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RESEARCH ARTICLE

Iron metabolism and metabolic dysfunction-associated fatty liver disease

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Objective: The primary objective of this study was to investigate the association between biomarkers of iron metabolism and metabolic dysfunction-associated fatty liver disease in individuals with type 2 diabetes and non-diabetic individuals compared to a control group. We also examined the possible association between estimated liver fibrosis and serum ferritin levels in all three groups. **Methods**: We conducted a descriptive, cross-sectional, comparative study involving subjects diagnosed with diabetes and/or metabolic dysfunction-associated fatty liver disease from an outpatient diabetology clinic and two general practices in Târgu Mureș. The patient population was divided into 3 groups: first group including diabetic patients suffering from fatty liver disease, second group including patients without fatty liver disease and third group with non-diabetic patients suffering from fatty liver disease. We compared the three groups based on specific laboratory tests. **Results:** Patients with fatty liver disease had significantly higher ferritin and transferrin saturation levels than non-fatty liver disease sufferers ($p<0.05$). Transferrin saturation of the first group was significantly ($p<0.05$) higher compared to the non-diabetic fatty liver disease group. Ferritin correlated well with Fibrosis-4 index level ($\tau= 0.193$, $p<0.01$) considering the whole sample and especially in the first group. **Conclusions**: In our study, there was a clear association between higher ferritin levels and the presence of metabolic dysfunction-associated fatty liver disease. The higher transferrin saturation observed in diabetic patients suffering from metabolic dysfunction-associated fatty liver disease may indicate the possible etiological significance of iron overload. Higher ferritin levels in diabetes increase the risk of liver fibrosis.

Keywords: fatty liver, iron biomarkers, liver fibrosis, metabolic syndrome

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Introduction

Metabolic associated fatty liver disease (MAFLD) according to new epidemiological data affects about a quarter of the world's population, representing a significant economic and health burden for our society. [1] The old term for this condition has been Non-alcoholic fatty liver disease (NAFLD) for a long time, but today fatty liver is considered the liver component of metabolic syndrome, a disorder closely related to type 2 diabetes, insulin resistance and cardiovascular disease, and many scientists have proposed this new and flexible term [2]. Based on the consensus accepted in recent years by international research groups, the diagnosis of MAFLD is based on the presence of steatosis and at least one additional factor from obesity/overweight or diabetes or two other elements of metabolic dysregulation simultaneously present from the following: waist circumference > 102/88 cm for Caucasian men and women, blood pressure > 130/85 mmHg or specific medication, plasma triglyceride level >150 mg/dl or specific medication, plasma HDL cholesterol <40 mg/dl in men and <50 mg/dl in women or specific medication, pre-diabetes (fasting glucose 100-125 mg/dl or post-exercise glucose 140-199 mg/dl or glycated haemoglobin 5.7-6.4% after 2 hours of exercise), Homeostatic Model Assessment for Insulin Resistance - insulin resistance score ≥2 5, plasma high-sensitivity C-reactive protein level >2 mg/l [3]. This new definition does not necessarily require exclusion criteria [1].

The effect of iron in the etiopathophysiology of fatty liver disease is based on the following: insulin resistance in the liver, insulin resistance in adipose tissue, oxidative stress, circadian rhythm. Regarding the relationship between MAFLD and iron metabolism, it can be affirmed that the harmful effects of iron are attributed to its ability to catalyse the formation of toxic hydroxyl radicals that cause damage to cell function [4]. Ferroptosis is a recently discovered iron-dependent programmed cell death that may play an important role in the development and progression of various chronic metabolic syndrome related diseases. Its main features in fatty liver disease are the accumulation of lipid peroxides and the presence of reactive oxygen species [5]. Moreover, another important related pathology is dysmetabolic iron overload syndrome, which is a disorder associated with a mild increase in liver and body iron stores associated with various components of metabolic syndrome, in which no specific etiological factor can be attributed to iron overload [6]. Finally, serum ferritin can be considered a non-invasive marker in identifying patients with MAFLD at high risk of disease progression. Serum ferritin value of 1.5-fold above the upper limit of normal is significantly associated with an increased risk of advanced fibrosis in patients with fatty liver [7].

The main objectives of our study were to investigate the possible association between biomarkers of iron metabolism and MAFLD in fatty liver disease patients with or without type 2 diabetes compared to a control group, and to examine the possible association between estimated liver fibrosis and serum ferritin.

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Methods

Our study was a descriptive, cross-sectional, comparative study including 131 participants diagnosed with diabetes and/or MAFLD. Participants were selected for our study using the following data collection sites and databases provided by these institutions: a diabetes outpatient clinic in Târgu Mureș, two family medicine practices in Târgu Mureș. Our research plan was approved by the Ethics Committee of University of Medicine, Pharmacy, Science and Technology of Târgu Mureș and written and oral informed consent was obtained from the study participants.

Participants were divided into three groups according to the following selection criteria based on diagnostic considerations:

- Group 1 included patients who simultaneously met diagnostic criteria for MAFLD and diabetes.
- Group 2 included patients with diabetes but without MAFLD.
- Group 3 included people diagnosed with MAFLD but without diabetes.

For all three groups, the following exclusion criteria were applied: alcohol consumption greater than 30 g per day, any chronic inflammatory disease or high Ultra-Sensitive C-Reactive Protein (>10). There was no significant difference between the groups in terms of age and gender distribution.

After grouping and applying the inclusion and exclusion criteria, our data elements were centralized using Microsoft Excel (Microsoft® Excel® 2016). The most relevant parameters monitored for all three groups were the following:

- laboratory markers on iron status (serum iron, ferritin, transferrin saturation)
- liver function markers (GOT, GPT, GGT, Fib4) – Fibrosis-4 index was calculated using the following known formula (age in years, GOT and GPT in IU/L and platelet count in 10^9 /L): Fib4 index=(age * GOT)/(platelet count *√GPT).

The statistical software used in our study was SPSS Statistics (IBM SPSS®). Prior to the actual statistical tests, we initially excluded inaccurate data, analysed outliers, and then performed the Kolmogorov-Smirnov test with Lilliefors significance correction and the Shapiro-Wilk test to examine the normality of the distribution of variables across each group. If for a variable, at least one distribution of the three group was not normal, we applied the Kruskall-Wallis test for the following examinations. For the analysis of contingency tables including categorical variables, the Chi-square test was performed. The results of the different statistical tests were considered statistically significant at $p<0.05$.

Results

For the whole sample, the average age is 61.4 years (standard deviation (SD)=12.85)) and the median age is 63 years. The oldest participant was 83 years old and the youngest was 25 years old. In terms of gender distribution, 77 female patients (59%) and 54 male patients (41%) were studied. The mean body mass index was 29.91 kg/m^2 (SD=5.25). The percentage of women was 53% in group 1, 65% in group 2 and 60% in group 3. The median ages and average body mass index in each group can be seen in *Table I*.

When comparing ferritin levels, the Kruskall-Wallis test indicates that there is a significant difference between the 3 groups ($p<0.05$). Based on the results of the post-hoc tests performed to compare the groups pairwise, it can be affirmed that the ferritin levels of those with MAFLD are significantly higher than of those without MAFLD (126.12 – median of group 1 and 136.24 – median of group 3 versus 37.4 group 2). There is a significant difference between group 1 and 2 ($p=0.0002$), and between group 3 and 2 ($p=0.00001$). There is also a significant difference among the groups in the prevalence of ferritin values above 200 ng/ml according to the Chi-square test ($p<0.05$), but Figure 1 shows that only between groups 1 and 2 there is a significant difference.

Similarly, there is a significant difference in transferrin saturation within the 3 groups ($p=0.004$). The results of post hoc tests performed to compare the pairwise groups indicate that the transferrin saturation of diabetics suffering from MAFLD is significantly higher compared to groups 2 and 3 (medians 31.6 vs. 19.37 vs. 20.75 respectively). There is a significant difference between groups 1 and 2 and between groups 1 and 3 ($p=0.09$ and $p=0.047$). In contrast to the previous variables, there is no significant difference between groups in serum iron ($p>0.05$). The distribution of ferritin, transferrin saturation and serum iron values is shown in Figure 2.

Ferritin correlates positively with FIB4 values in the whole sample ($\tau= 0.193$, $p<0.01$, $N=112$) as it can be seen graphically in Figure 3 too. Analysing this correlation by groups we observe that only in group 1 was a significant correlation ($\tau=0.225$, $p<0.05$, $N=41$) present. When the whole sample is divided into subgroups according to the two genders, while the correlation was significant for women ($p=0.012$, $\tau=0.214$, $n=65$), it was not significant for men ($p=0.058$). When looking at the whole sample, there is a significant difference between the FIB4 values of those with a ferritin level lower than 200 ng/ml and those with a ferritin level higher than 200 ng/ml ($p<0.05$, median values 1.06 vs 1.51). If we analyse this correlation in each group, we see that there is a significant correlation ($p<0.05$) only in groups 1 and 3.

Discussions

Our results indicate that participants diagnosed with MAFLD have significantly higher ferritin levels than those

Table I. Median ages and average body mass index in each group

Group	Median age (Interquartile range)	Mean Body Mass Index (Standard Deviation)
1	63 (17) years	31.25 (4.56) kg/m^2
2	61 (19) years	28.82 (5.78) kg/ m^2
3	68 (19) years	29.65 (5.32) kg/ m^2

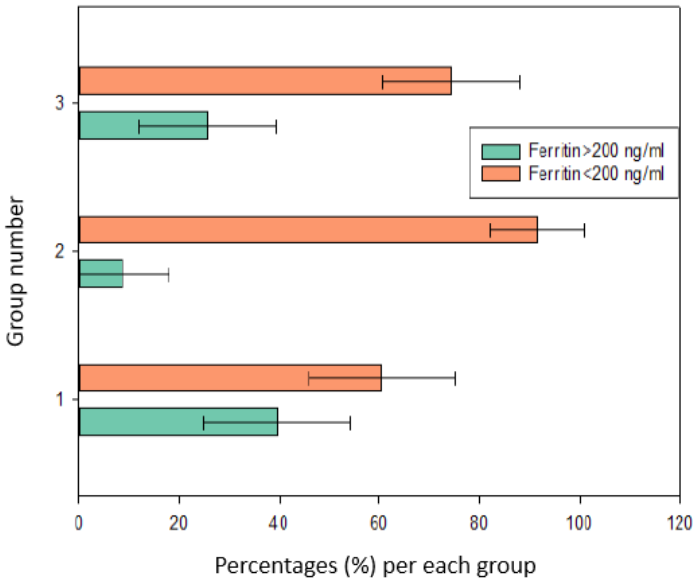

Fig. 1. Percentage and confidence intervals of ferritin values above 200 ng/ml in each group, source: own- Sigmaplot application

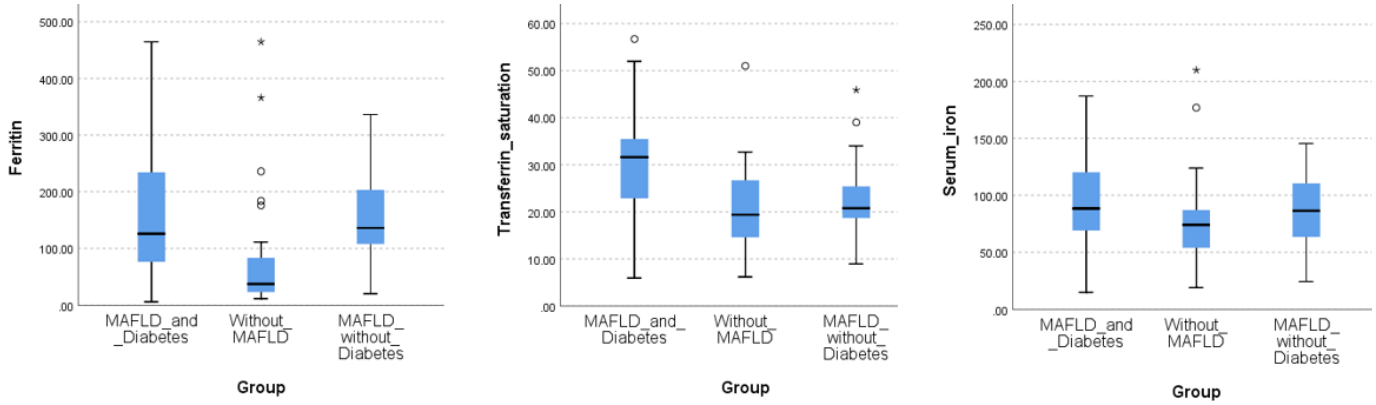

Fig. 2. Distribution of ferritin, transferrin saturation and serum iron values, Source:Own -SPSS application

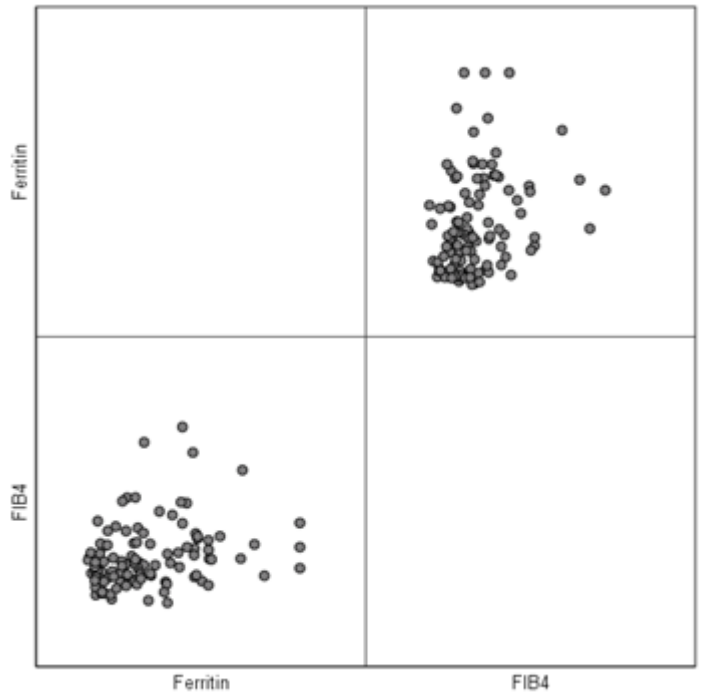

Fig. 3. Matrix representing the two variables as a function of each other for the whole sample, Source: Own -SPSS application

without fatty liver. A 2017 multivariate analysis identified hepcidin and intrahepatic iron as the main determinants of ferritin levels in fatty liver. The study also confirmed the association between ferritin and hepatic iron in a larger, independent cohort of patients with MAFLD. Consistent with previous findings, iron deposition in the fatty liver was predominantly non-parenchymal or mixed non-parenchymal and hepatocellular in distribution [8]. To exclude other causes of hyperferritinemia we used any chronic inflammatory disease or high US-CRP (>10) as exclusion criteria during data collection. Similarly, a study published in 2021 in the journal Endocrine Connections showed that diabetic patients with fatty liver had significantly higher serum ferritin levels compared to diabetic patients without fatty liver. This study concluded that serum ferritin is an independent risk factor for patients with MAFLD and type 2 diabetes [9].

Moreover, another important finding of our study is that the percentage of those with ferritin levels higher than 200 ng/ml is significantly greater in the group diagnosed with diabetes and fatty liver than in the group diagnosed with diabetes alone. Ferritin levels are mainly influenced by the body's iron stores, but liver inflammatory processes also increase ferritin levels, so high ferritin levels are common in fatty liver. In terms of comparison with previous literature, a study including 628 patients showed that a serum ferritin level greater than 1.5-fold above the upper normal limit was significantly associated with the following: increased likelihood of non-alcoholic steatohepatitis diagnosis, higher degree of steatosis, lobular inflammation and bloating of hepatocellular aetiology [10]. In contrast to the scientific article mentioned above, ferritin values in our study were in a smaller range (mean 131 ng/ml, standard deviation 103.56 ng/ml), so values above 200 ng/ml were considered as higher ferritin levels. In addition, several studies have concluded that hyperferritinemia, in combination with normal transferrin saturation, may be a marker of glucose or lipid metabolic disorders [9].

Another study claims that in patients diagnosed with MAFLD in general, hyperferritinemia is caused mainly by inflammation of the liver without iron overload. A smaller proportion is represented by dysmetabolic iron overload syndrome which can be defined in terms of laboratory parameters as hyperferritinemia with normal or only slightly elevated transferrin saturation. The smallest group is represented by fatty liver patients with hemochromatosis which can be characterised with a level of transferrin saturation usually increased (> 45%), therefore, screening for hemochromatosis mutations in MAFLD patients with hyperferritinemia is only recommended above this value of transferrin saturation [11]. In dysmetabolic iron overload syndrome, serum ferritin concentration overestimates iron overload in contrast to hemochromatosis, which can be probably due to the inflammatory state manifested in MAFLD [4].

Moreover, our results suggest that the group with diabetes and fatty liver has significantly higher transferrin saturation compared to the other two groups, which may indicate iron overload as a possible etiological factor. Several previous studies have shown that a transferrin saturation above 50% increases the risk of developing any form of diabetes, including type 1 and type 2 diabetes, by two to three times. Because treatment of iron over-saturation is simple (phlebotomy), 1-3% of future diabetics in the general population and 7% of cases among newly diagnosed diabetics could be easily prevented and managed if patients received such treatment earlier in life [12]. In our study, the proportion of subjects with transferrin saturation above 50% was much lower than in the previously mentioned study (only 7.54% in our research), but subjects with diabetes and liver disease in our case also had significantly higher transferrin saturation compared to the non-diabetic group with liver disease only.

There was no significant difference in serum iron among the three groups. Interestingly, a previous scientific study showed an inverse association between serum iron and the presence of non-alcoholic fatty liver disease, concluding that serum iron is inversely correlated with the risk of cardiovascular disease, chronic alcoholic liver disease and the presence of diabetic retinopathy. However, the pathogenetic mechanisms underlying this association are not well understood [13].

Based on the ferritin and estimated liver fibrosis correlations which can be seen in Results section, it is very likely that ferritin levels are a marker of liver fibrosis in patients diagnosed with both diabetes and MAFLD. Moreover, we observed that that high ferritin levels in patients with fatty liver disease are a marker of advanced fibrosis and that ferritin is a very accurate marker of liver fibrosis especially in women. A previous study involving 628 patients in a clinical trial showed that a serum ferritin level one and a half times higher than the upper normal limit is an independent marker of advanced fibrosis and found an odds ratio of 9.67 for increased FIB-4 in the highest quartile of ferritin in women and 2.47 in men [10].

Considering research limitations, had it been a higher number of sample size, we would have had more precise results, especially in subgroup level correlations. However, this objective requires more significant financial resource, as some of the iron metabolism laboratory markers can be categorized as expensive category for the general population. We are planning to find new types of sponsorship which will let us to continue the exploration of this research topic with a higher number of participants.

Conclusion

Our study is, in some respects, unique in the literature because we have not found a similar division based on a 3-group comparison that examines all important parameters of iron metabolism equally. Previous literature suggests

that both newly discovered iron deficiency and dysmetabolic iron overload syndrome may play an important role in the pathophysiology of MAFLD and that serum ferritin levels may independently predict severe fibrosis. Our present study aims to complement this pathological clarification by reaching the following conclusions:

- there is a clear association between higher ferritin levels and the presence of MAFLD
- patients with diabetes and MAFLD have a significantly higher transferrin saturation value and thus we can think of iron overload as a possible etiological factor
- elevated ferritin levels are a marker of liver fibrosis, and this biomarker is the most accurate predictor of liver fibrosis in people diagnosed with fatty liver disease and diabetes as well as in women.

The scientific value of our study and the prospect of pursuing this scientific topic lies in the need to clarify the etiopathogenesis before proposing appropriate and effective treatment for MAFLD. If iron metabolism disorders are accepted as a major pathogenic factor in the development of fatty liver disease, the scientific importance of several new intervention and prevention tools will be evaluated, such as: the future emergence of phlebotomy as a potential treatment and prevention tool for MAFLD.

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Authors' contribution

EZZS (Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Software; Visualization; Writing – original draft)

SZM (Conceptualization; Project administration; Resources; Supervision; Validation; Writing – review & editing)

Conflict of interest

None to declare.

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