ABSTRACT: Non-alcoholic fatty liver disease (NAFLD) is currently the most common liver disease that progressively develops in four stages (steatosis, non-alcoholic steatohepatitis-NASH, fibrosis, cirrhosis). In order to accurately evaluate the amount of fat content inside the hepatocytes, non-invasive imaging studies such as magnetic resonance spectroscopy (MRS) of the liver can be used as an alternative method to the traditional invasive liver biopsy. The main objective of our study was to quantitatively assess hepatic steatosis before and after treatment using liver MRS. The study group included 35 patients diagnosed with NAFLD who freely expressed their agreement to take part in this study. The treatment lasted 6 months and consisted of administering a local commercially available liver protection supplement combined with dietary and lifestyle adjustments. The initial quantitative assessment of the liver fat content using MRS revealed a hepatic lipid accumulation ranging from 15.70% to 68.03%, with a mean value of 41.26%. The subsequent liver MRS performed after 6 months of proper treatment indicated a fat content inside the liver parenchyma ranging from 15.47% to 58.98%, with a mean value of 36.07%. The therapeutic measures that were applied to the patients managed to reduce the amount of fat content inside the liver parenchyma in 24 patients (68.57%), while in 11 patients (31.43%) the results revealed an increase in hepatic lipid content. In conclusion, the current study reached its initial objective and offered a modern approach regarding the pre and post-treatment evaluation of hepatic steatosis using MRS.

KEYWORDS: Non-alcoholic fatty liver disease, magnetic resonance liver spectroscopy, liver biopsy

Introduction

Non-alcoholic fatty liver disease (NAFLD) is currently the most common liver disease worldwide. Regarding the worldwide prevalence of NAFLD, the mean values revolve around 20-30% in the general population, with slight regional differences. NAFLD refers to the hepatic changes that accompany the metabolic syndrome and progressively develops in four stages: 1. hepatic lipid accumulation (steatosis)-harmless build-up of fat content in the hepatocytes; 2. non-alcoholic steatohepatitis (NASH)-inflammation of the liver parenchyma is present; 3. fibrosis-prolonged inflammation of the liver parenchyma that leads to build-up of scar tissue; 4. cirrhosis-the most advanced stage that requires years to develop in the presence of chronic inflamed liver parenchyma and is associated with an improper liver function [1-5].

From a clinical standpoint, diagnosing NAFLD may prove challenging at times. An inflamed hepatic parenchyma is only sometimes associated with an increased size of the liver that can be detected during physical examination.

Liver parenchyma changes depicted on imaging studies such as ultrasonography (US), computed tomography (CT) and magnetic resonance imaging (MRI) combined with elevated values of the liver enzymes and a proper assessment of the NAFLD risk factors can provide a strong diagnostic algorithm. However, in order to accurately evaluate the amount of fat content inside the liver parenchyma, non-invasive imaging studies such as magnetic resonance spectroscopy (MRS) of the liver can be used as an alternative method for the traditional invasive liver biopsy [6,7].

Material and methods

The main objective of our study was to quantitatively assess hepatic steatosis before and after treatment using liver MRS. Over the course of two years (January 2017-January 2019), the current study included 35 patients (25 men, 10 women) diagnosed with NAFLD who freely expressed their agreement to take part in this study. All 35 patients were initially examined in the Medical Imaging Department of the University of Medicine and Pharmacy of Craiova, Romania.
of Medicine and Pharmacy of Craiova using a Philips Ingenia 3T MRI machine and liver MRS in order to accurately assess the amount of fat content inside the liver parenchyma before undergoing treatment.

A written informed consent was obtained from each patient, and the Ethics Committee of UMF Craiova approved the study.

The treatment lasted 6 months and consisted of administering a local commercially available liver protection supplement (two capsules per day) combined with dietary and lifestyle adjustments.

Regarding the liver protection supplement that was administered to all the patients in the study group, the following compounds were present in each capsule: choline (60mg), dry extract from Silybum marianum (150mg), dry extract from Phyllanthus niruri (225mg), microcrystalline cellulose, magnesium stearate, talcum.

The most important dietary and lifestyle adjustments included: no alcohol consumption, increased daily physical activity, weight loss, reduced intake of sugar and saturated fat, increased uptake of fruits and vegetables, normal blood sugar levels.

After following the six-month treatment, all 35 patients were subsequently evaluated using liver MRS in order to properly assess the post-treatment amount of fat content inside the hepatic parenchyma.

Both initial and follow-up liver MRS examinations involved a carefully selected region of interest (ROI) in the right lobe of the liver, without targeting bile ducts, vessels inside the liver parenchyma or the edge of the liver.

According the amount of fat content build-up in the hepatocytes, we used the following grading system: grade 0 (≤5%), grade 1 (6-33%), grade 2 (34-66%) and grade 3 (≥67%).

Results

The study group consisted of 35 patients (25 men, 10 women) aged between 35 and 70 years, with a mean age±standard deviation (SD) of 53.63±9.81 years.

The mean age recorded in men (56.84±8.68 years) was considerably higher compared to the mean age recorded in women (45.6±7.90 years) (Fig.1).

![Fig.1. Minimum, mean and maximum age of the patients included in the study group](image)

The initial quantitative assessment of the liver fat content using MRS revealed a hepatic lipid accumulation ranging from 15.70% to 68.03% (median 36.80%), with a mean value of 41.26±14.29% (Fig.2).

Regarding the pre-treatment grading of hepatic steatosis, the patients included in the study group were classified as follows: grade 0-0 patients, grade 1-9 patients, grade 2-23 patients, grade 3-3 patients.
Fig. 2. Liver MRS. The red square marks the ROI in all three-dimensional planes (axial, sagittal, coronal). The chart included in this image indicates the concentrations of the liver metabolites (water, choline, lipids).

The subsequent liver MRS performed after 6 months of properly conducted treatment indicated a fat content inside the hepatocytes ranging from 15.47% to 58.98% (median 35.04%), with a mean of 36.07±13.61%.

Regarding the post-treatment grading of hepatic steatosis, the patients included in the study group were classified as follows: grade 0-0 patients, grade 1-17 patients, grade 2-18 patients, grade 3-0 patients (Fig. 3).

Fig. 3. Before and after treatment grading of hepatic steatosis

The therapeutic measures that were applied to the patients managed to reduce the amount of fat content inside the liver parenchyma in 24 patients (68.57%), while in 11 patients (31.43%) the results revealed an increase in hepatic lipid content (Fig. 4).

All the 11 previously mentioned patients were men, aged between 51 and 70 years (median 66 years), with a mean age±SD of 61.82±7.48 years. The highest and lowest decrease regarding the percent of hepatic steatosis were 27.96% and 4.15% (median...
13.65%), respectively, with an average decrease of 13.49±7.24%. The highest and lowest increase regarding the percent of hepatic steatosis were 27.73% and 2.30% (median 12.45%), respectively, with an average increase of 12.92±8.76%. Furthermore, the grade of hepatic steatosis decreased in 16 cases (45.71%), remained the same in 14 cases (40%) and increased in 5 cases (14.29%).

**Fig.4 (A, B). Quantitative assessment of hepatic steatosis using liver MRS before (A) and after treatment (B). There is a significant decrease of lipid content inside the liver parenchyma**

**Discussion**

NAFLD is currently the most common liver disease and one of the biggest and underrated health concerns worldwide. The current literature data provides contradictory results regarding sex predilection for this disease [1-3].

The results provided by our study indicate a Male-to-Female ratio of 2.5:1 in the Romanian population included in the study group, but further local studies are required. Although NAFLD is a very common disease in the general population, the long duration of the treatment was the main limitation of our study.

Regarding the accurate assessment of liver fat content in hepatic steatosis, recent studies suggest that, if correctly conducted, liver MRS can provide realistic values of the intrahepatic fat fraction that correlates well with liver biopsy findings. Therefore, a non-invasive imaging method such as MRS can successfully replace the traditional invasive liver biopsy in evaluating the amount of fat content inside the hepatocytes [8-10].

A study conducted in the USA by An Tang et al. included 77 patients (children and adults) diagnosed with NAFLD who were recruited over the course of four years (2005-2009). All the patients included in the study underwent MRS and liver biopsy. The time interval between the two methods did not exceed 180 days (29 days on average). This study indicated a strong correlation between MRS and liver biopsy findings. Furthermore, the patients included in the study group were classified as follows: grade 0 -5 patients, grade 1-26 patients, grade 2 -27 patients, grade 3 -19 patients. In the same matter, the majority of the patients included in our local study group were classified as grade 1 or grade 2 liver steatosis. However, the number of grade 3 liver steatosis patients was significantly lower in our study mainly due to the fact that only adults aged between 35 and 70 years were admitted to our study group, while An Tang et al. included 65 children (84.4%) out of 77 patients [11].

Treating NAFLD should first reduce or completely eliminate the associated risk factors (no alcohol consumption, no sweetened drinks or juices, weight loss, reduced intake of saturated fats and sugar, normal serum glucose levels, increased daily physical activity).
Furthermore, physicians recommend an increased uptake of fruits and vegetables [12].

Weight loss is the most important goal of the entire treatment, but it can only be achieved through a combination of daily physical exercises and caloric restriction. However, dietary and lifestyle adjustments are not only hard to implement, but also difficult to maintain on the long term. Therefore, changes affecting the lifestyle and diet should always be personalized for each patient in order to benefit from a good compliance to treatment. Regarding the pharmacological treatment of NAFLD, several drugs were tested (omega-3 fatty acids, metformin, pentoxifylline) but their use yielded discordant results [13].

The therapeutic measures used in our study were successful in over two-thirds of the patients leading to a decreased fat content inside the liver parenchyma. Comparing the pre and post-therapeutic hepatic steatosis grade changes, the treatment was considered successful not only when the grade decreased, but also, in some cases, when the grade remained the same, given that the percent of liver fat content was reduced by a certain amount that was not enough for the patient to be classified into an inferior grade.

An important fact to take into account when analyzing the efficacy of the treatment measures used in this study is that all the 11 patients in which the results indicated an increase in hepatic lipid content were men, aged between 51 and 70 years, with a mean age of 61.82 years. Given the 6 month duration of the treatment, combined with an elevated mean age of these patients and an increased tendency for alcohol consumption in men, the treatment compliance in these cases is questionable at best.

Conclusion

In conclusion, the current study reached its initial objective and offered a modern approach regarding the pre and post-treatment evaluation of hepatic steatosis by fully exploiting the potential of liver MRS.

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Conflict of interests

The authors declare that they have no conflict of interests.

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