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Research Article

Anthropometric indices, biological samples and dietary hormones – could they be predictors of hepatic steatosis severity?

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Abstract. Objective: The aim of this study was to assess whether anthropometric indices, common laboratory samples, dietary hormones and RGC-32 correlate with the severity of ultrasonographically quantified hepatic steatosis. Methods: The study included 55 patients with varying degrees of hepatic steatosis. Patients were analyzed for anthropometric indices, and biological and serum samples were collected for ELISA determination of dietary hormones and RGC-32. Results: Anthropometric indices increased in direct proportion with the intensity of the severity of hepatic steatosis, the results being statistically significant. Regarding glucidic metabolism, statistically significant results were found regarding insulinemia, which was higher in patients with mild hepatic steatosis compared to those with advanced hepatic steatosis. Regarding inflammatory and immune system, higher serum WBC were found in those with advanced hepatic steatosis, results being statistically significant. Serum RGC-32 levels were higher in those with mild steatosis, but without statistical significance. Regarding dietary hormones, both ghrelin and leptin showed higher serum levels in patients with advanced hepatic steatosis compared to those with mild hepatic steatosis, the results being without statistical significance. Conclusions: Our study complets the data in the literature on the results obtained, nuanced the importance of anthropometric indices and certain common laboratory samples that could be valuable tools in diagnosing NAFLD and in selecting more accurately patients who require liver biopsy ant targeted therapies.

Key Words: liver steatosis, ghrelin, leptin, insulin resistance

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Introduction

Nonalcoholic fatty liver disease (NAFLD) and its subtype, nonalcoholic steatohepatitis (NASH) are beginning to become the most common diseases in the world (Mikami et al 2020). NAFLD is preceded by the presence of hepatic steatosis which is present when the liver contains more than 5% fat. Untreated, it can progress to fibrosis, cirrhosis, and hepatocellular carcinoma (Meex & Blaak 2021). Studies have shown worrying percentages (30-40%) of liver cirrhosis among patients with NAFLD and NASH. This metabolic liver disease is the second leading cause of liver transplantation in the United States (de Oliveira et al 2019; Yip et al 2017). NAFLD is associated with the other elements of the metabolic syndrome such as obesity, hypertension (HT), diabetes mellitus (DM) and dyslipidemia (Mikami et al 2020). Through various pathophysiological mechanisms, adipokines and dietary hormones contribute to the pathogenesis of NAFLD (Okamatsu et al 2009). Obesity is a major public health problem. Regarding NAFLD, the type of obesity (total or segmental) responsible for predicting the severity of hepatic steatosis is not certified, the studies being contradictory (Ho et al 2001). Some authors believe that BMI (body mass index) is a good predictor of the severity of hepatic steatosis (Fraum et al 2018). Other authors believe that abdominal circumference is a good predictor of steatosis and DM (Lee et al 2019; Lee et al 2010).

Leptin, produced by white adipose tissue, is a controversial element in the pathogenesis of NAFLD and MS, being considered by some to be adipokine and by others a food hormone (Bungau et al 2020; Huang et al 2008; Hynes and Jones 2001). Regarding the reporting of leptin to NAFLD, some authors believe that it has a dual activity. In the early stages of liver damage it has anti-steatotic properties and in advanced stages it has a proinflammatory and profibrotic activity (Inzaugarat et al 2017). Other authors consider that steatosis, inflammation and fibrosis are directly proportional to serum leptin levels,

incriminating the appearance of leptin resistance (MacHado et al 2012). Human studies have shown a direct correlation between increases in serum leptin levels and the severity of steatosis (Mikami et al 2020).

Ghrelin is a food hormone produced by the mucous of the digestive tract but also by other tissues, acting on its receptor at the level of adipose tissue, liver, pancreas (Ezquerro et al 2020; Gutierrez-Grobe et al 2010; Quiñones, Fernø, and Al-Massadi 2020). Regarding the action of ghrelin on hepatic steatosis, it seems to increase the content of hepatic triglycerides (TG) by activating acetyl-CoA carboxylase and fatty acid synthetase. Basically, it induces hepatic steatosis. Concerning liver inflammation, ghrelin seems to have a protective role (Gutierrez-Grobe et al 2010).

Another element in metabolic liver pathogenesis is the immune system through macrophages, liver Kupffer cells etc. Adipokines are a connecting bridge between adipose tissue, inflammation and immunity. The complement system is a component part of the immune system and intervenes in the regulation of the cell cycle. One of these elements of the complement is the response to gene complement-32 (RGC-32). It occurs in hepatic steatosis and in terms of liver inflammation studies have shown that deletion of RGC-32 has led to its regression (Fosbrink et al 2009; Papers and Doi 2015; Rus et al 2017).

Given that NAFLD is clinically asymptomatic condition, it is imperative to create diagnostic strategies (Mikami et al 2020). At the same time, although liver biopsy is the gold standard in the diagnosis of hepatic steatosis and its severe forms, it is important to be performed on selected groups of patients (Lee et al 2010). The objectives of this study are to establish a clearer relationship between the severity of steatosis detected ultrasonographically and the clinical and paraclinical elements of NAFLD subjects. At the same time, we investigate the possible connection between the degree of liver steatosis and food hormones, and the complement system represented by RGC-32 respectively.

Material and methods

Study population

The study included 55 subjects with hepatic steatosis. They participated voluntarily, being elected among the patients who periodically go to the family physician to be monitored for chronic diseases. This study was conduced at the 4th Medical Clinic, Cluj Napoca, Romania, following the approval the Ethics Committe of the "Iuliu-Haţieganu" University of Medicine and Pharmacy, number 122/15.04.2021. The introduction of patients in the study was performed after signing the informed consent. Heredo-colateral antecedents, personal pathologies and the chronic medication followed were taken from the observation documents of the family doctors. Throughout the study, all the Helsinki rules were observed.

Clinical and biological evaluation

High blood pressure has been defined as values above 140/90mmHg or the use of antihypertensive treatments. Patients were considered diabetic if they had serum blood glucose levels above 126 mg/dL or serum hemoglobin 1Ac (Hb1Ac) values above 6.5%, or the use of oral antidiabetic drug or insulin. Hyperlipemia was considered in the presence of serum cholesterol levels greater or equal to 220 mg/dL, triglycerides (TG) above 150 mg/dL or the use of antihyperlipemic drugs. We performed the following measurements: height, body weight, waist circumference, hip circumference, thigh circumference. Having these values, we performed BMI, waist to hip ratio (WHR), waist to thigh ratio (WTR). Alcohol consumption was determined from a questionnaire. Biological samples were taken from the patients in special devices. One of them was used to process routine analyses as well as ASAT, ALAT, FA, GammaGT, bilirubin, cholesterol, triglycerides, HDL (high-density lipoprotein), LDL (low density lipoprotein), C-reactive protein (CPR), uric acid, blood glucose, insulinemia. Another vacueta was centrifuged and its serum was stored in appropriate conditions, subsequently used to obtain serum levels of ghrelin, leptin and RGC-32, which were determined by enzyme-linked immunosorbent assay (ELISA).

Ultrasonographic evaluation of hepatic steatosis

Ultrasonographic evaluation of hepatic steatosis was performed with a Samsung RS85 device. The ultrasonographic assessment was performed by a single examiner with extensive experience who had no knowledge of the clinical and paraclinical data of the subjects. The ultrasound severity of steatosis was considered as follows:

- Mild: slight increase in fine echoes in liver parenchyma with normal visualization of diaphragm and intrahepatic vessel borders
- Moderate: moderate increase in fine echoes in liver parenchyma with slightly impaired visualization of hepatic vessel border and diaphragm
- Severe: marked increase in fine echoes with poor or non-visualization of the intrahepatic vessel borders, diaphragm and posterior right lobe of the liver (Saadeh et al 2002).

Statistical analysis

Statistical analysis was performed using MedCalc Statistical Software version 19.4.1 (MedCalc Software Ltd, Ostend, Belgium; https://www.medcalc.org;2020). Category variables were compared using the chi-square test. The comparisons were performed using Kruskal-Wallis test. Sperman's correlation coefficient was used to calculate the correlations between several results. Differences were considered to be significant with p-values of less than 0.05.

Results

Characteristics of the participants with NAFLD

The characteristics of patients with NAFLD are shown in table 1. The median age of patients with mild steatosis was 62 years, of those with moderate steatosis was 63 and of those with severe steatosis 55. From the point of view of BMI, all subjects, regardless of the severity of hepatic steatosis, were overweight and obese, the result being statistically significant. Most patients with steatosis did not have diabetes and therefore no treatment with insulin or oral antidiabetic drugs. Regarding the dysmetabolic profile, the majority received statin treatment, while others received fibrate treatment. From a cardiovascular point of view, most subjects with steatosis had a hereditary cardiovascular history, were hypertensive and most patients followed chronic treatment with thiazide diuretics.

Table 1. Baseline characteristics of the study population

Characteristic	Mild steatosis	Moderate steatosis	Severe steatosis	p value
Age	62 (53;70)	63 (59.25;69.00)	55 (35.25;60.50)	0.092
BMI	24.57 (21.13;26.81)	25.00 (22.91;26.35)	30.71 (26.62;33.88)	**0.01**
AHC	25 (75.8%)	12 (85.7%)	7 (97.5%)	0.626
AHT	20 (60.6%)	10 (71.74%)	6 (75.0%)	0.642
ICMP	15 (45.5%)	6 (42.9%)	1 (12.5%)	0.226
Angina pectoralis	6 (18.2%)	4 (26.6%)	0 (0%)	0.247
Gallstones	3 (9.4%)	0 (0%)	2 (25%)	0.150
Diabetes mellitus	8 (24.2%)	4 (28.6%)	4 (50%)	0.355
Antidiabetic agent	8 (24.2%)	3 (21.4%)	4 (50%)	0.290
Insulin treatment	1 (3%)	1 (7.1%)	0 (0%)	0.661
Statins	18 (54.5%)	8 (57.1%)	3 (37.5%)	0.368
Fibrates	1 (3.1%)	1 (7.1%)	3 (37.5%)	**0.01**
Beta-blocker treatment	16 (48.5%)	6 (42.9%)	3 (37.5%)	0.833
Thiazide diuretics	18 (54.5%)	8 (57.1%)	6 (75.0%)	0.572

Abbreviations: AHC, heredocolateral antecedents; AHT, arterial hypertension; BMI, body mass index; ICMP, ischemic cardiomyopathy. Boldface type indicates a significant p value ($p<0.05$); High blood pressure has been defined as values above 140/90 mmHg or the use of antihypertensive treatments. Diabetes has been defines as serum blood glucose levels above 126 mg/dL or serum Hb1Ac values above 6.5% or the use of oral antidiabetic drugs or insulin treatment

Table 2. Anthropometric indices and the severity of steatosis

Characteristic	Mild steatosis	Moderate steatosis	Severe steatosis	p value
Waist circumference	90 (93.50; 102.50)	100 (97.25; 110.0)	112 (106.25; 135.75)	**0.003**
Thigh circumference	54 (50.0;57.0)	56.0 (54.50;70.00)	63.50(54.50;72.75)	**0.02**
Hip circumference	103.0 (95.50; 108.0)	105.5 (100.0;116.75)	130.00(103.50;138.25)	**0.01**
WHR	0.961 (0.943;0.99)	0.961 (0.87;1.01)	0.97 (0.85;1.07)	0.867
WHT	1.83 (1.74;1.87)	1.76 (1.46;1.85)	1.86 (1.71;2.00)	0.06

Abbreviations: WHR, waist to hip ratio; WHT, waist to thigh ratio. Boldface type indicates a significant p value ($p<0.05$)

Table 3. Correlations between glycemic, lipidic profile and severity of hepatic steatosis

Characteristic	Mild steatosis	Moderate steatosis	Severe steatosis	p value
Glucose level	101 (93.5;113.5)	100.5 (94.7;137.50)	90.0 (83.0;106.7)	0.10
Cholesterol	209 (167;244.5)	216 (159.7;227.0)	256.5 (231.0;277.5)	**0.05**
HDL-cholesterol	50.0 (44.0;64.0)	55.50 (40.5;60.25)	56.8 (48.7;71.2)	0.389
TG	152.0 (99.5;211.0)	149.0 (112.5;197.0)	185.5 (120.0;315.5)	0.570
LDL	116.0 (102.5;152.0)	118.0 (95.0;143.0)	150.0 (92.2;157.7)	0.524
HOMA index	3.4 (2.9;4.0)	3.3 (2.9;4.2)	3.1 (2.6;3.6)	0.433
Insulinemia	1.31 (1.2;1.4)	1.2 (1.2;1.2)	1.30 (1.2;1.5)	**0.02**

Abbreviations: HDL, high density lipoprotein; HOMA, homeostatic assay; LDL, low density lipoprotein; TG, triglyceride. Boldface type indicates a significant p value ($p<0.05$)

Correlations between liver steatosis severity and anthropometric indices

In terms of anthropometric indices, BMI correlated with the severity of hepatic steatosis, with an average of 30 in those with severe hepatic steatosis, compared to 24 in those with mild hepatic steatosis, the results being statistically significant (Tab. 1). Also, statistically significant was the correlation between abdominal, thigh and hip circumference with the severity of hepatic steatosis (Tab. 2).

The relationship between the severity of steatosis with the lipid, glycemic profile and insulin resistance

From the point of view of the lipid profile, we found that patients with advanced hepatic steatosis had higher serum cholesterol levels compared to the mild form of steatosis, the results being statistically significant (Tab. 3). The same can be said for the serum triglyceride and LDL cholesterol levels, but the results are statistically insignificant.

Regarding metabolism, we can see that the HOMA index has higher values in subjects with mild steatosis compared to those

Table 4. Correlations between dietary hormones, inflammatory profile and steatosis severity

Characteristic	Mild steatosis	Moderate steatosis	Severe steatosis	p value
Ghrelin	28.4 (24.2;62.2)	24.7 (24.0;32.6)	45.1 (28.4;51.8)	0.430
Leptin	58.0 (31.3;102.0)	94.7 (32.5;132.5)	112.8 (30.6;145.8)	0.27
RGC-32	0.4 (0.4;0.5)	0.4 (0.4;0.4)	0.4 (0.4;0.4)	0.805
CRP	3.0 (2.0;4.0)	3.0 (0.54;4.0)	3.0 (0.8;3.7)	0.47
WBC	7.0 (6.0;8.4)	9.0 (7.6;9.2)	8.5 (7.2;8.9)	**0.02**

Abbreviations: CRP, C reactive protein; RGC-32, response to gene complement-32; WBC, white blood cells. Boldface type indicates a significant p value ($p<0.05$)

with advanced steatosis. Regarding serum insulin levels, it shows higher serum levels in patients with mild steatosis compared to those with severe steatosis, the results being statistically significant (Tab. 3).

The relationship between food intake hormones and the severity of steatosis

Both ghrelin and leptin levels showed higher serum levels in patients with severe steatosis compared to those with mild steatosis, the results not being statistically significant (Tab. 4).

The relationship between the severity of steatosis and the immune system, respectively the inflammatory syndrome

RGC-32 showed higher values in subjects with mild steatosis compared to subjects with severe steatosis. Regarding inflammatory syndrome, serum leukocyte levels were significantly higher in patients with moderate and severe steatosis compared to patients with mild steatosis, the results being statistically significant. Serum PCR values remained constant, regardless of the severity of ultrasound-detected steatosis (Tab. 4).

Discussion

This study evaluated the relationship between the severity of steatosis detected ultrasonographically and anthropometric indices, lipid, carbohydrate, immune and inflammatory profile. Recent, studies have attempted to create scores to quantify visceral adiposity and its link to the severity of hepatic steatosis using anthropometric indices (Borruel et al 2014).

In connection with these anthropometric indices, our study showed a directly proportional relationship between steatosis severity and BMI. The same directly proportional relationship is observed between the degrees of severity of steatosis and the perimeter of the waist, hip and thigh. Our results were statistically significant. Our findings are consistent with the studies cited in the literature. A similar study to ours was performed on brain-dead patients who had liver biopsies and found the same relationship directly proportional to the severity of steatosis and BMI (Kyoung et al 2020). Others have postulated the idea that the severity of hepatic steatosis increases in intensity with each additional unit of BMI. The authors reached this conclusion following an observational study on subjects with autoimmune hepatitis and found a directly proportional relationship between the intensity of hepatic steatosis and BMI (Chalasani et al n.d.). The same directly proportional relationship was found in another observational study in which hepatic steatosis was detected by MRI. The authors hypothesized that only BMI is a good predictor of the severity of liver steatosis. They consider this to be of immense clinical utility, helping the clinician to take appropriate therapeutic attitudes (Fraum et al 2018). Other authors have postulated that hepatic steatosis occurs not only in obese people with two other risk factors for SM, but also in overweight people with the same cardiovascular risk factors (Clarke et al 2019).

Abdominal circumference directly reflects abdominal adiposity, being closely related to cardiovascular complications and other complications given by MS (Lee & Jeong 2017; Ozturk et al 2020). Some have postulated that abdominal circumference could be a strong mediator between inappropriate eating habits and the occurrence of NAFLD (Ghaemi et al 2018). The relationship between abdominal circumference, visceral and hepatic adiposity was assessed in an observational study in adolescents. The authors found a directly proportional relationship between the previously mentioned elements. They postulated that the abdominal circumference could be a strong predictor of hepatic steatosis (Lee et al 2010). Others postulate that abdominal circumference is not only a good predictor for the appearance of hepatic steatosis, but also for the appearance of diabetes, another element of SM. The authors concluded this in an observational study on prediabetic subjects (Lee et al 2019). We found the same directly proportional relationship between abdominal circumference and the severity of liver steatosis, our results were statistically significant. In addition to the directly proportional relationship between abdominal circumference and hepatic steatosis, another observational study found the same directly proportional relationship between abdominal circumference and one of the complications of hepatic steatosis, fibrosis (Khamseh et al 2021). Abdominal circumference has been shown to be beneficial in screening for NAFLD in an observational study of obese adolescents. It is practically a low cost evaluation, easy to perform by any medical practitioner (Clemente et al 2016). Other authors concluded that BMI and abdominal circumference would be the best indicators of visceral adiposity and the best predictors of insulin resistance and hepatic steatosis (Borruel et al 2014).

Regarding the hip circumference, our study showed a directly proportional relationship, statistically significant, with the severity of hepatic steatosis. Regarding the ratio between the waist circumference and hip circumference, our study shows that patients with severe steatosis have a higher ratio compared to patients with mild and moderate steatosis, the results not being statistically significant. A comparative observational study that included adults with healthy liver, steatosis, steatohepatitis and fibrosis biopsy diagnosed found that hip circumference

and waist to hip ratio were significantly higher in patients with hepatic steatosis compared to adults with healthy liver. The same directly proportional relationship between the previously mentioned parameters is maintained in patients with steatohepatitis compared to adults with healthy liver (Reis et al 2021). The same directly proportional relationship between steatosis severity and hip circumference was demonstrated in an observational study of the Chinese pediatric population, the results being statistically significant on hip circumference and statistically insignificant when referring to the ratio of waist circumference to hip circumference (Chan et al 2004). Some authors have evaluated the relationship between anthropometric indices and the degree of disturbance of cellular mechanisms involved in the pathogenesis of NAFLD, such as mitochondrial dysfunction. The authors found that there was a directly proportional relationship between the intensity of the mitochondrial dysfunction and hip circumference, along with abdominal circumference (Uğuz et al 2020).

Regarding the lipid profile, our study showed statistically high serum levels of cholesterol in patients with advanced steatosis, compared to those with mild steatosis. And triglyceride levels were higher in patients with advanced steatosis compared to those with mild steatosis, but our results were not statistically significant; however, the results were in accordance with date from the literature that found that component serum levels of the lipid profile correlated in direct proportion to the severity of hepatic steatosis (Briseño-Bass et al 2019). The results of our study are also consistent with the data from the literature regarding the pediatric population. In this regard, we mention a study on the pediatric population that found a directly proportional relationship between serum levels of triglycerides, total cholesterol, HDL and LDL cholesterol as being higher in pediatric patients with advanced steatosis compared to those with mild steatosis. Of all the above mentioned, the only statistically significant one is the correlation between HDL and steatosis severity (Soydan et al 2021). Another study in pediatric patients found an inversely proportional relationship between serum HDL cholesterol levels and the degree of hepatic steatosis assessed by the ratio of hepatic to renal echogenicity (Damar et al 2021). The studies focus on creating scores that predict as accurately as possible the appearance of hepatic steatosis. One of these is the ratio of uric acid and HDL cholesterol (Zhu et al 2022). Another observational study in children evaluated the relationship between the ratio of triglyceride to HDL cholesterol, the ratio of total cholesterol to HDL cholesterol, the ratio of LDL and HDL cholesterol and the intensity of hepatic steatosis. They found a directly proportional relationship, statistically significant, postulating that the lipid profile is a strong predictor of the severity of metabolic liver injury (Nobili et al 2010).

Regarding the glycemic profile, our study showed discreetly higher values of glycemic values and HOMA index in those with a mild form of steatosis, without statistical significance. The same previously mentioned study regarding patients in brain death, found a directly proportional relationship between serum glycemic levels (as a marker of insulin resistance) and the presence of hepatic steatosis (Kyoung et al 2020). The same higher serum glucose levels in patients with steatosis were found in another observational study. Moreover, glucose was introduced into a calculation formula, called TgG index. Applying this formula, the authors found statistically significant correlations between its areas and the presence of hepatic steatosis, being higher in those with steatosis, compared to those without steatosis (Khamseh et al 2021). Regarding the severity of ultrasonographically quantified steatosis, the same index showed higher values in those with severe steatosis compared to those with mild steatosis, the results being statistically significant (Guo et al 2020). Also, related to the relationship between glycemic profile and hepatic steatosis, other authors considered that glycated hemoglobin showed a higher predictive value for the occurrence of NAFLD compared to abdominal circumference (Masroor and Haque 2021). Another comparative study between patients with steatosis and adults without steatosis found higher serum blood glucose, insulinemia and HOMA index levels, postulating that HOMA values greater than 2 have the potential to certify the presence of NAFLD among patients (Salgado et al 2010). In relation to serum insulinemia, an observational study found that excessive experimental administration correlated with the severity of hepatic steatosis (Jolliet, Leverve, and Pichard 2001). Regarding serum insulin levels, our study found that it had an inversely proportional correlation, statistically significant, being higher in patients with mild steatosis, compared to those with severe steatosis.

The literature has shown conflicting results regarding the relationship between serum insulin levels and the severity of hepatic steatosis. An observational study found that serum insulin levels correlated directly with the severity of steatosis, suggesting that NAFLD also reflects the degree of pancreatic β-cell dysfunction (Miya et al 2020), while two other observational studies did not find this relationship. One of them, which was performed on the obese juvenile population, found that the severity of steatosis was associated only with lipid profile, not with the glycemic one, and, therefore no correlations between the severity of hepatic steatosis and serum insulin levels (Guzzaloni et al 2000). The same absence of correlation between serum insulin levels and the relationship between hepatic and renal echogenicity ratio was found in another observational study. The same study found that serum insulin levels were only associated with the amount of abdominal adipose tissue that was also detected ultrasonographically (Damar et al 2021).

From an immune and inflammatory point of view, our study showed that serum CRP levels were constant, regardless of the severity of steatosis, and the results were not statistically significant. An observational study found increases in CRP levels with the steatosis severity, the results being statistically significant (Chowdhury & Chakraborty 2017). It is known that the increase in serum leukocyte levels is modulated by proinflammatory cytokines, their number being a routine determination during clinical activity. It has a role both in assessing the severity of hepatic steatosis and the risk of NAFLD (Chao et al 2022). Regarding serum leukocyte levels, our study showed that it was significantly higher in patients with moderate steatosis. A directly proportional relationship between leukocyte counts and steatosis severity was found in another observational study in approximately 2000 patients. Probably these associations are also due to the pathophysiological mechanisms that underline the appearance and progression of hepatic steatosis to its more severe forms such as insulin resistance, fatty acid oxidation, hepatic apoptosis, adipose tissue dysfunction etc. (Chao

et al 2022). The increase in leukocyte counts may be closely related to NAFLD, independent of the other pathophysiological mechanisms underlying the etiological basis of NAFLD. This was documented in another observational study, concluding that hepatic steatosis is not a simple hepatic lipid accumulation but also a trigger of other mechanisms that can lead to both hepatic and extrahepatic complications (Lee et al 2010). Other authors, after conducting a 6-year study, concluded that leukocytes could be viewed as an independent predictor of NAFLD (Wang et al 2016). Regarding RGC-32, observational studies on the association with the severity of hepatic steatosis are limited. Our study found higher serum levels of RGC-32 in patients with mild steatosis compared with those with severe steatosis, but the results were not statistically significant. An experimental murine study that looked at the inhibitory effects of RGC-32 on hepatic steatosis induced by caloric diets found regression of liver damage (Papers & Doi 2015). Regarding the other elements that underlie the appearance of NAFLD, another experimental study, also in a murine model, found that inhibition of RGC-32 at the adipocyte level was associated with improved insulin sensitivity and improved lipid profile. They considered that it could be a therapeutic target in limiting the complications of obesity (Cui et al 2015).

There are few studies that have determined serum RGC-32 levels by ELISA in various diseases. A search in PubMed using the keywords ”RGC-32” and ”ELISA” found two published studies. One of these evaluated the relationship between RGC-32 and the balance between Treg and Th17 in patients with dilated cardiomyopathy. They found a directly proportional relationship between RGC-32 and Th17 and inversely proportional relationship between RGC-32 and Treg, and, implicitly, the ratio between Th17 and Treg (Li et al 2017). The disturbance of the balance between Th17 and Treg was also found in the pathogenesis of NAFLD, in an observational study on the severity of liver damage diagnosed by liver biopsy in both adults and children. However, RGC-32 was not evaluated (Cairoli et al 2021). The second study indexed in PubMed, which assessed serum RGC-32 levels detected by ELISA technique in children with chronic kidney disease, postulated that RGC-32 could be a biomarker for them (Liu et al 2014).

Regarding ghrelin, our study found higher serum levels in patients with advanced steatosis. Experimental studies found that the administration of ghrelin had led to increased levels of exogenous liver triglycerides and also de novo hepatic lipogenesis (Rasineni et al 2019). A comparative observational study in patients with hepatic impairment and adults without hepatic impairment found higher serum levels of ghrelin in those with hepatic steatosis, compared to the healthy group, and postulated the idea that the intervention of proteases released by NAFLD had an inhibitory role on ghrelin (Gutierrez-Grobe et al 2010).

Regarding leptin, our study found higher serum levels in patients with severe hepatic steatosis compared to those with mild hepatic steatosis. Although our results were not statistically significant, they are consistent with data from the literature that found the same relationship directly proportional to serum leptin levels and the severity of hepatic steatosis (Nobili et al 2006). Another study postulated that leptin could be a mediator between insulin resistance and hepatic steatosis (detected by elastography). This was due to the findings of a directly proportional relationship between its levels, of insulin resistance levels and the severity of hepatic steatosis (Ramírez-Vélez et al 2021). Others found that serum leptin levels were higher in patients with hepatic steatosis, regardless of the presence or absence of overweight, obesity or other elements related to adiposity (Kim et al 2008). Other studies have attempted to create scores and calculation formulas that include adipokines and dietary hormones with a role in differentiating the severe form of NALD, NASH respectively. They found a good accuracy of this formula in predicting the occurrence of NASH (MacHado et al 2012). Regarding dietary hormones and their relationship with NAFLD, the results are controversial and further studies are needed to accurately detect their roles in the pathogenesis of NAFLD (Ramírez-Vélez et al 2021).

Due to the epidemiological context of the SARS-COV2 infection that Romania in the last two years, the number of hospital presentations for other pathologies decreased drastically, which determined the inclusion of a small number of patients into our study. Probably the statistical significance of our results could not be reached because of the small number of patients.

Another limitation of our study was that the severity of hepatic steatosis was quantified ultrasonographically, and not by the gold standard, liver biopsy.

Conclusions

Our study found a directly proportional relationship between anthropometric indices and the severity of hepatic steatosis, practically, between cutaneous, visceral adiposity and the severity of hepatic steatosis. At the same time, our study found that certain routine tests were directly correlated with the severity of steatosis.

We consider them valuable tools in diagnosing steatosis and indicating the patients who require liver biopsy.

We found the same directly proportional relationship in terms of dietary hormones and hepatic steatosis. Given the results obtained, even if some were not statistically significant, we consider it imperative to conduct large population studies to validate the results of our study and other authors and to create the necessary scores to quantify as accurately as possible the severity of hepatic steatosis, so that they can be easily performed by the practitioners, regardless of their medical specialty.

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