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Retrospective Analysis of the Relationship of Serum Magnesium, Vitamin D and Non-HDL Cholesterol Values with Hepatosteatosis, Fibrosis-4 Index and Aspartate Aminotransferase to Platelet Ratio Index

Serum Magnezyum, D Vitamini ve Non-HDL Kolesterol Düzeylerinin Hepatosteatoz, Fibrozis-4 İndeksi ve Aspartat Aminotransferaz/ Trombosit Oranı İndeksi ile İlişkisinin Retrospektif Analizi

İsmail SELİMOĞLU[1], Hüseyin DÖNGELLİ[1], Mehmet Emin ARAYICI[2], Özlem GÜRSOY DORUK[3], Canan ALTAY[4], Tevfik DEMİR[5]

[1]Dokuz Eylül University Hospital, Department of Internal Medicine, İzmir, Türkiye
[2]Dokuz Eylül University Hospital, Department of Biostatistics and Medical Informatics, İzmir, Türkiye
[3]Dokuz Eylül University Hospital, Department of Biochemistry, İzmir, Türkiye
[4]Dokuz Eylül University Hospital, Department of Radiology, İzmir, Türkiye
[5]Dokuz Eylül University Hospital, Department of Internal Medicine, Division of Endocrinology, İzmir, Türkiye

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ABSTRACT

Objective: Non-alcoholic fatty liver disease (NAFLD) is a common chronic liver condition with an increasing global prevalence. Magnesium and vitamin D are essential for various physiological functions. However, their potential relationships with NAFLD and liver fibrosis remain unclear. This study aimed to assess the correlation between serum magnesium and vitamin D, and liver fibrosis indices, including the aspartate aminotransferase to platelet ratio index (APRI) and the fibrosis-4 (FIB-4) index.

Methods: In this retrospective study, 414 patients underwent abdominal ultrasound for hepatic steatosis assessment. Data on demographics, laboratory parameters, and imaging findings were recorded. Patients were categorized by hepatosteatosis presence, and severity. Biochemical fibrosis scores and their relationships with NAFLD were evaluated.

Results: Serum magnesium levels were inversely correlated with the FIB-4 index (r=-0.101, p=0.045). Magnesium levels were higher in low-risk groups (FIB-4 <1.45) (p=0.006). Vitamin D was inversely associated with hepatic steatosis severity (r=-0.107, p=0.031) and with APRI scores in non-diabetic/non-hyperlipidemic patients (r=-0.383, p=0.044). Low magnesium levels were linked to increased hepatic steatosis in prediabetic patients (p=0.013). Non-high-density lipoprotein cholesterol is positively correlated with steatosis severity (p<0.001).

Conclusion: Magnesium and vitamin D may have protective roles against hepatic steatosis and fibrosis. Their inverse correlations with fibrosis indices suggest potential antifibrotic effects, and magnesium could play a role in NAFLD pathogenesis, particularly in prediabetic patients. These findings support further investigation of magnesium and vitamin D in fibrosis scoring and NAFLD management.

Keywords: Non-alcoholic fatty liver disease, magnesium, vitamin D, HOMA-IR index, fibrosis-4 index

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Corresponding Author/ Sorumlu Yazar:
İsmail SELİMOĞLU, MD,
Dokuz Eylül University Hospital, Department of Internal Medicine, İzmir, Türkiye
ismailselm@gmail.com
ORCID: 0000-0003-2803-3456

ÖZ

Amaç: Alkole bağlı olmayan yağlı karaciğer hastalığı (NAFLD), küresel yaygınlığı giderek artan yaygın bir kronik karaciğer hastalığıdır. Magnezyum ve D vitamini birçok fizyolojik işlev için gereklidir. Ancak bu elementlerin NAFLD ve karaciğer fibrozisi ile olası ilişkileri hala net değildir. Bu çalışmanın amacı, serum magnezyum ve D vitamini düzeyleri ile aspartat aminotransferaz/trombosit oranı (APRI) ve fibrosis-4 (FIB-4) indeksi gibi karaciğer fibrozis göstergeleri arasındaki ilişkiyi değerlendirmektir.

Yöntem: Bu retrospektif çalışmada, hepatik steatoz değerlendirmesi için karın ultrasonu yapılan 414 hastanın verileri incelendi. Demografik veriler, laboratuvar bulguları ve görüntüleme sonuçları kaydedildi. Hastalar, karaciğerde yağlanma varlığı ve şiddetine göre sınıflandırıldı. Biyokimyasal fibrozis skorları ile NAFLD arasındaki ilişkiler değerlendirildi.

Bulgular: Serum magnezyum düzeyleri, FIB-4 indeksi ile ters yönde ilişkiliydi (r=-0,101, p=0,045) ve düşük risk grubunda (FIB-4 <1,45) daha yüksekti (p=0,006). D vitamini, hepatik steatoz şiddetiyle (r=-0,107, p=0,031) ve diyabetik olmayan/hiperlipidemik olmayan hastalarda APRI skorlarıyla ters ilişki gösterdi (r=-0,383, p=0,044). Prediyabetik hastalarda düşük magnezyum düzeyleri, artmış karaciğer yağlanmasıyla ilişkiliydi (p=0,013). Non-yüksek yoğunluklu lipoprotein kolesterol, steatoz şiddetiyle pozitif korelasyon gösterdi (p<0,001).

Sonuç: Magnezyum ve D vitamini, karaciğer yağlanması ve fibrozisine karşı koruyucu etkilere sahip olabilir. Fibrozis indeksleriyle olan ters ilişkileri, potansiyel anti-fibrotik etkilerini düşündürmektedir. Özellikle prediyabetik hastalarda magnezyum, NAFLD patogenezinde rol oynayabilir. Bu bulgular, magnezyum ve D vitamininin fibrozis değerlendirmesi ve NAFLD yönetimindeki potansiyel rollerinin daha fazla araştırılmasını desteklemektedir.

Anahtar Kelimeler: Alkole bağlı olmayan yağlı karaciğer hastalığı, magnezyum, D vitamini, HOMA-IR indeksi, fibrozis-4 indeksi

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease worldwide, with a prevalence of approximately 25%, and its incidence is increasing. It is characterized by excessive fat accumulation in the liver in the absence of significant alcohol consumption, where significant alcohol consumption is defined as less than 21 units (30 g/day) per week in men and 14 units (20 g/day) per week in women. Several risk factors contribute to the development of NAFLD, including advanced age, male sex, ethnicity, obesity, hypertriglyceridemia, low levels of high-density lipoprotein (HDL) cholesterol, hypertension, type 2 diabetes mellitus (T2DM), and various genetic predispositions.[1]

NAFLD is now recognized as a hepatic manifestation of insulin resistance (IR) and a component of metabolic syndrome. Increased IR leads to increased lipid influx into the liver, which, combined with inflammatory cytokines and oxidative stress factors, triggers inflammation and subsequent fibrosis.[1-4]

Currently, NAFLD is most commonly detected using ultrasonography (USG) during routine examinations or screening because of its feasibility and accessibility. This assessment is based on differences in echogenicity between the liver, kidney, and portal vein walls.[5] However, it does not provide histological evidence of disease activity. Therefore, several scoring systems have been developed that incorporate biochemical parameters to estimate histological activity, without the need for liver biopsy. Among these, the fibrosis-4 (FIB-4) index and the aspartate aminotransferase to platelet ratio index (APRI) are widely used in clinical practice.[6-8] Although these scores are not fully sufficient to differentiate simple steatosis from steatohepatitis, one study has reported that the APRI score correlates with the severity of NAFLD as determined by radiological findings.[9] Magnesium (Mg^{2+}) is the most abundant intracellular divalent cation and plays a crucial role in many physiological processes. Due to its involvement in enzymatic reactions, Mg^{2+} has been reported to play a protective role in hepatic inflammatory processes and hepatocyte damage, which are fundamental histological features of NAFLD.[10,11]

Vitamin D-related receptors are known to affect metabolic pathways in skeletal muscle, liver, adipose tissue, and pancreas.[12,13] Through these mechanisms, vitamin D deficiency contributes to IR. In addition, inadequate anti-inflammatory responses have been associated with increased hepatocyte inflammation, the development of hepatic steatosis or steatohepatitis, and an increased risk of metabolic syndrome.[14,15]

Based on these data, we aimed to investigate the relationship between biochemical parameters and NAFLD, as well as the aspartate APRI and the FIB-4 index, to identify thresholds that may indicate a protective role against steatosis and fibrosis.

METHODS

Patients and Data Collection

A total of 691 patient records were reviewed. Among them, the following cases were excluded based on predefined exclusion criteria: 38 patients with liver cirrhosis; 65 patients with a history of past or chronic hepatitis; 25 patients who had received pioglitazone or liraglutide therapy; 67 patients with malignancy; 14 patients with a history of inflammatory bowel disease; 9 patients with autoimmune hepatitis; 2 patients with celiac disease; 10 patients with a history of chronic alcohol use; 43 patients using medications known to induce hepatic steatosis (anti-tumor necrosis factor therapy, corticosteroids, chemotherapeutic agents, etc.); and 4 patients diagnosed with Cushing's syndrome.

As a result, 414 patients (230 females and 184 males) were included in this study.

Inclusion Criteria

The study included male and female patients aged 18 years and older who presented to our outpatient clinic between March 2012 and September 2022. Participants were required to have undergone abdominopelvic USG and to have relevant laboratory parameters available for evaluation. Individuals were only included if they did not meet any of the exclusion criteria listed below at the time of their hospital visit.

Exclusion Criteria

Patients with any of the following conditions during the data collection period were excluded from the study: Age under 18, alcohol consumption, presence of autoimmune hepatobiliary disease, history of viral hepatitis carriage or active hepatitis, diagnosis of cirrhosis of the liver or inflammatory bowel disease, presence of active malignancy or history of malignancy, or Cushing's syndrome. Excessive alcohol consumption was defined as an average intake of more than 30 grams per day for men and more than 20 grams per day for women.

Hepatosteatosis Assessment

Ultrasound assessment of hepatic steatosis was performed using a LOGIQ S7 Expert ultrasound machine. A convex probe was used for B-mode imaging, scanning from the superior to inferior through the subcostal and intercostal regions. IR, non-HDL/HDL cholesterol ratio, and biochemical fibrosis parameters, including the APRI and FIB-4 scores, were calculated. The presence and severity of hepatic steatosis were assessed using USG. Hepatic steatosis was graded according to increased echogenicity, as follows:[16]

- **Grade 1:** Mild diffuse increase in echogenicity with normal visualization of the diaphragm and intrahepatic vessel walls.
- **Grade 2:** Moderate increase in echogenicity with slight opacity of the diaphragm and intrahepatic vessel walls.
- **Grade 3:** Severe enhancement of echogenicity with marked blurring of the diaphragm and intrahepatic vessel walls, and loss of visualization of the posterior segment of the right hepatic lobe.

Indices

The non-HDL/HDL cholesterol ratio was calculated by subtracting HDL cholesterol from total cholesterol to obtain non-HDL cholesterol, which was then divided by HDL cholesterol. The aspartate APRI, one of the indices used to assess liver fibrosis, was calculated by dividing the AST elevation ratio by the platelet count and multiplying the result by 100. The AST elevation ratio was determined by dividing the patient's AST level by the upper laboratory limit (35 U/L for women and 50 U/L for men). The FIB-4 index was calculated using the formula: (age × AST) / (platelet count × √ALT).

Patients were also categorised according to

- Vitamin D levels: ≥20 ng/mL vs. <20 ng/mL
- Magnesium levels: ≤0.75 mmol/L vs. >0.75 mmol/L
- Non-HDL cholesterol levels: ≤150 mg/dL vs. >150 mg/dL

Ethics

Approval for this retrospective study was obtained from Dokuz Eylül University Non-Interventional Research Ethics Committee (decision number: 2023/37-17, date: 23.11.2022).

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics 24.0. Descriptive statistics are presented as mean ± standard deviation (median, minimum, and maximum) and percentages. Shapiro-Wilk and Kolmogorov-Smirnov tests were used to assess the normality of the data distribution. As the data did not follow a normal distribution, the Mann-Whitney U test was used for pairwise comparisons of numerical variables. The Kruskal-Wallis test was used for comparisons involving more than two groups. Subgroup analyses in the parametric tests were interpreted using the Bonferroni correction. Categorical variables were analyzed using the chi-square test, and correlations between two numerical variables were assessed using the Spearman correlation test. A p value <0.05 or a 95% confidence interval that does not include the null value was considered statistically significant.

RESULTS

Demographic and Clinical Characteristics of the Patients

The mean age of all patients was 53.2±17.1 years (minimum: 18, maximum: 91 years). Descriptive statistics of the patients' biochemical parameters are presented in Table 1. On ultrasonographic evaluation, 166 patients (40.1%) did not have hepatic steatosis, whereas 248 patients (59.9%) had varying degrees of steatosis (Table 1).

The correlations between biochemical parameters and hepatic steatosis when patients were evaluated as a whole, without stratification by comorbidities or sex, are summarized in Table 2. Non-HDL cholesterol levels and non-HDL/HDL cholesterol ratios were inversely correlated with the degree of hepatic steatosis ($p<0.001$ and $p<0.001$, respectively). In non-diabetic patients, non-HDL cholesterol and the non-HDL/HDL ratio were significantly

higher in those with steatosis (Table 3). In the entire study population, a positive correlation was observed between non-HDL cholesterol levels and the APRI scores (r=0.126, p=0.012). In patients without diabetes and hyperlipidemia, non-HDL cholesterol levels showed a positive correlation with the steatosis grade (r=0.415, p=0.025), whereas the non-HDL/HDL ratio did not show a significant correlation (r=0.289, p=0.128).

Factors Related to Hepatosteatosis

When patients were evaluated as a whole, without stratification by comorbidities or sex, the correlations between biochemical parameters and hepatic steatosis were summarized in Table 2. Non-HDL cholesterol levels and non-HDL/HDL cholesterol ratios were inversely correlated with the degree of hepatic steatosis (p<0.001 and p<0.001, respectively).

Table 1. Clinical characteristics of study group

	No hepatosteatosis (n=166)	**Hepatosteatosis (n=268)**	**Total (n=414)**	**p**
Age mean ± SD	53.6±20.3	53.0±14.6	53.2±17.1	0.774
Gender n (%) Female Male	 104 (62.7) 62 (37.3)	 126 (50.8) 122 (49.2)	 230 (55.6) 184 (44.4)	 **0.017**
Comorbidities n (%) None Dyslipidemia DM	 19 (11.4) 136 (81.9) 83 (50.0)	 10 (3.7) 220 (88.7) 187 (75.4)	 29 (7.0) 356 (86.0) 270 (65.2)	 **0.002** 0.051 **<0.001**
Hepatosteatosis n (%) Grade 0 Grade 1 Grade 2 Grade 3	 166 (100.0) 0 (0) 0 (0) 0 (0)	 0 (0) 94 (37.9) 123 (49.6) 31 (12.5)	 166 (40.1) 94 (22.7) 123 (29.7) 31 (7.5)	 N/A
Fibrosis indicis median (IQR) FIB-4 APRI	 0.93 (0.86) 0.20 (0.11)	 0.87 (0.61) 0.20 (0.13)	 0.88 (0.69) 0.20 (0.12)	 0.217 0.979
Laboratory findings median (IQR) Creatinine mg/dL BUN mg/dL AST U/L ALT U/L GGT U/L ALP U/L LDL mg/dL HDL mg/dL VLDL mg/dL Triglyceride mg/dL T. cholesterol mg/dL Albumin g/dL Calcium mg/dL Magnesium mmol/L Vitamin D ng/mL Ferritine ng/mL CRP mg/dL FBG mg/dL HbA1c% Lymphocyte 10^9/L Thrombocyte 10^9/L HOMA-IR	 0.71 (0.32) 13.8 (10.2) 19 (9) 16 (12) 18 (16) 68 (28) 120 (53) 52 (22) 21 (12) 105 (59) 199 (64) 4.2 (0.6) 9.5 (0.7) 0.84 (0.11) 18 (15) 35 (72) 2.1 (6.2) 91 (17) 5.7 (0.7) 1.9 (0.9) 246 (77) 2.0 (2.5)	 0.80 (0.20) 14 (7) 21 (10) 22 (17) 27 (21) 76 (27) 139 (53) 48 (18) 29 (18) 144 (92) 217 (72) 4.3 (0.5) 9.6 (0.6) 0.84 (0.10) 17 (13) 46 (69) 4.5 (6.4) 97 (29) 6.0 (1.1) 2.2 (0.9) 263 (94) 3.5 (3.1)	 0.79 (0.31) 10 (9) 20 (10) 19 (16) 22 (20) 72 (28) 131 (55) 50 (18) 25 (17) 124 (87) 207 (71) 4.3 (0.5) 9.6 (0.7) 0.84 (0.10) 17 (13) 40 (69) 3.6 (7.0) 94 (26) 5.9 (0.9) 2.1 (0.9) 257 (89) 2.9 (2.8)	 0.562 0.652 **0.003** **<0.001** **<0.001** **<0.001** **<0.001** **0.032** **<0.001** **<0.001** **<0.001** **0.017** **0.007** 0.842 0.237 0.235 **<0.001** **<0.001** **<0.001** **<0.001** **0.026** **0.001**
Vitamin D deficiency n (%)	10 (6.0)	23 (8.6)	33 (7.9)	0.360
Hypomagnesemia n (%)	29 (17.5)	35 (13.1)	64 (15.5)	0.354

ALP: Alkaline phosphatase, ALT: Alanine transferase, APRI: Aspartate transferase to platelets ratio index, AST: Aspartate transferase, BUN: Blood urea nitrogen, CRP: C-reactive protein, DM: Diabetes mellitus, FBG: Fasting blood glucose, FIB-4: Fibrosis 4 score, GGT: Gamma-glutamyl transferase, HbA1c: Hemoglobin A1c, HDL: High-density lipoprotein, HOMA-IR: Homeostasis model assessment of insulin resistance, LDL: Low-density lipoprotein, N/A: Not applicable, IQR: Interquartile range, VLDL: Very low-density lipoprotein, IQR: Interquartile range, SD: Standard deviation

In non-diabetic patients, non-HDL cholesterol and non-HDL/HDL ratio were significantly higher in those with steatosis (Tables 2 and 3). In the entire study population, a positive correlation was observed between non-HDL cholesterol levels and APRI scores (r=0.126, p=0.012). In patients without diabetes and hyperlipidaemia, non-HDL cholesterol levels showed a positive correlation with the steatosis grade (r=0.415, p=0.025), whereas the non-HDL/HDL ratio did not show a significant correlation (r=0.289, p=0.128).

Serum vitamin D levels were inversely correlated with the degree of steatosis in the overall population and in patients with both hyperlipidemia and T2DM/preDM. Non-HDL cholesterol levels and non-HDL/HDL ratio were positively correlated with steatosis grade in the overall population, independent of comorbidities. In addition, these parameters were positively correlated with steatosis grades in patients with both hyperlipidemia and diabetes/prediabetes (Table 2).

Fibrosis Indices

A significant negative correlation was observed between serum magnesium levels and the FIB-4 index (r=-0.101, p=0.045). Furthermore, when the FIB-4 score was stratified into two groups based on a cutoff value of 1.45, magnesium levels were significantly higher in the low-risk FIB-4 group (Table 4). In addition, in patients with hyperlipidemia, magnesium levels were significantly increased in the FIB-4 <1.45 group (p<0.05) (Table 4).

Subgroup Analysis

When patients were divided into two groups based on a serum magnesium threshold of 0.75 mmol/L, no correlation between vitamin D levels and hepatic steatosis severity was observed in the group with higher magnesium levels. However, in patients with lower magnesium levels, there was a significant inverse correlation between serum vitamin D levels and hepatic steatosis severity according to Spearman's rho test (p<0.01; r=0.385). Furthermore, chi-squared analysis showed that hepatic steatosis was more common in patients with both serum magnesium levels ≤0.75 mmol/L and vitamin D levels <20 ng/mL (Table 5).

No significant correlation was found between vitamin D levels and another fibrosis index, APRI, in the whole population. However, in patients without diabetes and hyperlipidaemia, there was a negative correlation between vitamin D levels and APRI (r=-0.383, p=0.044). We also analysed the diabetic/prediabetic group separately. After excluding patients receiving any diabetes treatment and/or those with HbA1c ≥6.5% and fasting blood glucose ≥126 mg/dL, 45 prediabetic patients remained.

Table 2. Relationship between hepatosteatosis degree and biochemical parameters

	r	p*
All patients		
Magnesium	-0.200	0.678
Vitamin D	-0.107	0.031
Non-HDL cholesterol	0.277	<0.001
Non-HDL/HDL cholesterol ratio	0.323	<0.001
Patients with DM and hyperlipidemia		
Magnesium	-0.030	0.620
Vitamin D	-0.128	0.048
Non-HDL/HDL ratio	0.313	<0.001
Non-HDL cholesterol	0.274	<0.001

*Determined by Spearman's rho correlation test. r: Correlation coefficient.
HDL: High-density lipoprotein, DM: Diabetes mellitus

Table 3. Comparison of non-high-density lipoprotein cholesterol and non-high-density lipoprotein/high-density lipoprotein cholesterol ratios with hepatosteatosis in non-type 2 diabetes mellitus/pre-diabetes mellitus patients

	Hepatosteatosis status (n)	Median (25%-75%)	p*
Non-HDL (mg/dL)	Absent (83)	148 (112-179)	**0.007**
	Present (61)	163 (141.5-192.5)	
Non-HDL/HDL ratio	Absent (83)	2.63 (1.97-3.64)	**0.023**
	Present (61)	3.18 (2.40-3.74)	

*Calculated using Mann-Whitney U test.
HDL: High-density lipoprotein, T2DM: Type 2 diabetes mellitus

Table 4. Comparison of magnesium levels in patients with fibrosis-4 scores below and above 1.45			
	Sample size (n)	**Magnesium (mmol/L) Median (25-75%)**	**p**
Total			
FIB-4 <1.45	316	0.85 (0.792-0.90)	**0.006**
FIB-4 ≥1.45	80	0.82 (0.762-0.887)	
Patient with hyperlipidemia			
FIB-4 <1.45	264	0.845 (0.79-0.89)	**0.026**
FIB-4 ≥1.45	74	0.820 (0.76-0.88)	
*p values were calculated using the Mann-Whitney U test. FIB-4: Fibrosis-4			

Table 5. Association between vitamin D levels and the presence of hepatic steatosis in patients with serum magnesium levels ≤0.75 mmol/L				
		Present n (%)	**Absent n (%)**	**p***
Magnesium ≤0.75 mmol/L	Vitamin D <20 ng/mL	23 (69.7)	10 (30.3)	0.013
	Vitamin D ≥20 ng/mL	12 (38.7)	19 (61.3)	
*Chi-square test				

In this subgroup of exclusively prediabetic patients, we observed a significant inverse correlation between serum Mg^{+2} levels and HOMA-IR (Spearman's rho, p<0.01, r=-0.396). In prediabetic but non-diabetic patients, serum magnesium levels were significantly higher in those with HOMA-IR <2 compared to those with HOMA-IR ≥2 (median 0.90 vs. 0.87 mmol/L; p=0.008), suggesting a potential inverse association between IR and magnesium levels.

DISCUSSION

Although the relationship between magnesium and the inflammatory response remains unclear, adequate serum and intracellular magnesium levels are thought to have a protective role against inflammation.[17] In a study by Rayssiguier et al.[18], in rats, hepatic magnesium depletion was associated with increased collagen accumulation in the liver. In addition, data suggest that both serum and intracellular magnesium levels are significantly lower in cirrhotic patients.[19] Furthermore, studies have shown that each 100 mg increase in magnesium intake is associated with a 49% reduction in the risk of death from all causes of liver disease, and increased magnesium intake is associated with a reduced risk of advanced decompensated liver disease.[20,21] Although no similar study has been reported in the literature, our study found a weak inverse correlation between serum magnesium levels and the FIB-4 index, which is considered a predictive index of liver fibrosis, in the entire population evaluated (p<0.05, r=-0.101). In addition, when patients were grouped based on a FIB-4 threshold of 1.45, serum magnesium levels were significantly lower in the higher FIB-4 group (p<0.01). Due to the absence of patients with cirrhosis in our population, the APRI and FIB-4 scores were generally low. Nevertheless, the negative correlation between serum magnesium and the FIB-4 index suggests that magnesium may be directly or indirectly involved in antifibrotic and anti-inflammatory pathways.

Another study investigated the relationship between serum and intracellular magnesium levels, IR, and severity of hepatic steatosis in patients with metabolic syndrome. Patients with grades 2 and 3 steatosis, as assessed by ultrasound, were found to have lower magnesium levels than those without steatosis. In the same study, individuals with a HOMA-IR above 2.7 also had lower serum magnesium levels compared to those with a HOMA-IR <2.7.[22] It should be noted that maintaining a high HOMA-IR threshold may have contributed to this finding. In an obesity study of patients with liver biopsy-confirmed NASH, patients with NASH had lower magnesium levels than those without NASH. Furthermore, low magnesium concentrations were found to be associated with high HOMA-IR.[23] A meta-analysis of randomized controlled trials showed that magnesium supplementation improved IR in patients with type 2 diabetes, further supporting these findings.[24] As IR is a fundamental mechanism of NAFLD, it plays a crucial role in the disease's progression. Our study also provided data consistent with the literature. In the prediabetic patient population, a significant inverse correlation was found between serum magnesium and HOMA-IR levels. When patients were divided into two groups based on a HOMA-IR threshold of 2.00, those with a higher HOMA-IR had lower magnesium levels. This suggests that even in the absence of diabetes, serum magnesium levels may be affected under conditions of high IR. For example, a study comparing 50 patients with glucose intolerance or

diabetes with 50 healthy controls found no statistically significant difference in serum magnesium levels between the groups.[25]

Vitamin D regulates several genes that are widely expressed in the liver, some of which are involved in glucose and lipid metabolism.[26] A study by Chung et al.[27] of 6,055 healthy individuals diagnosed with steatosis by ultrasound found an inverse correlation between vitamin D levels and the severity of hepatic steatosis. Individuals with vitamin D levels >20 ng/mL were found to have a lower prevalence of steatosis. Similarly, a meta-analysis of 15 studies involving 20,096 individuals showed that patients with NAFLD of Western origin tended to have lower vitamin D levels. In the general population, low vitamin D levels have been associated with an increased risk of NAFLD.[28] A cohort study of 10,960 cases also found an inverse association between ultrasonographic steatosis severity and vitamin D levels, and between NAFLD fibrosis scores and vitamin D levels. Another study of biopsy-confirmed cases of liver fibrosis reported an inverse association between vitamin D levels and the severity of fibrosis. However, a separate biopsy-based study found no association between vitamin D levels and the severity of hepatic steatosis.[29-31]

In a study that examined 614 patients by biopsy, without stratifying them by comorbidities such as hyperlipidemia and diabetes, patients with advanced steatosis had significantly lower vitamin D levels. There was also an inverse association between vitamin D levels and steatosis severity. However, the same study found no association between serum magnesium levels and the presence or severity of steatosis.[32] Notably, these studies examined vitamin D and magnesium levels separately, rather than assessing their combined effects.

Consistent with the literature, we found an inverse association between vitamin D levels and the severity of hepatic steatosis in the whole population. Furthermore, when we analyzed patients with both hyperlipidemia and diabetes/prediabetes separately from those without these comorbidities, we found a significant inverse association between the steatosis severity and vitamin D levels in these patients. However, no such correlation was observed in the patients without these comorbidities.

Studies in the literature have separately evaluated the relationships between serum magnesium levels, vitamin D levels, and NAFLD. However, in our analysis, when patients were divided into two groups based on a threshold serum magnesium level of 0.75 mmol/L, a significant inverse association between vitamin D levels and the degree of hepatic steatosis was observed in those within the lower magnesium group. Furthermore, when the group with low magnesium levels was subdivided based on a vitamin D threshold of 20 ng/mL, hepatic steatosis was more prevalent in patients with both low vitamin D and low magnesium levels (p=0.013). No significant association was found in the higher magnesium group. Based on the available literature, if low serum magnesium levels are accepted as being associated with NAFLD, it can be suggested that in patients with hypomagnesemia or those who are unable to maintain optimal magnesium levels for any reason. Ensuring adequate vitamin D levels or preventing vitamin D deficiency may have a protective effect against the occurrence and severity of NAFLD.

Vitamin D and APRI levels were examined in a study of 3,972 patients categorized into diabetes, pre-diabetes, and normal glucose tolerance groups. Despite a significant p value for correlation, no significant difference in mean APRI scores was found between different categories of vitamin D levels.[33] Another study of 58 HCV cases divided into two groups based on vitamin D levels found that APRI scores were higher in the low vitamin D group, with an inverse correlation between vitamin D levels and APRI.[34] Similarly, a study of 185 patients with primary biliary cholangitis and 141 healthy controls found an inverse association between vitamin D levels and APRI.[35]

In our study, no association was found between vitamin D and APRI when the entire population was analyzed. However, in patients without hyperlipidemia and diabetes/pre-diabetes comorbidities, a significant inverse association was found between vitamin D levels and APRI. This suggests that vitamin D levels may not be sufficient to predict fibrosis in patients with metabolic comorbidities. However, in patients without metabolic comorbidities, incorporating vitamin D levels into the APRI score may help predict liver fibrosis.

In a study that followed 147 patients without initial NAFLD for seven years, 19% developed NAFLD. However, none of the patients with a baseline non-HDL level of <130 mg/dL developed NAFLD. The incidence of NAFLD increased with increasing non-HDL-C levels, leading to the conclusion that non-HDL-C levels may be a strong predictor of NAFLD development.[36] Similarly, in a study of 2,717 cases with a mean follow-up of 1.6 years, 9.1% (264 cases) developed NAFLD, and the non-HDL/HDL ratio was significantly higher in those who developed NAFLD than in those who did not. Statistical analysis identified cut-off values for the non-HDL/HDL cholesterol ratio of 2.4 in women and 2.3 in men.[37] Another clinical study of 265 cases found that non-HDL/HDL cholesterol levels were positively associated with the degree of hepatosteatosis and the FIB-4 index.[38]

Our analyses showed that both non-HDL cholesterol and non-HDL/HDL cholesterol ratio were positively correlated with the degree of steatosis. When patients were

divided into groups based on the presence or absence of hepatosteatosis, the parameters of non-HDL levels and non-HDL/HDL ratios showed significant differences between groups, with higher values observed in those with steatosis.

Although recent studies have begun to highlight the superiority of the non-HDL/HDL cholesterol ratio over non-HDL cholesterol alone, our findings suggest that non-HDL cholesterol levels may be more indicative than the non-HDL/HDL cholesterol ratio. For example, in individuals with neither diabetes/prediabetes nor hyperlipidemia, no association was found between the non-HDL/HDL cholesterol ratio and the degree of steatosis, whereas in the same group, non-HDL cholesterol levels were correlated with the degree of steatosis. Another indication of the functional superiority of non-HDL cholesterol over the non-HDL/HDL ratio is its relationship with the APRI score. In the whole population, there was a significant positive correlation between the APRI and non-HDL cholesterol levels. The correlation between biochemical fibrosis parameters and radiological steatosis has also been investigated. In a study by Sahin et al.[9] in which 205 organ transplant donors were evaluated, a significant correlation was found between the severity of steatosis detected by CT and the APRI score. However, in our study, these parameters did not provide any data regarding the presence or severity of hepatosteatosis.

Study Limitations

This study has several limitations that should be acknowledged. First, the retrospective and cross-sectional design precludes the establishment of causal relationships between serum magnesium, vitamin D levels, and hepatic fibrosis or steatosis. Second, the study was conducted at a single center, which may limit the generalizability of the findings to broader populations. Third, some laboratory and clinical parameters had missing data, potentially affecting the completeness and accuracy of the analyses. Additionally, hepatic steatosis was assessed using abdominal ultrasonography, which, while non-invasive and widely available, is less sensitive and specific compared to histopathological evaluation. Lastly, liver biopsy, the gold standard for diagnosing and staging non-alcoholic fatty liver disease and fibrosis, was not performed, which limits the precision in determining the severity of hepatic pathology.

CONCLUSION

In light of this information and the data obtained, serum vitamin D levels and non-HDL/HDL cholesterol ratios could be included in the modification of the APRI score, which is currently used to predict liver fibrosis. Magnesium and vitamin D appear to exert protective effects against hepatic steatosis and fibrosis. The observed inverse association with fibrosis indices may indicate potential anti-fibrotic properties. Moreover, magnesium may contribute to the pathophysiological mechanisms underlying NAFLD, particularly in individuals with prediabetes. These findings underscore the need for further research on the roles of magnesium and vitamin D in fibrosis assessment and clinical management of NAFLD.

Ethics

Ethics Committee Approval: Approval for this retrospective study was obtained from Dokuz Eylül University Non-Interventional Research Ethics Committee (decision number: 2023/37-17, date: 23.11.2022).

Informed Consent: Retrospective study.

Footnotes

Authorship Contributions

Concept: İ.S., H.D., M.E.A., Ö.G.D., C.A., T.D., Design: İ.S., H.D., M.E.A., Ö.G.D., C.A., T.D., Data Collection or Processing: İ.S., H.D., Analysis or Interpretation: İ.S., H.D., M.E.A., T.D., Literature Search: İ.S., H.D., Writing: İ.S., H.D.

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