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Assessment of Plasma Vitamin D Levels in Diabetic Patients With and Without Hepatic Steatosis at Imam Khomeini Hospital, Urmia, Iran

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ABSTRACT

Introduction: Metabolic dysfunction-associated steatotic liver disease (MASLD) represents a significant public health challenge, particularly in populations with high rates of obesity and type 2 diabetes. The association between vitamin D deficiency and MASLD in diabetic patients remains incompletely characterized. This study aimed to assess plasma vitamin D levels in type 2 diabetes patients with and without MASLD at Imam Khomeini Hospital, Urmia.

Methods: In this cross-sectional study, diabetic patients with and without MASLD were enrolled. Demographic and clinical data, including BMI, lipid profile, liver enzymes, blood pressure, and plasma vitamin D levels, were collected. MASLD was diagnosed using ultrasonography. Exclusion criteria included other chronic liver diseases, alcohol consumption, and hepatotoxic medications. Statistical analyses were performed using SPSS 27.

Results: Among 128 participants (97 men, 31 women), the mean vitamin D level was 24.5 ± 10.4 ng/mL, with 72% exhibiting deficiency. Hepatic steatosis was present in 50% of patients, who demonstrated significantly lower vitamin D levels. Vitamin D deficiency correlated positively with higher BMI and poorer glycemic control in non-steatotic patients, though this relationship was absent in those with steatosis. Steatotic patients also showed elevated triglycerides, total cholesterol, ALT, and higher rates of hypertension and cardiovascular disease.

Conclusion: Vitamin D deficiency is highly prevalent in diabetic patients, particularly those with MASLD, and is associated with obesity and inadequate glycemic control. These findings support targeted screening and vitamin D supplementation in high-risk populations to potentially improve metabolic and hepatic outcomes.

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Introduction

The prevalence of chronic diseases is increasing worldwide, and due to their long-term impacts on individual health and substantial economic burden, they represent a major public health challenge (Mohammadi et al., 2025; Hajiesmaello et al., 2019; Mohammadi and Abdi, 2025). Metabolic dysfunction-associated steatotic liver disease (MASLD), formerly known as non-alcoholic fatty liver disease (NAFLD), encompasses a broad spectrum of hepatic injuries ranging from simple steatosis to steatohepatitis, fibrosis, and cirrhosis (Eslam et al., 2020). Today, MASLD is recognized as the most common cause of chronic liver disease in both developed and developing countries and significantly overlaps with the high prevalence of obesity and type 2 diabetes (Younossi et al., 2016). MASLD is strongly associated with metabolic risk factors, including obesity—particularly central obesity insulin resistance, type 2 diabetes, dyslipidemia, and hypertension (Cai et al., 2020). Given the global upward trends in obesity and diabetes, the incidence of MASLD is expected to rise correspondingly (Rabiee et al., 2017).

Vitamin D, a fat-soluble steroid hormone, plays a wide range of roles in regulating calcium and phosphate metabolism, immune function, and metabolic health processes, including insulin sensitivity and inflammation control (Olsson et al., 2016; Rossberg et al., 2016). Vitamin D deficiency represents a significant global public health challenge, with prevalence influenced by factors such as sunlight exposure, diet, skin pigmentation, obesity, and overall health status (Tilg et al., 2017; Anderson et al., 2010).

The relationship between vitamin D deficiency and metabolic disorders particularly type 2 diabetes and obesity has been well-documented. Insulin resistance, a key feature in the pathogenesis of type 2 diabetes and MASLD, has been linked to low vitamin D levels. Vitamin D may improve insulin sensitivity and glycemic control through effects on insulin signaling pathways, pancreatic beta-cell insulin secretion, and systemic inflammation modulation.

Recent studies suggest that vitamin D deficiency may also play a role in MASLD pathogenesis. Proposed mechanisms include modulation of lipid metabolism, reduction of oxidative stress, and regulation of inflammatory responses in the liver (Cashman, 2022). Evidence indicates that patients with MASLD have a higher prevalence of vitamin D deficiency compared to healthy individuals, with lower vitamin D levels associated with greater steatosis and hepatic fibrosis severity (Chagas et al., 2012). However, study results are not entirely consistent, with some reporting no significant association between vitamin D deficiency and MASLD, likely due to differences in study populations, inclusion and exclusion criteria, measurement methods, and confounding factors (Chagas et al., 2012).

Given the high prevalence of diabetes in Iran and the rising incidence of MASLD, investigating the relationship between vitamin D status and MASLD in diabetic patients has important clinical implications. Diabetic patients may be at increased risk for both MASLD and vitamin D deficiency due to underlying metabolic disturbances and lifestyle factors (Dutra et al., 2021). A better understanding of this association can aid in identifying high-risk individuals and developing effective preventive and therapeutic strategies (Ha et al., 2017).

The primary objective of this study was to investigate serum vitamin D levels in diabetic patients attending Imam Khomeini Hospital in Urmia and to assess their association with the presence or absence of metabolic dysfunction associated steatotic liver disease (MASLD). In addition, related clinical parameters including body mass index (BMI), glycated hemoglobin (HbA1c), lipid profile, and blood pressure were examined to provide a more comprehensive understanding of the potential role of vitamin D in the clinical outcomes of diabetic patients.

Materials and Methods

Study Design and Population

This was a cross-sectional analytical study. The study population comprised patients with type 1 or type 2 diabetes attending the internal medicine and endocrinology clinics at Imam Khomeini Hospital, Urmia, who met the study inclusion criteria.

Inclusion Criteria:

The inclusion criteria comprised a confirmed diagnosis of diabetes according to the American Diabetes Association (ADA) criteria, an age range of 18 to 75 years, provision of signed written informed consent, and the availability of standard abdominal ultrasonography in the medical record or the ability to perform it at the time of the study.

Exclusion Criteria:

Patients were excluded if they had chronic liver diseases other than MASLD, such as hepatitis B or C, autoimmune liver diseases, Wilson's disease, or

hemochromatosis. Exclusion also applied to individuals with alcohol consumption exceeding 20 g/day, as well as those who had used medications influencing vitamin D metabolism or liver function including corticosteroids, antiepileptics, certain antiretrovirals, or ketoconazole within the past three months. Additional exclusion criteria included a history of bariatric surgery within the previous six months, pregnancy or breastfeeding, and the presence of severe renal or cardiac disease that could affect nutrition or metabolism.

Sample Size and Sampling Method:

Based on previous studies and the prevalence of vitamin D deficiency and MASLD, 64 diabetic patients with MASLD and 64 diabetic patients without MASLD were selected, totaling 128 participants. Sampling was used during the study period.

Data Collection Tools

Clinical Form:

Collected demographic information (age, sex, residence), medical history (diabetes type and duration, family history of diabetes or liver disease), medications, dietary habits, physical activity, and comorbidities (e.g., hypertension, cardiovascular disease, dyslipidemia).

Lifestyle Questionnaire:

Included alcohol and tobacco use and other health-related behaviors.

Biochemical Tests:

Plasma 25-hydroxyvitamin D [25(OH)D] levels were measured using standard immunoassay kits (ELISA or chemiluminescence). Other parameters included fasting blood glucose, HbA1c, lipid profile (triglycerides, total cholesterol, LDL, HDL), liver enzymes (AST, ALT), and serum creatinine.

Abdominal Ultrasonography:

Performed by a radiologist using standard equipment to detect hepatic steatosis and grade severity where possible. MASLD was defined based on ultrasonographic evidence of steatosis with exclusion of other chronic liver disease causes.

Operational Definitions:

In this study, vitamin D deficiency was defined as a serum level below 30 ng/mL, insufficiency as 30–50 ng/mL, and sufficiency as greater than 50 ng/mL. MASLD was diagnosed based on the presence of hepatic steatosis on abdominal ultrasonography, following the exclusion of other chronic liver diseases. Obesity was defined as a body mass index (BMI) of ≥30 kg/m^2, while overweight was classified as a BMI of 25–29.9 kg/m^2. Poor glycemic control was determined by an HbA1c level of ≥8%. Hypertension was defined as systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg, or the use of antihypertensive medications.

Statistical Analysis

Data were analyzed using SPSS version 27 (IBM Corp., Armonk, NY, USA). Descriptive statistics included mean ± standard deviation for continuous variables and frequency (percentage) for categorical variables.

Inferential statistics:

- Continuous variables were compared using independent t-tests or Mann–Whitney U tests if non-normally distributed.
- Categorical variables were compared using Chi-square tests or Fisher's exact test when expected counts were <5.
- Plasma vitamin D levels across different groups (e.g., steatosis grades, glycemic control) were compared using one-way ANOVA or Kruskal–Wallis test.
- Correlations between continuous variables and vitamin D levels were evaluated using Pearson or Spearman correlation coefficients.
- Statistical significance was set at $p < 0.05$.

Results

Descriptive Findings

A total of 128 diabetic patients were included in the study, comprising 97 men (75.8%) and 31 women (24.2%). The mean age was 52 ± 12 years, with the highest frequency observed in the 60–69-year age group (26.6%). Most participants resided in urban areas (81.3%). Regarding diabetes type, 107 patients (83.6%) had type 2 diabetes, while 21 (16.4%) had type 1 diabetes. Adequate glycemic control (HbA1c ≤7%) was achieved in 31 patients (24.2%).

The mean body mass index (BMI) was 29.1 ± 4.1 kg/m^2, with 43.8% being overweight and 38.3% classified as obese; only one patient (0.8%) was underweighted. The most common therapeutic regimen was oral medications (57.8%), followed by combination therapy of oral agents and insulin (28.1%), and insulin-only therapy (14.1%).

Vitamin D Status

The mean serum vitamin D concentration across all participants was 24.5 ± 10.4 ng/mL, ranging from 8.1 to 64.8 ng/mL (Table 1). Vitamin D deficiency,

defined as <30 ng/mL, was observed in 92 patients (71.9%), while only approximately one-third of participants had sufficient levels (Table 1). These results indicate that the mean vitamin D level in this population was below the optimal range and that deficiency is highly prevalent. This high prevalence suggests that inadequate vitamin D status may play a significant role in the development or exacerbation of metabolic complications associated with diabetes and hepatic steatosis.

Table 1: Distribution of serum vitamin D levels among patients

Parameter	Value / n	Percentage
Mean ± SD	24.5 ± 10.4 ng/mL	–
Range	8.1–64.8 ng/mL	–
Deficient (<30 ng/mL)	92	71.9%
Sufficient (≥30 ng/mL)	36	28.1%

Hepatic Steatosis

Half of the study participants (n=64) were diagnosed with hepatic steatosis, indicating a high prevalence of this disorder within the studied population (Table 2). Among these patients, 27 (21.1%) were classified as grade 3 and 7 (5.5%) as grade 4, whereas grading data were unavailable for the remaining 64 patients. These findings highlight the common occurrence of hepatic steatosis among diabetic patients and underscore the importance of examining associated factors, including vitamin D status.

Table 2: Prevalence and grading of hepatic steatosis

Hepatic Steatosis	n	%
Present	64	50
Absent	64	50
Steatosis Grade (Ultrasound)	n	%
Grade 1	10	7.8
Grade 2	20	15.6
Grade 3	27	21.1
Grade 4	7	5.5
Not reported	64	50

Analytical Findings

As shown in Table 3, patients with hepatic steatosis had significantly lower mean vitamin D levels compared to those without steatosis (22.4 vs. 26.6 ng/mL, p=0.005). In addition, vitamin D deficiency was more prevalent among patients with steatosis (79.7% vs. 61.4%, p=0.049) (Table 3). These findings indicate a significant inverse association between vitamin D status and the presence of hepatic steatosis in the studied population, suggesting that vitamin D deficiency may contribute to the development or progression of fatty liver disease.

Table 3: Comparison of vitamin D status in patients with and without hepatic steatosis

Group	Deficient n (%)	Sufficient n (%)	Mean ± SD (ng/mL)	P-value
Steatosis present	51 (79.7%)	13 (20.3%)	22.4 ± 10.7	0.049* (deficiency), 0.005* (mean)
Steatosis absent	41 (61.4%)	23 (35.9%)	26.6 ± 9.6	–

*: Chi-square test: 0.049; Mann–Whitney U test: 0.005

Statistically significant

Sex and Age Differences

Although steatosis was more frequent in men than women (68.8% vs. 31.3%), this difference was not statistically significant (p=0.063). Mean age did not differ between the two groups (p=0.592). Among patients without steatosis, women had significantly higher vitamin D levels than men (36.7 vs. 24.5 ng/mL, p<0.001); however, no sex-based differences were observed in the steatosis group. No significant associations between vitamin D levels and age groups were detected in any subgroup.

Body Mass Index (BMI)

Vitamin D levels declined with increasing body mass index (BMI). In both the steatosis and non-steatosis groups, obese patients had significantly lower vitamin D levels compared to individuals with normal weight or overweight (p=0.019 and p=0.010, respectively) (Table 4). These findings suggest that obesity is a key factor associated with vitamin D deficiency and may contribute to an elevated risk of developing hepatic steatosis.

Table 4: Vitamin D levels according to BMI in patients with and without hepatic steatosis

BMI	Steatosis present (Mean ± SD)	Steatosis absent (Mean ± SD)	P-value
Underweight	34.0 (-)	– (-)	0.019*, 0.010*
Normal	28.6 ± 19.1	31.6 ± 9.2	–
Overweight	24.8 ± 10.0	27.2 ± 9.7	–
Obese	18.1 ± 7.6	22.3 ± 8.4	–

: Kruskal–Wallis H test; In patients with steatosis: 0.019; In patients without steatosis: 0.01.

Diabetes Type and Residence

No significant associations were observed between diabetes type (type 1 vs. type 2) or place of residence (urban vs. rural) and vitamin D levels.

Glycemic Control (HbA1c)

Among patients without hepatic steatosis, those with poor glycemic control (HbA1c >7%) had significantly lower vitamin D levels compared to patients with adequate glycemic control (26.6 vs. 30.5 ng/mL, p=0.008) (Table 5). This association was not observed in patients with hepatic steatosis, suggesting that the effect of vitamin D status on glycemic control may be attenuated in the presence of hepatic steatosis.

Table 5: Vitamin D levels according to HbA1c in patients with and without hepatic steatosis

HbA1c	Steatosis present (Mean ± SD)	Steatosis absent (Mean ± SD)	P-value
≤7%	32.0 ± 18.7	30.5 ± 10.6	0.129, 0.008*
>7%	20.6 ± 7.5	26.6 ± 9.6	–

*: Mann–Whitney U test; In patients with steatosis: 0.129; In patients without steatosis: 0.008.

Physical Activity

Patients with higher levels of physical activity tended to have a lower prevalence of hepatic steatosis, although this association was not statistically significant (p=0.367) (Table 6). These findings suggest that physical activity may have a protective role in reducing the risk of hepatic steatosis, but in this sample, the results were not strong enough to draw definitive conclusions.

Table 6: Association between physical activity and hepatic steatosis

Weekly Activity	Steatosis n (%)	No Steatosis n (%)	P-value
<30 min	14 (51.9%)	13 (48.1%)	0.367
30–150 min	25 (58.1%)	18 (41.9%)	–
150–300 min	16 (48.5%)	17 (51.5%)	–
>300 min	9 (36%)	16 (64%)	–

Laboratory Parameters

Patients with hepatic steatosis had significantly higher triglycerides (220 vs. 182 mg/dL, p=0.004), total cholesterol (214 vs. 197 mg/dL, p=0.003), and ALT (41 vs. 36 U/L, p=0.042) compared to those without steatosis, whereas differences in LDL, HDL, and AST were not statistically significant (Table 7). These findings indicate that hepatic steatosis is associated with metabolic disturbances and elevated liver enzymes, highlighting the importance of biochemical assessment in diabetic patients.

Table 7: Biochemical parameters in patients with and without hepatic steatosis

Test	Steatosis (Mean ± SD)	No Steatosis (Mean ± SD)	P-value
LDL (mg/dL)	123 ± 23	115 ± 22	0.076
HDL (mg/dL)	44 ± 8	46 ± 7	0.185
TG (mg/dL)	220 ± 87	182 ± 54	0.004
Cholesterol (mg/dL)	214 ± 30	197 ± 31	0.003
AST (U/L)	35 ± 11	34 ± 11	0.421
ALT (U/L)	41 ± 13	36 ± 13	0.042

Duration of Diabetes and Comorbidities

The mean duration of diabetes did not differ significantly between groups (9.9 vs. 10.7 years, p=0.393). However, the prevalence of hypertension (69% vs. 31%, p=0.020) and cardiovascular disease (83.3% vs. 16.7%, p=0.030) was significantly higher among patients with hepatic steatosis, whereas other comorbidities, including dyslipidemia, hypothyroidism, arthritis, renal failure, and rheumatoid arthritis, did not differ significantly (Table 8). These findings suggest that hepatic steatosis in diabetic patients is associated with a higher burden of cardiovascular disease and hypertension.

Table 8: Diabetes duration and comorbidities in patients with and without hepatic steatosis

Variable	Steatosis Mean ± SD / n (%)	No Steatosis Mean ± SD / n (%)	P-value
Diabetes duration (years)	9.9 ± 5.5	10.7 ± 5.4	0.393
Hypertension	20 (69%)	9 (31%)	0.020
Dyslipidemia	8 (53.3%)	7 (46.7%)	0.783
Hypothyroidism	5 (31.3%)	11 (68.7%)	0.109
Osteoarthritis	4 (50%)	4 (50%)	1.0
Cardiovascular disease	10 (83.3%)	2 (16.7%)	0.030
Renal failure	0 (0%)	1 (100%)	1.0
Rheumatoid arthritis	0 (0%)	1 (100%)	1.0

Vitamin D Levels by Steatosis Grade

Vitamin D levels tended to decrease with increasing grades of hepatic steatosis, ranging from a mean of 24.4 ng/mL in grade 1 to 18.4 ng/mL in grade 4; however, this trend was not statistically significant (p=0.411) (Table 9). These findings suggest that, although vitamin D deficiency may be related to the severity of steatosis, the study sample lacked sufficient power to detect this association significantly.

Table 9: Vitamin D levels according to hepatic steatosis grade

Steatosis Grade	Mean Vitamin D (ng/mL)	SD	P-value
1	24.4	7.8	0.411
2	23.5	12.4	–
3	22.0	11.1	–
4	18.4	8.3	–

Discussion

This study aimed to investigate the association between plasma vitamin D levels and hepatic steatosis in diabetic patients attending Imam Khomeini Hospital. Our results demonstrate a significant relationship between vitamin D deficiency and the presence of hepatic steatosis.

Among the 128 diabetic patients evaluated, 75.8% were men and 24.2% were women, with a mean age of 52 ± 12 years; the highest prevalence was observed in the 60–69-year age group. Most participants were overweight (43.8%) or obese (38.3%), indicating a population with clear metabolic risk factors. The prevalence of vitamin D deficiency (71.9%) was considerably higher than that reported in the general population. For instance, Kumar et al. (2023) reported a 45% prevalence in India. Such differences may reflect geographic factors, sun exposure, dietary habits, lifestyle, or variations in laboratory methods (Cashman, 2022).

The principal finding of our study was that patients with hepatic steatosis had lower vitamin D levels compared to those without steatosis (22.4 vs. 26.6 ng/mL, p=0.005). Vitamin D deficiency was also more prevalent in the steatosis group (79.7% vs. 61.4%, p=0.049). These results are consistent with the findings of Xing et al. (2022). Similarly, Xiu et al. (2021) demonstrated significantly lower vitamin D levels in diabetic patients with MASLD, with a negative correlation between vitamin D and fibrosis severity. Zhang et al. (2021) reported that 25(OH)D levels below 20 ng/mL were associated with increased fibrosis risk. The inverse gradient between vitamin D levels and steatosis severity supports the proposed protective role of vitamin D in liver disease progression (Wang et al., 2015).

The precise mechanism underlying this association remains unclear. Some studies suggest that vitamin D exerts hepatoprotective effects via inhibition of inflammatory signaling and reduction of oxidative stress (Cai et al., 2020). Conversely, advanced hepatic steatosis may itself impair vitamin D metabolism. Monitoring vitamin D levels in diabetic patients, especially those with hepatic steatosis, holds clinical importance. Considering the high prevalence of deficiency, screening and supplementation may form part of a comprehensive management strategy. Liu et al. (2019) proposed a potential critical threshold below which vitamin D deficiency increases steatosis risk.

In steatosis patients, obese individuals had lower vitamin D levels compared to those with normal weight (18.1 vs. 28.6 ng/mL, p=0.019). A similar trend was observed in non-steatosis patients (22.3 vs. 31.6 ng/mL, p=0.010) (Hosny et al., 2019; Liu et al., 2019). This may be due to sequestration of vitamin D in adipose tissue, reducing its bioavailability (Cai et al., 2020). Among patients without steatosis, women exhibited higher vitamin D levels than men (36.7 vs. 24.5 ng/mL, p<0.001), possibly reflecting hormonal or cultural factors, whereas no sex differences were observed in the steatosis group (Kumar et al., 2023). In non-steatosis patients, poor glycemic control (HbA1c >7%) was associated with lower vitamin D levels (p=0.008), consistent with Xing et al. (2022).

Patients with hepatic steatosis had a higher prevalence of hypertension (69% vs. 31%, p=0.020) and cardiovascular disease (83.3% vs. 16.7%, p=0.030), emphasizing steatosis as part of a systemic metabolic disorder (Liu et al., 2019). However, some studies (Dutra et al., 2021; Ha et al., 2017) did not observe these associations, likely reflecting differences in populations, lifestyle, diagnostic methods, and confounding factor control.

This study has several limitations that should be considered when interpreting the findings. First, the cross-sectional design precludes any inference of a causal relationship between vitamin D status and MASLD in diabetic patients. Second, hepatic steatosis was diagnosed using ultrasonography, which, although practical and widely available, has lower accuracy compared with gold-standard methods such as liver biopsy or advanced magnetic resonance–based imaging techniques. Third, certain potential confounders, including seasonal variations in vitamin D levels, supplement intake, and sunlight exposure, were not fully controlled, which may have influenced the observed associations. Therefore, prospective studies employing more precise diagnostic tools and more comprehensive control of confounding factors are warranted.

Conclusion

Vitamin D deficiency is significantly associated with hepatic steatosis in diabetic patients, particularly among obese individuals and those with poor glycemic control (Xing et al., 2022; Xiu et al., 2021; Cai et al., 2020). Screening for and correcting vitamin D deficiency could be integrated into comprehensive patient management strategies. Nevertheless, prospective interventional studies are needed to confirm the efficacy of supplementation.

Declarations

Conflict of interest

The authors declare there is no competing interests.

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Consent for publications

The authors gave approval for the publication of the manuscript.

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Authors' contributions

EM contributed to conceptualization, visualization, and writing of the original draft. SN performed formal analysis, supervision, and validation. MH participated in all aspects of data curation, investigation, methodology, project administration, resources, software, and review & editing. All authors read and approved the final manuscript.

Ethical considerations

The study protocol was approved by the Ethics Committee of Urmia University of Medical Sciences (Approval No.: 1403-046). All participants provided written informed consent after receiving full information about the study objectives, procedures, and potential benefits and risks. Participant data were maintained confidentially.

The authors have fully adhered to ethical standards, ensuring no issues related to plagiarism, misconduct, data fabrication, falsification, duplicate publication or submission, or redundancy.

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