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ORIGINAL ARTICLE

Value of Serum Ferritin in Combination with Alanine Aminotranseferase and Glucose Levels in Assessment of Non-Alcoholic Fatty Liver Disease in Obesity

Hala Abd_Allah Mahmoud [1], Yaser Abd_Elmohsen Diab[1], Aya Shehata Waheib[1*], Abdallah Mahmoud Abdallah[2], Weaam Mohamed Mohamed Ali[1]

[1]Department of Internal Medicine, Faculty of Medicine, Aswan University

[2]Department of Biochemistry, Faculty of Medicine, Aswan University

ABSTRACT

Keyword: Alanine Aminotranseferase, Glucose Levels, Non-Alcoholic Fatty Liver Disease, Serum Ferritin

[*] **Corresponding author**: Aya Shehata Waheib
Mobile: 01028358505
E-mail: ayashehata499@gmail.com

Background: Possible substitutes for liver biopsies in this setting include noninvasive biomarkers of the liver and repeatable surrogate regular laboratory tests. The study aimed to determine whether ferritin, on its own or in conjunction with other common biochemical markers, might be a helpful diagnostic tool for non-alcoholic fatty liver disease. **Methods:** This cross-sectional study was carried out on 100 patients aged from 18 to 70 years old, with metabolic syndrome. Patients were divided into three groups in accordance with serum ferritin levels: T1: between 6.00 and 67.00 (ng/ml). T2: between 68.00 and 177.00 (ng/ml). T3: between178.00 and 560.00 (ng/ml) **Results: There** was a significant difference among groups in liver function tests. GGT levels also increased significantly from T1 to T2 and T2 to T3. There was no statistically significant difference between these two groups for F1 degree, whereas both show significant differences when compared to T3. There was a negative correlation between serum ferritin and BMI, waist circumference, systolic blood pressure, diastolic blood pressure, and glucose levels, and significant positive correlations was noted with liver enzymes: ALT, AST, and particularly GGT. **Conclusions:** Serum ferritin may serve as a non-invasive diagnostic for evaluating different stages of NAFLD. The majority of individuals with NAFLD have increased levels of serum ferritin. However, the degree of elevation is not indicative of the severity of the underlying liver disease.

INTRODUCTION:

Nowadays, the majority of people throughout the globe suffer from non-alcoholic fatty liver disease (NAFLD). A significant cause of hepatocellular carcinoma (HCC) and one of the leading indications for liver transplantation is NAFLD [1]**.** Screening for problems and providing liver-specific medications would be futile in patients who are unlikely to develop cirrhosis and HCC, as only a small percentage of NAFLD patients will experience these outcomes [2].

Hypertriglycerideemia (the buildup of lipids in the liver cells) is a hallmark of NAFLD, which is related to insulin resistance, obesity, and excess body fat. NAFLD is marked by oxidative stress and inflammation. Lipid peroxidation, which compromises membrane function and structure, can result from an elevation in reactive oxygen species (ROS) [3].

It may lead to the oxidation of nucleic acids and oxidize proteins that are essential for cellular metabolism and function [4].

Because the liver can only store so many triglycerides, lipid deposition in conditions of overfeeding, like NAFLD, leads to the buildup of excessive amounts of fatty acids, most of which are saturated, which can cause cell dysfunction [5].

When inflammation is present, ferritin, the principal protein for storing iron, is often found in the blood and the liver. An increased risk of NAFLD has been associated with elevated serum ferritin levels [6].

In both clinical and epidemiological settings, liver ultrasonography is the imaging method of choice for the initial diagnosis of NAFLD due to its safety, low cost, widespread availability, and good tolerability [7]. I find it interesting that abnormalities in lipid metabolism, which are common in NAFLD, can be used as a standalone indicator of hyperechoic liver on ultrasonography. One study found that obese people with semi-quantitative ultrasonography assessments of fatty liver had higher quantities of non-esterified fatty acids. In addition, in non-obese, non-diabetic participants, the ultrasound-based diagnosis of NAFLD was more predictive of insulin resistance than the ATPIII criteria [8].

The aim of this work was to determine whether ferritin, on its own or in conjunction with other common biochemical markers, might be a helpful diagnostic tool for NAFLD.

PATIENTS AND METHODS:

This cross sectional study was carried out on 100 patients aged from 18 to 70 years old, with metabolic syndrome, no history of chronic viral hepatitis and primary liver disease, non - alcohol abuse, non-hepatotoxic drugs, BMI equal to or greater than 30, type II diabetes mellitus], from February 2023 to September 2023.

The study was done after approval by the Ethical Committee Aswan University Hospitals. An informed written consent was obtained from the patients.

Exclusion criteria were cardiovascular disease, hepatocellular carcinoma, history of alcohol abuse, history of chronic viral hepatitis and primary liver disease, liver cirrhosis, BMI less than 30 and, use of hepatotoxic drugs.

Patients were divided into 3 groups in accordance with serum ferritin levels (Tertiles):

- T1: ferritin levels between 6.00 and 67.00 (ng/ml).
- T2: ferritin levels between 68.00 and 177.00 (ng/ml).
- T3: ferritin levels between178.00 and 560.00 (ng/ml)

All patients were subjected to: Assessment of nonalcoholic fatty liver disease in very obese patients by imaging and noninvasive testing, History taking (gender, age, blood pressure, weight, BMI and medical History), laboratory investigations [Seum Ferritin, tests for glucose and glycosylated hemoglobin A1c (HbA1c), uric acid, serum lipids, and liver function, including aspartate aminotransferase, alkaline phosphatase, S-albumin, prothrombin time, and alkaline chloride. Blood tests for hepatitis B surface antigen (HbsAg) and anti-hepatitis C virus will also be administered to all individuals whose livers show abnormalities on ultrasound]

Ultrasonographic examination

Two out of the three following criteria were present in the diagnosis of fatty liver.

1) The liver is more echogenic than the kidney or spleen.

2. Vascular opacification of the liver.

3. The ultrasonographic signal is significantly muted.

Statistical analysis

Statistical analysis was done by SPSS v25 (Armonk, NY: IBM Corp). Quantitative variables were presented as mean and standard deviation (SD) and were compared by Student's T- test for the same group. Qualitative variables were presented as frequency and percentage (%) and were compared by Mann Whitney Test (U test). Shapiro-Wilk test was used for normality of data distribution, the Kruskal-Walli's test used to evaluate the disparity among many research groups' non-parametric variables. one-

way ANOVA test used for multiple parametric variables in different study groups. The Chi-Square test was employed to analyze the association between two categorical variables. A two tailed P value < 0.05 was considered significant.

RESULTS:

There were no statistically significant variations between three study groups regarding age, gender and physical activity. The pairwise comparisons for demographic data and physical activity between the groups also indicate no significant differences. Table 1

Table 1: Comparison between examined groups consistent with demographic data, Physical activity.

		T1	T2	T3	Test Result	Pairwise Comparisons
		n=36	n=34	n=30		
Age (years)	Mean ± SD	43.89 ± 5.26	42.91 ± 5.46	44.43 ± 5.54	F=1.363, p=0.506	p2 = 0.429 p3 = 0.624 p4 = 0.280
	Median (Min-Max)	44 (36- 52)	42 (36 -52)	45 (36 -52)		
Gender	Female	13(36.1%)	12(35.3%)	10(33.3%)	X2=0.057, p=0.972	p2: 1.000 p3: 1.000 p4: 1.000
	Male	23(63.9%)	22(64.7%)	20(66.7%)		
Physical activity	Elevated	3(8.3%)	3(8.8%)	2(6.7%)	X2= 3.766, p=0.708	p2= 0.306 p3= 0.638 p4= 0.912
	Mild	10(27.8%)	8(23.5%)	7(23.3%)		
	Moderate	5(13.9%)	11(32.4%)	8(26.7%)		
	Never	18(50.0%)	12(35.3%)	13(43.3%)		

F: One way ANOVA test, X2: Chi square test, p2: comparison between T1 and T2, p3: comparison between T1 and T3, p4 comparison between T2 and T3

There was a significant difference regarding BMI, p-values are less than 0.001. Regarding waist circumference, T1 again shows the highest mean value, with T3 having the lowest, similar to the BMI results with a highly significant difference across the groups. Pairwise comparisons indicate significant distinctions between T1 and the other two groups, but not between T2 and T3. Table 2

Table 2: Comparison between studied groups according to anthropometric measurements.

		T1	T2	T3	Test Result	Pairwise Comparisons
		n=36	n=34	n=30		
BMI (kg/m2)	Mean ± SD	34.63 ± 2.04	33.26 ± 2.27	32.02 ± 1.9	H= 29.330, p<0.001*	p2 <0.001* p3 <0.001* p4 <0.001*
	Median (Min-Max)	34.77 (32.10-40.75)	32.86 (31-40.82)	31 (31.2-39.81)		
Waist circumference (cm)	Mean ± SD	113.81 ± 2.34	108.56 ± 2.15	107.83 ± 2.74	F= 62.763, p<0.001*	p2 <0.001* p3 <0.001* p4 = 0.248
	Median (Min-Max)	113.50 (107 -119)	108.5 (103 -113)	107.5 (102 -116)		

The systolic blood pressure results show that Group T1 has the highest mean value, followed by T2 and T3, with each group showing statistically significant variances from the others with all p-values indicating significant differences (p=0.028 for T1 vs T2, p<0.001 for T1 vs T3, and p=0.045 for T2 vs T3). For diastolic blood pressure, similar trends are observed. T1 has the highest diastolic pressure, though the distinction between T1 and T2 is not statistically significant (p=0.429). However, significant differences are found between T1 and T3 (p<0.001), and between T2 and T3 (p=0.012), indicating that

T3 generally has lower diastolic blood pressure compared to the other two groups. Statistical test results for fasting glucose showed highly significant differences. T3 has notably lower fasting glucose levels compared to T1 and T2. In contrast, the HbA1c levels across the groups showed no significant differences.

There were no significant differences for lipid profile including total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides, across study groups. According to liver functions, significant differences among groups in liver function tests (ALT, AST, GGT) with p-values less than 0.001. Specifically, ALT and AST levels indicate that T3 has significantly higher levels compared to T1 and T2, with T3 showing a clear upward trend in enzyme levels across all parameters. GGT levels also increased significantly from T1 to T2 and T2 to T3. Table 3

Table 3: Comparison between groups studied according to blood pressure, glucose metabolism variables, lipid profile and liver functions.

	T1	T2	T3	Test Result	Pairwise Comparisons
	n=36	n=34	n=30		
Systolic blood pressure (mmHg)	134.06 ± 5.72	130.82 ± 6.25	127.70 ± 5.69	H= 16.02, p<0.001*	p2 = 0.028* p3<0.001* p4 = 0.045*
	134 (124 -143)	130 (121 -142)	126 (120 -138)		
Diastolic blood pressure (mmHg)	87.14 ± 3.58	86.38 ± 2.70	84.07 ± 4.27	H= 10.314, p<0.001*	p2 = 0.429 p3<0.001* p4 = 0.012*
	87 (82 -93)	86 (83 -91)	83 (79 -91)		
Fasting Glucose (mg/dL)	102.25 ± 2.55	103.18 ± 2.14	97.17 ± 2.67	H=47.359, p<0.001*	p2 = 0.103 p3 <0.001* p4 <0.001*
	102 (95 -106)	103 (98 -108)	97 (93 -103)		
HbA1c (%)	6.37 ± 1.64	6.06 ± 1.30	6.38 ± 1.78	H=0.272, p=0.873	p2 = 0.668 p3 = 0.685 p4 = 0.800
	5.7 (5 -11.3)	5.55 (5 -9.4)	5 (5 -9.7)		
Total cholesterol (mg/dL)	194.69 ± 16.69	193.35 ± 22.99	197.70 ± 22.96	H=0.742, p=0.690	p2 = 0.585 p3 = 0.562 p4 = 0.476
	196.5 (167 -223)	191.5 (159 -230)	197.5 (162 -231)		
LDL cholesterol (mg/dL)	115.89 ± 15.65	118.35 ± 16	116.7 ± 17.37	H=0.272, p=0.873	p2 = 0.569 p3 = 0.867 p4 = 0.845
	114 (94-142)	120.5 (93 -143)	110.5 (91 -144)		
HDL cholesterol (mg/dL)	52.08 ± 5.85	54.47 ± 5.13	54.47 ± 6.21	H=3.912, p=0.141	p2 = 0.086 p3 = 0.101 p4 = 0.798
	52 (43 -61)	56 (46 -61)	55.5 (41-64)		
Triglyceride (mg/dL)	119 ± 24.37	119.38 ± 16.13	120 ± 14.59	H=0.068, p=0.967	p2 = 0.967 p3 = 0.847 p4 = 0.808
	118 (82- 155)	121.5 (93 -143)	114.5 (92-144)		
ALT (IU/L)	29.42 ± 2.08	30.00 ± 1.39	36.87 ± 2.86	H=55.919, p<0.001*	p2 = 0.385 p3 <0.001* p4 <0.001*
	30 (25 -33)	29 (29-34)	37 (27 -41)		
AST (IU/L)	20.72 ± 2.02	20.35 ± 2.20	25.33 ± 2.78	H=43.648, p<0.001*	p2 = 0.506 p3 <0.001* p4 <0.001*
	20.5 (18-24)	20 (17 -24)	25 (21 -32)		

GGT (IU/L)	24.33 ± 2.14	29.18 ± 0.58	38.30 ± 4.10	H=80.275, p<0.001*	p2 <0.001* p3 <0.001* p4 <0.001*
	24 (20 -30)	29 (29 -32)	38.5 (22- 45)		

H: Kruskal Wallis test, * for significant p value (<0.05), p2: comparison between T1 and T2, p3: comparison between T1 and T3, p4 comparison between T2 and T3

Regarding ultrasound degrees there was a highly significant p-value (<0.001), indicating substantial differences in steatosis degree distributions among the groups. The data showed that for the mild steatosis degree (F1), the percentages are fairly similar among T1 and T2 (44.4% and 47.1%, respectively) and slightly lower for T3 (36.7%). The pairwise comparison between T1 and T2 (p2=1.000) supports no statistically significant difference between these two groups for F1 degree, whereas both show significant differences when compared to T3 (p3 and p4 < 0.001). For moderate steatosis degree (F2), the differences are less pronounced, with T1 showing 55.6%, T2 at 52.9%, and a notable decrease to 30.0% for T3. The most striking difference is observed in the severe steatosis degree (F3), where neither T1 nor T2 have any cases (0%), but T3 has a substantial 33.3%. Table 4

Table 4: Comparison between study groups according to ultrasound steatosis degree.

		Group T1	Group T2	Group T3	Test Result	Pairwise Comparisons
		n=36	n=34	n=30		
Ultrasound steatosis degree	F1	16(44.4%)	16(47.1%)	11(36.7%)	X2= 26.422, p<0.001*	p2= 1.000 p3< 0.001 p4< 0.001
	F2	20(55.6%)	18(52.9%)	9(30.0%)		
	F3	0(0.0%)	0(0.0%)	10(33.3%)		

There was a strong negative correlation between serum ferritin and BMI, waist circumference, systolic blood pressure, diastolic blood pressure, and glucose levels, all indicating that higher ferritin levels are associated with lower values of these metabolic health indicators. Conversely, significant positive correlations are noted with liver enzymes: ALT, AST, and particularly GGT, suggesting that higher ferritin levels may be linked to increased liver activity or inflammation. Other parameters studied didn't show significant correlation. Table 5-Figure 1

Table 5: Correlation between ferritin and other parameters studied.

	r_s	P-Value
Age (years)	0.004	0.966
BMI (kg/m2)	-0.521	<0.001*
Waist circumference (cm)	-0.673	<0.001*
Systolic blood pressure (mmHg)	-0.369	<0.001*
Diastolic blood pressure (mmHg)	-0.320	<0.001*
Glucose (mg/dL)	-0.505	<0.001*
HbA1c (%)	-0.049	0.630
Total cholesterol (mg/dL)	-0.048	0.637
LDL cholesterol (mg/dL)	-0.006	0.956
HDL cholesterol (mg/dL)	0.129	0.199
Triglyceride (mg/dL)	-0.036	0.724
ALT (IU/L)	0.643	<0.001*
AST (IU/L)	0.473	<0.001*
GGT (IU/L)	0.857	<0.001*

rs: Spearman correlation coefficient, * for significant p value (<0.05)

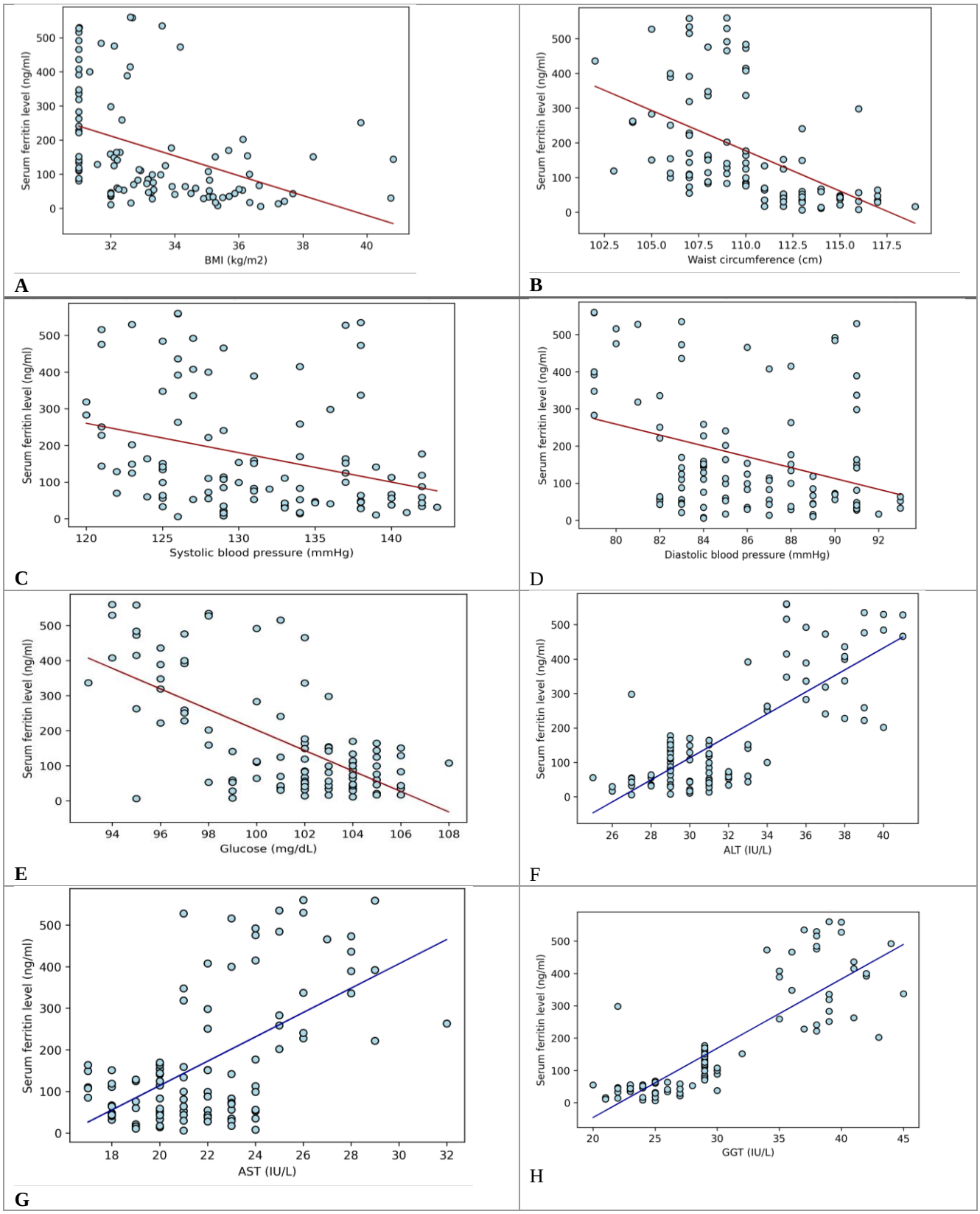

Figure 1: Correlation between serum ferritin and (A) BMI, (B) waist circumference, (C) SBP, (D) DBP, (E) fasting glucose, (F) ALT, (G) AST and (H) GGT.

DISCUSSION

The current gold standard for diagnosing NASH is a liver biopsy, which is still necessary despite the fact that it is an intrusive, costly, and potentially dangerous operation that only accounts for about 1/50,000 of the total hepatic volume. Possible substitutes for liver biopsies in this setting include noninvasive biomarkers of the liver and repeatable surrogate regular laboratory tests. Consequently, there has been a recent uptick in the search for better diagnostic and predictive biomarkers to spot NAFLD symptoms in their earliest stages [9].

The current study estimates that for age, the mean values across the groups are fairly close, with T3 having a slightly higher mean age compared to T1 and T2, though this difference is not statistically significant as indicated by the overall test results. In terms of gender, the proportion of females to males is consistent across the groups, with about one-third females and two-thirds males in each group. The statistical analysis confirms that there were no significant differences in gender composition. The pairwise comparisons for demographic data between the groups also indicate no significant differences. In this context, Galarregui et al., estimated that the mean age of the study groups was 51 ± 9 years old, and 42% were women. In which the mean age of T1, T2 and T3 groups was 53 (48; 64), 47 (43; 52) and 51 (46; 56) respectively. Also, the gender of the three study groups were distributed as male to female ratio by 22\16, 22\16 and 21\15 for groups T1,2 and 3 respectively. Also, there were no statistically differences between the three groups in relation to the sex and age [10].

While this result is in contradiction with Zhang et al., who mentioned that A total of 221 children with obesity were categorized as having NAFLD. The mean age of these children was 11.52 ± 2.39 years, with 164 males and 57 females. Additionally, there were 126 obese children without NAFLD, with a mean age of 10.42 ± 2.62 years, consisting of 84 men and 42 females.

The current study mentions that a higher percentage of participants in Group T2 report Moderate activity compared to T1 and T3. Conversely, the highest percentage of participants never engaging in physical activity is seen in Group T1. Despite these differences, the overall statistical test indicates that there are no significant differences in the distribution of physical activity levels across the groups. The pairwise comparisons between groups for physical activity levels further support the test results [11].

This outcome is consistent with Galarregui et al., who found that higher percentage of participants in Group T2 report Moderate activity compared to T1 and T3. Conversely, the highest percentage of participants never engaging in physical activity is seen in Group T1 [10].

The current study mentioned that the results Group T1 has the highest mean BMI, followed by T2, and then T3, which has the lowest. This descending pattern is statistically significant, with each group statistically different from the others, as shown by pairwise comparisons where all p-values are less than 0.001. This study result is in agreement with Galarregui et al., who found that the mean BMI of the individuals was 34 ± 4 kg/m2, with a waist circumference of 110 ± 8 cm for all the study groups in which BMI for the three groups were 34.0 (32; 36), 33.6 (31; 36) and 31.9 (31; 35) respectively [10].

For waist circumference, T1 again showed the highest mean value, with T3 having the lowest, similar to the BMI results. The statistical analysis reveals a highly significant difference in waist circumferences across the groups. Pairwise comparisons indicate significant differences between T1 and the other two groups, but not between T2 and T3. In contradiction with Galarregui et al., who found that the waist circumference mean of all the study groups was 108.9 (104; 116) while it was 114.1 (105; 118), 108.8 (101; 115) and 106.8 (102; 112) for group T1,2 and 3 respectively. They also, estimated that no significant variances were seen among the serum ferritin groups with respect to anthropometric and body composition factors [10].

The present study found that ferritin concentration was positively correlated with body mass index and waist circumference, it stands to reason that a higher ferritin level would be associated with a higher prevalence of NALFD. Nonetheless, results also demonstrated that serum ferritin was persistently

associated with NAFLD in models adjusted for body mass index, fasting hyperglycemia, and other potential covariates [12].

In relation to Comparison between studied groups according to glucose metabolism variables the current study illustrates that T3 has notably lower fasting glucose levels compared to T1 and T2, as evidenced by both mean and median values. The pairwise comparisons further illuminate these differences, with T3 significantly differing from both T1 ($p < 0.001$) and T2 ($p < 0.001$), while the difference between T1 and T2 is not statistically significant ($p = 0.103$). In contrast, the HbA1c levels across the groups do not show significant difference. This result is in agreement with Galarregui et al., people who hypothesized that additional investigation into the correlation between ferritin levels in the blood and metabolic processes involving glucose, lipids, and the liver was conducted. Regarding glucose metabolism, there were positive correlations between serum ferritin and HOMA-IR and the TyG index [10].

As regard to Comparison between studied groups according to lipid profile. The current study mentions that Statistical tests used to compare these groups indicate no significant differences for lipid profile. In this context, Galarregui et al., found that We dug more into the connection between ferritin levels in the blood and metabolic processes involving the liver, lipids, and glucose. When looking at lipid characteristics, we found that serum ferritin levels were positively correlated with TG and the TG/HDL index, although HDL-c was inversely correlated with ferritin [10].

Concerning comparison between studied groups according to liver functions, the current study shows that there were significant differences among groups in liver function tests (ALT, AST, GGT) with p-values less than 0.001. Specifically, ALT and AST levels indicate that T3 has significantly higher levels compared to T1 and T2, with T3 showing a clear upward trend in enzyme levels across all parameters. GGT levels also increased significantly from T1 to T2 and T2 to T3. In this context, Galarregui et al., estimated that Hepatic iron, hepatic fat, liver volume, steatosis degree, ALT, AST, and GGT were all positively connected with serum ferritin when it came to hepatic condition. When looking at cytokines, ferritin was found to have strong positive relationships with both DPP4 and RBP-4 [10].

Further-more, Zhang et al., estimated that in their study in which Using statistical software, all subjects were separated into three groups according to ferritin tertiles (1st Q: ferritin < 39.0 μg/L, 2nd group: ferritin ≥ 39.1 – 63.3 μg/L, and 3rd Q: ferritin > 63.4 μg/L) In every model, it was found that medium and high serum ferritin levels were significantly unrelated to NAFLD [13].

Limitations: The sample size was relatively small. There was a lack of a control group. NASH was diagnosed using noninvasive criteria such as NLFS, HSI, and other methods, rather than relying on liver biopsy.

CONCLUSIONS:

Serum ferritin may serve as a non-invasive diagnostic for evaluating different stages of NAFLD. The majority of individuals with NAFLD have increased levels of serum ferritin. However, the degree of elevation is not indicative of the severity of the underlying liver disease.

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