steatosis) with or without varying degrees of inflammation, to hepatocyte necrosis phenomena, fibrosis and ultimately cirrhosis [1]. Various studies have established that traditional cardiovascular risk factors are commonly encountered in subjects with various degrees of liver steatosis [11, 12]. However, there are parameters that are typically included

in cardiovascular risk assessment scales, such as insulin resistance, obesity and elevated serum triglyceride levels, which are obviously changing the development of non-alcoholic fatty liver [13].

Intrahepatic triglyceride accumulation causes hepatic steatosis and this phenomena is favored by the presence of obesity. Obesity, defined by the body mass index is directly proportional to the prevalence of non-alcoholic fatty liver [14]. In our study, we found that obesity has a high prevalence in the group we had considered. Only two patients with non - alcoholic liver steatosis had a BMI in the normal range, 23 patients with liver steatosis entering in those overweighted or obese groups. It is important that structural and metabolic changes of liver to be captured from a young age so that targeted complex interventions can have the ability to correct the errors in the carbohydrate and lipid metabolisms. In a recent study, Deivanayagam et al. found significant association between weight status and altered fatty liver from adolescence with promoting insulin resistance and increased cardiovascular risk [15]. Weight reduction is beneficial for improving the liver histology (not for fibrosis).

Brutal reduction in body weight may be associated with a transient increase in fatty infiltration of the liver in parallel with transient increases in ALAT [16].

It has been shown that fatty liver is associated with changes in carbohydrate and lipid metabolisms. Furthermore, in vivo experiments have shown that hepatic steatosis causes insulin resistance [17]. In obese subjects, increased fat tissue determines the excretion of non - esterified fatty acids, glycerol, hormones, pro-inflammatory cytokines and other factors involved in the determinism of insulin resistance. Association with beta-pancreatic islets' dysfunction results the impossibility of glucose homeostasis, developing a type 2 diabetes [18]. Pro-inflammatory status conferred by the key link of non-alcoholic hepatic steatosis – the insulin resistance - underpins the development and evolution of the atherosclerotic process until the appearance of clinical events. In our study, type 2 diabetes was present almost exclusively in subjects with weight status modified: only one diabetic patient had normal weight. 9 of the 14 patients had been diagnosed with diabetic fatty liver by abdominal ultrasonography, results which overlap with data shown above in the current literature.

Recently it was pointed out that there is epidemiological evidence supporting that elevated levels of serum transaminases, elevations of gamma-glutamyl transpeptidase, documented morphological (by ultrasound assessment) of fatty liver and the presence of surrogate markers of liver fat infiltration may be associated with the development of type 2 diabetes [19].

Referring to the entire group of patients studied, mild hypertriglyceridemia was found to be a basic feature. The risk of developing non-alcoholic steatosis in hypertriglyceridemia basal conditions is over 4 times higher compared to the one in subjects without hypertriglyceridemia, with statistical significance. Recent studies have proven the validity of this relationship: hypertriglyceridemia is present in 64% of subjects with non-alcoholic liver steatosis and reduced HDL-cholesterol levels in 30-42% of subjects with non-alcoholic fatty liver [20, 21]. 90% of the subjects with non-alcoholic fatty liver have at least one risk factor for metabolic syndrome, and 33% are meeting all the criteria for the metabolic syndrome. [22]. As a result of these associations, it is reasonable to extrapolate the relationship between the non-alcoholic fatty liver and the cardiovascular risk. It was demonstrated an independent association between the presence of non-alcoholic fatty liver and the presence of carotid atherosclerotic plaques, endothelial dysfunction, relationship that retains statistical significance after adjusting for factors of metabolic syndrome components [23,24].

Ratios between serum levels of triglycerides and HDL and cholesterol with values of more than 3 are indicators of insulin resistance in subjects with weight status modified (in excess). In a recent study, in subjects of this type, the ratio TG / HDL ≥ 3 was associated with the risk of coronary events (r^2 = 0.227) in a much higher proportion than the components of metabolic syndrome taken separately or as a whole. The logarithm of the ratio TG / HDL (expression rate of the esterification of HDL - cholesterol) is in connection with the risk of coronary artery (r^2 = 0.252) [25]. Previously establishing the relationship between obesity and non-alcoholic hepatic steatosis, the ratio TG / HDL ≥ 3 can be used as a predictive factor for cardiovascular risk in such patients. The subjects in our study who had high ratio (over 3) have been also identified with fatty liver (OR = 4.422, 95 % CI, 1.352 to 15.57, p = 0.0065).

A recent study demonstrated that the increased ratio TG / HDL upraises the risk of first event coronary in each category of body mass index [26].

It is interesting to note that non-alcoholic fatty load of the liver in subjects taken in our study is related to the non-HDL-cholesterol: OR = 2.727 (95 % CI, 0.8431 to 9.326, p = 0.0047). Non - HDL cholesterol (calculated mathematically as the difference between total cholesterol and HDL-cholesterol) is a single measure of expression of the total atherogenic particles: lipoproteins containing apolipoprotein B (LDL, VLDL, IDL). It turned out that setting numerical value of non-HDL cholesterol is cost-effective compared to the direct determination of apolipoproteins B in determining the cardiovascular risk, especially in patients with levels of LDL cholesterol which are classifying the cardiovascular risk as "moderate" [27] impacting the practice of proper management of the cardiovascular risk factors and cardiovascular risk itself. According to recent recommendations [28], the non-HDL cholesterol is suitable to be calculated in the following situations:
- Patients with moderate hypertriglyceridemia (200-500 mg/dL);
- Patients with diabetes and/or coronary artery disease;
- Patients with insulin resistance syndrome.

4. CONCLUSIONS

In the light of these recommendations, assessment of non-HDL cholesterol levels contributes to a more accurate stratification of cardiovascular risk than the calculation of LDL cholesterol. The target level of non-HDL-cholesterol is with 30 mg/dL higherr than the corresponding target level of LDL - cholesterol.

In our study, no significant relationship was found between the absolute SCORE cardiovascular risk and the non-alcoholic liver steatosis. Identification of the non-alcoholic fatty liver (by abdominal ultrasonography) in hypertensive subjects with atherothrombotic cardiovascular disease should guide the practitioner to quantify potential secondary lipid targets that are directly related and independently with the absolute cardiovascular risk.

Further studies are needed to enroll a significant number of patients with non-alcoholic fatty liver disease, with a more diverse range of co-morbidities, to validate the hepatic morphological change as a cardiovascular risk marker.

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