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Correlation between Serum Zinc Level and Hepatic Fibrosis in Type 2 Diabetic Patients with Non-Alcoholic Fatty Liver Disease

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Abstract:

Background: Zinc is an important trace element involved in metabolic regulation, insulin signaling, antioxidant and anti-inflammatory pathways. Zinc deficiency has been associated with insulin resistance (IR), oxidative stress, liver inflammation, and non-alcoholic fatty liver disease (NAFLD) progression. This study aimed to investigate the correlation between serum zinc levels and hepatic fibrosis in type 2 diabetes mellitus (T2DM) patients with NAFLD. **Patients & Methods:** This cross-sectional study included 84 T2DM patients with NAFLD attended the National Nutrition Institute (NNI) outpatient clinics, from June 2022 to June 2023. Participants underwent clinical, anthropometric, and biochemical assessments, including complete blood count (CBC), liver function, fasting glucose, lipid profile, fasting insulin, Homeostatic Model Assessment for Insulin Resistance (HOMA-IR), and serum zinc. NAFLD was diagnosed using the NAFLD-liver fat score and abdominal ultrasound, and hepatic fibrosis was assessed with the NAFLD fibrosis score (NFS). **Results:** Among the participants, 34.5% had advanced fibrosis (NFS > 0.676), and 17.9% had zinc deficiency (Zn < 80 μg/dL). Patients with advanced fibrosis were significantly older, had a higher BMI, elevated AST, and lower serum zinc levels. Serum zinc was negatively correlated with NFS (r = −0 .44, p < .001) and age, and positively with albumin. Patients with lower zinc levels showed significantly higher NFS compared to those with higher zinc levels. In multivariate analysis, age, BMI, AST, and serum zinc were independent predictors of advanced fibrosis. **Conclusion:** Low serum zinc levels were correlated with advanced hepatic fibrosis in T2DM patients with NAFLD, highlighting zinc's potential as a biomarker and therapeutic target

Keywords: Insulin Resistance; Liver disease progression; Zinc deficiency

Introduction

Type 2 diabetes mellitus (T2DM) and non-alcoholic fatty liver disease (NAFLD) are increasingly prevalent worldwide, primarily due to the rising incidence of obesity and insulin resistance (IR) [1]. A bidirectional relationship between these two conditions has been described, where T2DM exacerbates hepatic steatosis, inflammation, and fibrosis [2]. In parallel, NAFLD affects a large proportion (45–80%) of individuals with diabetes [3].

Zinc (Zn) is a trace element that plays an essential role in many aspects of metabolic function and hepatic health. Zn deficiency has been shown to impair lipid metabolism [4], and contribute to NAFLD progression [5]. Moreover, zinc modulates oxidative stress [6] and regulates hepatic zinc transporters such as Zip14 [7]. It also plays an antifibrotic role through the modulation of matrix metalloproteinases (MMPs) [8], and contributes to hepatoprotection by reducing inflammatory signaling and oxidative stress [9].

In the context of T2DM, Zn deficiency further worsens IR by impairing insulin synthesis and signaling [10]. An inverse association between serum Zn levels, IR, and liver fibrosis was reported by Ito et al.[11], suggesting a potential mechanistic link worth exploring.

Aim of the work

This study aimed to investigate the correlation between serum Zn levels and hepatic fibrosis in T2DM patients with NAFLD.

Patients and Methods

This cross-sectional study enrolled 84 clinically stable patients diagnosed with both T2DM and NAFLD. Participants were recruited from the National Nutrition Institute (NNI) outpatient clinics, Cairo, Egypt, between June 2022 and June 2023. Eligible participants were adults aged over 18 years, of both sexes, ambulatory, orally fed.

Exclusion criteria included individuals with significant alcohol intake, chronic liver diseases (e.g., viral hepatitis, autoimmune hepatitis, Wilson's disease), cirrhosis or liver decompensation, malignancy, severe comorbidities (e.g., heart failure, kidney disease), acute diabetic complications, pregnancy, lactation, history of surgeries leading to secondary NAFLD, malabsorption syndromes, or current use of steroids or zinc supplements.

Sample size calculation: The sample size was determined using OpenEpi software, based on methodologies described by Kelsey et al. [12] and data from related studies (Ciardullo et al. [13] ; García-Compeán et al. [14]), provided a final sample of 84 patients.

Study procedure: Each participant underwent comprehensive medical history and clinical examination, with emphasis on lifestyle habits, diabetes duration, comorbidities, and medication history. Blood pressure was measured and systemic hypertension was defined as ≥140/90 mmHg following European Society of Cardiology and European Society of Hypertension (ESC/ESH) guidelines by Williams et al. [15]. Anthropometric assessment included weight, height, body mass index (BMI) classification and waist circumference (WC) measurement according to WHO criteria [16], [17].

Laboratory workup: Blood samples were obtained after a 12-hour fast for biochemical analysis at the NNI laboratory. Tests included complete blood count (CBC), liver enzymes (AST, ALT, GGT), serum albumin, fasting glucose, lipid profile (total cholesterol, triglycerides, HDL-C, LDL-C calculated using the Friedewald formula [18] , plasma insulin, and serum zinc. Plasma insulin was measured via ELISA (DRG Instruments, Germany), and IR was calculated using the HOMA-IR index according to Matthews et al. [19]. Zn deficiency was defined as <80 μg/dL [20], [21]. Metabolic syndrome was identified based on AHA/NHLBI criteria requiring at

least three of five specific risk factors, following Grundy et al. [22]. NAFLD-LFS, calculated using metabolic syndrome status, T2DM, fasting insulin, AST, and AST/ALT ratio, was applied to confirm hepatic steatosis as proposed by Kotronen et al. [23]. NAFLD diagnosis was confirmed by ultrasound as described by Saadeh et al. [24] and a NAFLD Liver Fat Score (NAFLD-LFS) ≥ −0.640 [23].

Participants were categorized into two groups based on the NAFLD Fibrosis Score (NFS), a validated non-invasive fibrosis marker, following Angulo et al. [25]: the non-advanced fibrosis group (NFS ≤ 0.676; n = 55) and the advanced fibrosis group (NFS > 0.676; n = 29). The cutoff value of 0.676 was selected in accordance with prior literature [25].

Ethical consideration: The study received ethical approval from the Research Ethics Committee of Benha Faculty of Medicine and the General Organization for Teaching Hospitals and Institutes (GOTHI) under approval number IN000118. A written informed consent was obtained from all individual participants included in the study."

Statistical analysis: was conducted using SPSS version 21. Quantitative data were expressed as means and standard deviations, while categorical variables were reported as frequencies and percentages. Independent t-tests or Mann-Whitney U tests were used to compare continuous variables, and Chi-square or Fisher's exact tests were applied for categorical comparisons. Correlations were assessed using Pearson's or Spearman's coefficients, with partial correlation to control for confounders. Logistic regression (both univariate and multivariate) was employed to identify significant risk factors. Statistical significance was defined as a p-value < 0.05 in two-tailed tests.

Results

Baseline Characteristics of T2DM Patients with NAFLD:

As shown in (**Table 1)**, the mean age of the study population was 50.71 ± 8.86 years, with a mean BMI of 37.51 ± 8.34 kg/m². The average diabetes duration was 6.23 ± 5.67 years. Hypertension (HTN) affected 47.6% of participants, while metabolic syndrome was highly prevalent (92.9%).

Biochemical Parameters:

(Table 2) presented the mean values for liver enzymes: ALT (26.22 ± 13.29 U/L), AST (25.47 ± 9.35 U/L), and GGT (26.48 ± 7.82 U/L). The mean NAFLD Fibrosis Score (NFS) was 0.245 ± 1.18. Serum Zn averaged 148.05 ± 68.71 μg/dL, and 17.9% of patients were zinc-deficient (Zn < 80 μg/dL).

Fibrosis Classification:

As shown in **(Figure 1)**, 65.5% of patients had non-advanced fibrosis (NFS ≤ 0.676), while 34.5% had advanced fibrosis (NFS > 0.676).

Comparison between Fibrosis Groups:

(Table 3) highlighted that patients with advanced fibrosis were significantly older (54.37 vs. 48.78 years, p = .002), had higher body weight (103.94 vs. 90.63 kg, p = .001), waist circumference (119.84 vs. 112.29 cm, p = .005), and BMI (40.26 vs. 36.06 kg/m², p = .005). HTN was more frequent in this group (65.5% vs. 38.2%, p = .017). Biochemically, they had higher AST (29.76 vs. 23.22 U/L), GGT (p = .04), lower albumin (3.85 vs. 4.14 g/dL, p < .001), and significantly lower serum Zn levels (106.71 vs. 169.85 μg/dL, p < .001). No significant differences were found in HOMA-IR, NAFLD liver fat score, or diabetes duration.

(Figure 2) and (**Figure 3)** showed higher obesity (93.1% vs. 83.6%) and HTN (65.5% vs. 38.2%, p = .017) prevalence in advanced fibrosis.

Zinc Deficiency and Fibrosis Severity:

(Figure 4) demonstrated that Zn deficiency was significantly more prevalent in the advanced fibrosis group (37.9%) compared to non-advanced fibrosis (7.3%, p = .001). Females comprising 80% of Zn-deficient patients.

Correlation Analysis:

(Table 4), serum Zn negatively correlated with NFS (r = -0.44, $p < .001$) and age (r = -0.28, $p = .01$), and positively with albumin (r = 0.43, $p < .001$). **(Figure 5**) presented a significant inverse partial correlation between Zn and NFS (r = -0.33, $p = .004$) after adjusting for confounders (e.g. age, gender, BMI, AST, ALT, albumin, TC, TG, HDL, LDL, FBG, metabolic syndrome). When serum Zn levels stratified into quartiles (Q1: <80 μg/dL, Q2: 80–150 μg/dL, Q3: 150–190 μg/dL, Q4: ≥190 μg/dL), the mean NFS values were 0.909, 0.393, 0.507, and -0.626, respectively, showing a significant graded inverse relationship between Zn levels and fibrosis severity ($p = .000$) (**Figure 6**).

Risk Factors for Advanced Fibrosis: (Table 5) showed univariable logistic regression identified age, BMI, waist circumference, AST, albumin, and serum Zn as significant risk factors for advanced Fibrosis**.** In the multivariable model, independent predictors of advanced fibrosis included age (OR: 1.15, $p = .013$), BMI (OR: 1.50, $p = .005$), AST (OR: 1.11, $p = .013$), and serum Zn (OR: 0.98, $p = .008$).

(Table 6) confirmed that lower serum zinc levels are independently associated with increased fibrosis severity (OR: 0.98; 95% CI: 0.96–0.99; $p = .008$) after adjusting for age, BMI, waist circumference, AST, GGT, and albumin.

Table 1: Demographic & Clinical characteristics of T2DM patients with NAFLD

Variables	**Patients (N=84)**
Age (years)	50.71 ± 8.86
Weight (kg)	95.23 ± 18.20
Gender (M/F)	14/70 (17% /83%)
Height (cm)	159.28 ± 8.34
BMI (Kg/m2)	37.51 ± 6.58
– **Overweight (25 – <30)**	11(13.1%)
– **Obesity class I (30 – < 35**	27 (32.1%)
– **Obesity class II (35 – < 40)**	19 (22.6%)
– **Obesity class III (≥ 40)**	27 (32.1%)
WC (cm)	114.89 ± 11.81
SBP (mmHg)	123.27 ± 16.25
DBP (mmHg)	82.20 ± 10.15
Duration of diabetes (Years)	6.23 ± 5.67
HTN	40 (47.6%)
Metabolic syndrome	78 (92.9%)

Quantitative variables were expressed as (mean ± SD) & Qualitative variables as n (%). SBP: systolic blood pressure; DBP: diastolic blood pressure; BMI: body mass index; WC: waist circumference; HTN; hypertension.

Table 2: Biochemical characteristics of T2DM patients with NAFLD

Variables	Patients (N=84)
Hb (g/dl)	12.48 ± 1.41
TLC	6.74 ± 2.33
Platelets	23^.20 ± 63.14
FBG (mg/dl)	144.88 ± 65.29
Insulin (µU/ml)	11.41 ± 4.59
HOMA-IR	3.57 ± 1.28
TC (mg/dl)	215.57 ± 48.64
TG (mg/dl)	149.31 ± 63.43
LDL (mg/dl)	148.74 ± 45.20
HDL (mg/dl)	35.45 ± 9.23
ALT(U/L)	26.22 ± 13.29
AST(U/L)	25.47 ± 9.35
GGT(U/L)	26.48 ± 7.82
Albumin(g/dl)	4.04 ± 0.31
Zinc(µg/dl)	148.05 ± 68.71
Zinc (< 80µg/dl)	15 (17.9 %)
NFS	0.245 ± 1.18
NAFLD-LFS	0.84 ± 1.11

Quantitative variables were expressed as (mean ± SD) & Qualitative variables as n (%).
Hb: hemoglobin; TLC: total leucocytic count; FBG: fasting blood glucose; ALT: alanine aminotransferase; AST: aspartate aminotransferase; GGT: gamma-glutamyl-transpeptidase; TG: triglycerides; TC: total cholesterol; LDL: low density lipoprotein; HDL: high density lipoprotein; HOMA-IR: homeostasis model assessment for insulin resistance; NAFLD-LFS: NAFLD Liver Fat Score; NFS: NAFLD fibrosis score

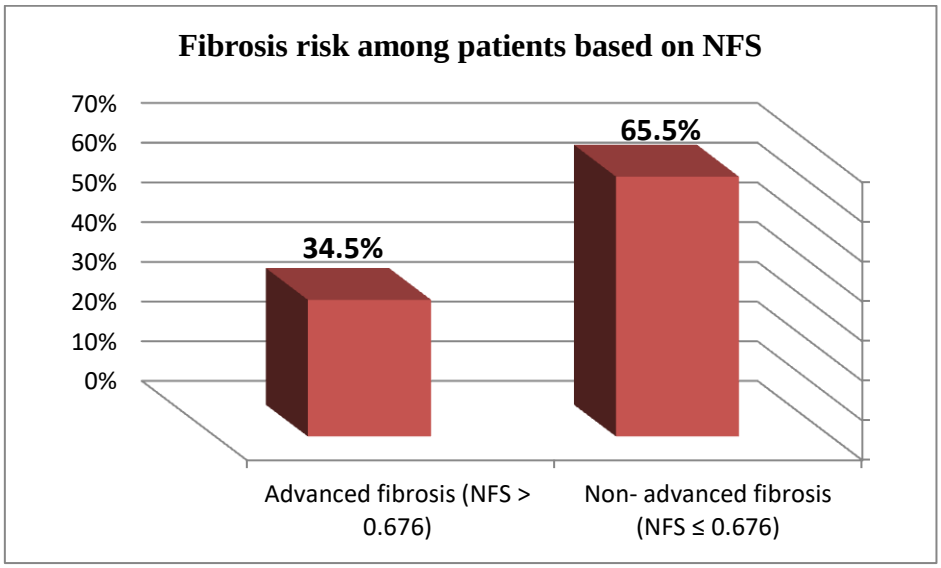
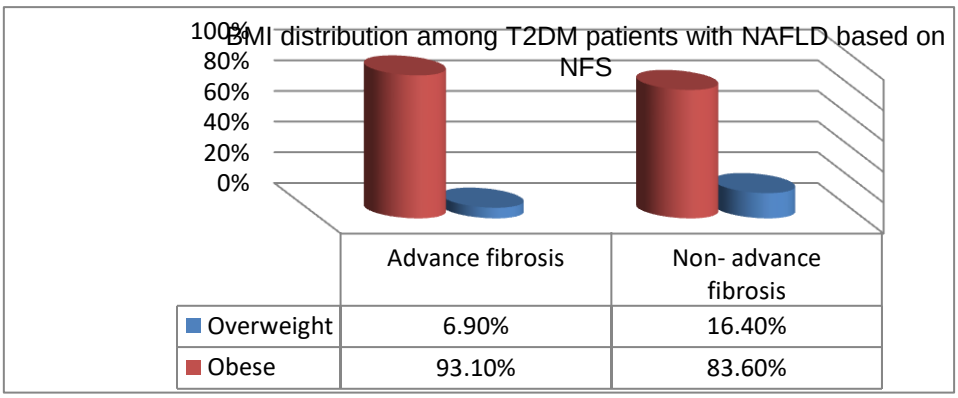


Figure 1: Classification of the patients based on fibrosis risk score (NFS)

Table 3: Clinical &biochemical characteristics of the patients based on NFS

Variables	Non-advanced fibrosis (n=55)	Advanced fibrosis (n=29)	P. value
Age (years)	48.78 ± 9.44	54.37 ± 6.31	0.002*
Gender (M/F)	8/47 (14.5% /85.5%)	6 / 23 (20.7% /79.3%)	0.47*
Duration of diabetes(yr)	5.03 ± 5.16	6.85 ± 5.87	0.16
Weight(kg)	90.63 ± 17.98	103.94 ± 15.44	0.001*
Height(cm)	158.37 ± 8.10	161.00 ± 8.65	0.17
BMI (Kg/m2)	36.06 ± 6.36	40.26 ± 6.19	0.005*
WC (cm)	112.29 ±10.66	119.84 ±12.47	0.005*
SBP(mmHg)	121.63 ± 17.07	126.37 ± 14.32	0.20
DBP(mmHg)	81.67± 10.73	83.20 ± 9.04	0.51
Hb (g/ dL)	12.29 ±1.28	12.83 ± 1.60	0.10
TLC	7.16 ± 2.15	5.94 ± 2.47	0.02*
Platelets	255.20 ± 66.53	205.96 ± 40.46	0.000*
ALT (U/L)	26.43 ±13.77	25.81 ±12.55	0.83
AST (U/L)	23.22 ± 8.13	29.76 ± 10.13	0.002*
GGT (U/L)	25.25 ± 7.67	28.82 ± 7.70	0.04*
Albumin mg/dl	4.14 ± 0.27	3.85 ± 0.29	0.000*
TC (mg/dl)	216.49 ± 49.76	213.82 ± 47.25	0.81
TG (mg/dl)	145.44 ± 51.89	151.35 ± 69.10	0.66
HDL (mg/dl)	36.58 ±10.13	33.30 ±6.87	0.12
LDL (mg/dl)	147.46 ±46.95	151.18 ±42.38	0.72
FBG (mg/dl)	136.69 ± 40.63	145.47 ± 73.65	0.25
Insulin (µIU/ml)	11.16 ± 4.88	11.89 ± 4.02	0.49
HOMA-IR	3.63 ±1.41	3.45 ± 1.0	0.49
Zinc (µg/dl)	169.85 ± 65.11	106.71 ± 55.83	0.000*
NAFLD fat score	0.86 ± 1.14	0.82 ± 1.08	0.87
HTN	21 (38.2%)	19 (65.5%)	0.017*
Metabolic syndrome	50 (90.9%)	28 (96.٦%)	0.42

Quantitative variables were expressed as (mean ± SD) & Qualitative variables as n (%). * Significant (p< 0.05)
SBP: systolic blood pressure; DBP: diastolic blood pressure; Hb: hemoglobin; TLC: total leucocytic count; BMI: body mass index; WC: waist circumference; HTN; hypertension. ALT: alanine aminotransferase; AST: aspartate aminotransferase; GGT: gamma-glutamyl-transpeptidase; TG: triglycerides; TC: total cholesterol; LDL: low density lipoprotein; HDL: high density lipoprotein; FBG: fasting blood glucose; HOMA-IR: homeostasis model assessment for insulin resistance; NAFLD-LFS: NAFLD Liver Fat Score.



(Figure 2): Prevalence of overweight and obesity among patients based on NFS

Table 4: Correlation between serum zinc levels with clinical and laboratory parameters

Variable	Correlation coefficient	P.value
Age (years)	-0.28	0.01*
Weight (kg)	0.027	0.80
BMI (kg/m2)	0.081	0.46
WC (cm)	0.056	0.61
SBP (mmHg)	-0.056	0.61
DBP (mmHg)	-0.111	0.31
AST (U/L)	-0.107	0.33
ALT (U/L)	-0.049	0.66
GGT (U/L)	-0.030	0.78
Albumin (g/dl)	0.43	0.000*
TC (mg/dl)	-0.131	0.23
LDL (mg/dl)	-0.184	0.09
HDL (mg/dl)	0.113	0.30
TG (mg/dl)	0.067	0.54
FBG (mg/dl)	0.001	0.99
HOMA-IR	-0.140	0.20
NFS	-0.443	0.000*

***** Significant (p< 0.05)

SBP: systolic blood pressure; DBP: diastolic blood pressure; BMI: body mass index; WC: waist circumference; HTN; hypertension. ALT: alanine aminotransferase; AST: aspartate aminotransferase; GGT: gamma-glutamyl-transpeptidase; TG: triglycerides; TC: total cholesterol; LDL: low density lipoprotein; HDL: high density lipoprotein; FBG: fasting blood glucose; HOMA-IR: homeostasis model assessment for insulin resistance; NFS: NAFLD fibrosis score

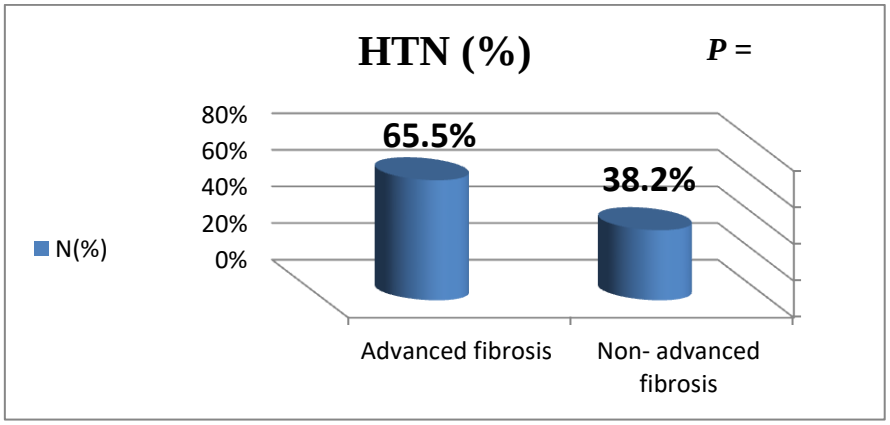


Figure 3: Hypertension (HTN) prevalence (%) among patients based on NFS

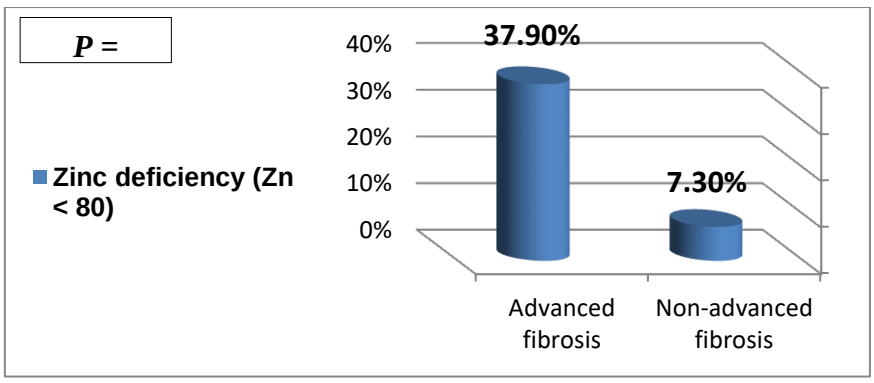


Figure 4: Prevalence of zinc deficiency among patients based on NFS

Table 5: Univariable and multivariable logistic regression analysis of hepatic fibrosis (based on NFS)

Univariable regression analysis				**Multivariable regression analysis**		
Variable	**OR**	**CI**	**P. value**	**OR**	**CI**	**P. value**
Sex	1.53	0.48–4.94	0.474			
Age (years)	1.085	1.022–1.152	0.008	1.158	1.032 – 1.299	**0.013***
BMI (Kg/m2)	1.109	1.028–1.197	0.008	1.503	1.128 –2.001	**0.005***
WC (cm)	1.060	1.016–1.107	0.007	0.894	0.77 – 1.03	0.132
SBP (mmHg)	1.019	0.990–1.049	0.205			
DBP (mmHg)	1.015	0.971-1.062	0.509			
ALT (U/L)	0.996	0.963–1.031	0.837			
AST (U/L)	1.082	1.024-1.143	0.005	1.114	1.023 – 1.214	**0.013***
GGT (U/L)	1.061	1.000–1.125	0.050	1.102	0.985 – 1.23	0.89
Albumin (g/dl)	0.018	0.002–0.157	<0.001	0.66	0.004- 1.10	0.059
TC (mg/dl)	0.999	0.990–1.008	0.810			
TG (mg/dl)	0.998	0.991–1.006	0.684			
HDL (mg/dl)	0.958	0.907–1.012	0.126			
LDL (mg/dl)	1.002	0.992–1.012	0.718			
FBG (mg/dl)	0.992	0.984–1.001	0.071			
Insulin	1.036	0.938–1.145	0.487			
HOMA-IR	0.892	0.622–1.278	0.532			
NAFLD-LFS	0.968	0.645–1.454	0.877			
Zinc (µg/dl)	0.983	0.974–0.992	<0.001	0.980	0.965 –0.995	**0.008***

* Significant (p< 0.05)

SBP: systolic blood pressure; DBP: diastolic blood pressure; BMI: body mass index; WC: waist circumference; HTN; hypertension. ALT: alanine aminotransferase; AST: aspartate aminotransferase; GGT: gamma-glutamyl-transpeptidase; TG: triglycerides; TC: total cholesterol; LDL: low density lipoprotein; HDL: high density lipoprotein; FBG: fasting blood glucose; HOMA-IR: homeostasis model assessment for insulin resistance; NFS: NAFLD fibrosis score; NAFLD-LFS: NAFLD Liver Fat Score; OR: odd ratio; CI: confidence interval.

Table 6: Stepwise regression model after adjustment of other confounders

	OR	**CI**	**P. value**
Zinc	0.983	0.974 - 0.992	<0.001*
Zinc with age adjustment	0.984	0.974 - 0.993	0.001*
Zinc with age and BMI adjustment	0.979	0.968 - 0.991	0.001*
Zinc with age, BMI, WC and adjustment	0.979	0.968 - 0.991	0.001*
Zinc with age, BMI , WC and AST adjustment	0.976	0.962 - 0.989	0.001*
Zinc with age, BMI, WC, AST and GGT adjustment	0.975	0.961 - 0.989	0.001*
Zinc with age, BMI, WC, AST, GGT and albumin adjustment	0.980	0.965 - 0.995	0.008*

* Significant (p< 0.05)

BMI: body mass index; WC: waist circumference; AST: aspartate aminotransferase; GGT: gamma-glutamyl-transpeptidase; OR: odd ratio; CI: confidence interval.

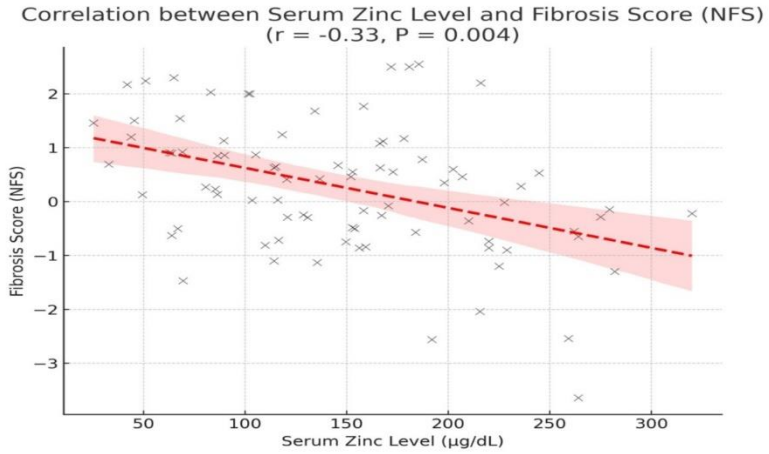


Figure 5: Partial correlation between serum zinc level and NFS

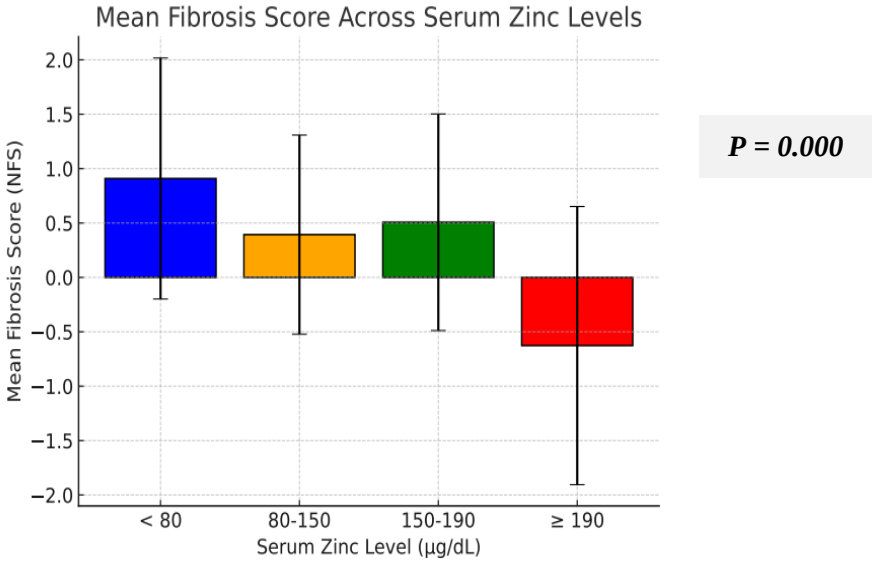


Figure 6: Comparisons of mean NFS across different serum zinc levels

Discussion

The primary objective of this study was to investigate the relationship between serum zinc and hepatic fibrosis in the high-risk population (T2DM with NAFLD).

The current study revealed a significant inverse correlation between serum Zn levels and the severity of liver fibrosis, as assessed by the NAFLD Fibrosis Score (NFS), even after adjusting for potential confounders including age, gender, BMI, liver enzymes, lipid profile, glycemic parameters, and the presence of metabolic syndrome (partial $r = -0.33$, $p = .004$) **(Figure 5).** Notably, zinc deficiency was five times more prevalent among patients with advanced fibrosis (37.9% vs. 7.3%, $p = .001$) **(Figure 4).** Multivariate regression analysis identified Zn deficiency (<80 µg/dL) as an independent risk factor for hepatic fibrosis in patients with T2DM and NAFLD (OR = 0.98, $p = .008$) **(Tables 5 & 6).**

The current results were in agreement with previous studies. Ito et al. [11] reported that lower serum Zn levels were associated with more severe liver histology and fibrosis progression in NAFLD. Similarly,

Akdaş and Yazihan [26] highlighted the protective role of Zn in liver health. Experimental studies by Himoto and Masaki [27] further support this, showing that Zn depletion impairs hepatocyte regeneration and promotes oxidative stress, thus exacerbating hepatic fibrogenesis. Conversely, zinc supplementation has been shown to reduce fibrosis markers such as procollagen III and collagen IV-7S, as demonstrated by Moriya et al. [28].

However, not all studies align. Kosari et al. [29] found no significant association between serum zinc and liver fibrosis after adjusting for confounders such as BMI and IR, suggesting potential attenuation of zinc's effect by metabolic factors. Similarly, Wong et al. [30] reported that the protective effect of zinc became non-significant after adjusting for IL-6, indicating a possible modulatory role of inflammatory cytokines.

These discrepancies may arise from methodological differences across studies. First, heterogeneity in the study populations is a key factor; some studies include patients with liver disease of mixed etiology (e.g., NAFLD and viral hepatitis), while others like those by Younossi et al. [31] and Loomba and Sanyal [32] focus strictly on metabolic-associated liver fibrosis. Liver stiffness values differ significantly based on coexisting metabolic or viral risk factors, which may obscure Zn specific associations as reported by Petta et al. [33]. Second, variability in Zn deficiency thresholds complicates comparisons; studies use different cutoffs ranging from <65 to <80 μg/dL [26], [28], leading to inconsistent prevalence estimates (28–62%) and differing odds ratios for fibrosis (OR: 1.3–2.1).

Mechanistically, Zn appears to influence fibrosis via several interconnected pathways. As a cofactor for antioxidant enzymes such as superoxide dismutase, Zn may mitigate oxidative stress—a known activator of hepatic stellate cells (HSCs) [34]. This study was found a positive correlation between Zn and serum albumin (r = 0.43, p < .001) **(Table 4)** supporting the hypothesis that zinc also contributes to hepatic synthetic function, potentially via modulation of IL-6-driven albumin catabolism [35]. Furthermore, Wei et al. [36] showed that Zn can inhibit TGF-β1/Smad3 signaling, a key driver of hepatic fibrogenesis. Supporting this, animal studies demonstrated that hepatic fibrosis was 2.1 times higher in zinc-deficient mice, while Zn supplementation reduced collagen deposition by 55% [36].

A dose-dependent relationship was observed in the present study: the lowest Zn quartile (<80 μg/dL) had the highest mean NFS (0.909 ± 1.1), while the highest Zn quartile (≥190 μg/dL) had the lowest NFS (−0.626 ± 1.27) (p = .000) **(Figure 6).** This gradient reinforces the potential protective role of Zn in fibrosis attenuation.

The prevalence of Zn deficiency in the current study (17.9%) **(Table 2)** was comparable to previous reports in NAFLD populations. Himoto et al. [37] found a 21% deficiency rate in biopsy-confirmed NASH, rising to 34% in F3–F4 stages compared to 12% in F0–F2. Similarly, Ye et al. [38] reported 18% prevalence overall, with rates increasing alongside disease severity (e.g., 9% in mild steatosis vs. 27% in NASH, p = .003).

The pathophysiology of Zn deficiency in T2DM and NAFLD appears multifactorial. In diabetes, hyperglycemia-induced hyperzincuria leads to renal zinc wasting, thereby Jayawardena et al. [39] recorded a 42% increase in urinary zinc loss in diabetics versus controls (p < .001). In NAFLD, hepatic Zn sequestration occurs via upregulation of metallothioneins, as described by Ozeki et al. [40]. Chronic inflammation also plays a role; King et al. [41] found an inverse relationship between serum Zn and inflammatory markers such as CRP. This "triad" of renal loss, hepatic trapping, and systemic inflammation forms a vicious cycle where metabolic

dysfunction fuels Zn deficiency, which in turn worsens liver pathology. An age-related decline in serum Zn ($r = -0.28$) **(Table 4)** was also observed, consistent with Prasad [42], who highlighted aging and chronic inflammation as contributors to Zn depletion. Albumin's role as the main plasma Zn carrier was evident in the strong positive Zn –albumin correlation ($r = 0.43$, $p < .001$) **(Table 4),** corroborated by Tan et al. [43], who reported improved albumin levels after Zn supplementation in cirrhotic patients.

No significant correlations were found between serum Zn and metabolic markers such as HOMA-IR ($r = -0.14$, $p = .20$) or lipid profile variables (TC, TG, LDL-C, HDL-C) **(Table 4).** This may reflect the overwhelming metabolic burden in our study (mean BMI = 37.5 kg/m²; 92.9% with metabolic syndrome) **(Table 1),** where severe IR and obesity-related inflammation may reduce zinc's protective effects [44]. In contrast to that of Ito et al. [11] and Fathi et al. [45], who reported that Zn correlated inversely with HOMA-IR and IR improved with Zn supplementation. Similarly, the absence of correlation between Zn and liver enzymes (ALT/AST) aligns with Himoto et al. [37], who noted zinc's hepatoprotective effects were more evident in NASH subgroups. Grüngreiff et al. [46] found ALT/AST reductions only in Zn -deficient patients.

The present data also identified key metabolic risk factors for hepatic fibrosis. Hypertension was significantly more prevalent in the advanced fibrosis group (65.5% vs. 38.2%, $p = .017$) **(Table 3, Figure 3),** consistent with Loomba et al. [47], who reported a 1.4-fold increase in fibrosis risk in hypertensive individuals. However, Petäjä and Yki-Järvinen [48] suggested hypertension's predictive value may diminish after adjusting for IR, indicating that overlapping metabolic syndrome components may confound fibrosis associations.

BMI was an independent predictor of fibrosis, with each 1-unit increase associated with a 50% higher risk (OR = 1.50, $p = .005$) **(Table 5),** supported by observed BMI differences between fibrosis groups (40.3 vs. 36.1 kg/m²) **(Table 3, Figure 2).** This mirrors the results of Younossi et al. [49], who identified BMI >35 kg/m² as a strong fibrosis risk factor (OR = 2.1). Wong et al. [30] highlighted the importance of visceral adiposity (MRI-assessed) as a more precise predictor than BMI (OR = 2.28 vs. 1.62 per SD), emphasizing the role of adipose tissue inflammation and insulin resistance (HOMA-IR; $r = 0.63$).

No significant association was found between diabetes duration and fibrosis severity ($p = .16$) **(Table 3),** aligning with a recent meta-analysis by Wongtrakul et al. [50], but contrasting with Chhabra et al. [51], who identified longer diabetes duration as an independent predictor of fibrosis. This discrepancy can arise from the advanced metabolic profile of our study, where visceral obesity and metabolic syndrome components may override duration effects.

Patients with advanced fibrosis also showed higher AST levels (29.76 ± 10.13 vs. 23.22 ± 8.13, $p = .002$) and lower albumin (3.85 ± 0.29 vs. 4.14 ± 0.27, $p = .000$) **(Table 3),** indicating hepatocytes injury and reduced synthetic function. These results were found in Alboraie et al. [52] and Takahashi et al. [53], who reported a similar association between AST, albumin, and fibrosis severity.

Strengths of the Study:

This study is one of the few that examine the association between serum Zn levels and hepatic fibrosis, especially in patients with T2DM and NAFLD—a high-risk group for fibrosis progression. The application of rigorous statistical analysis, including correlation and multivariable logistic regression, enabled the identification of Zn deficiency as an independent predictor of advanced fibrosis. Moreover, the study highlights the potential role of Zn as a modifiable clinical factor, supporting future exploration of Zn

supplementation as a therapeutic approach in this population.

Potential weaknesses of the study

When assessing these results, some key limitations must be acknowledged. First, hepatic fibrosis was assessed using the NAFLD Fibrosis Score (NFS) rather than liver biopsy—the diagnostic gold standard—although NFS is a validated, non-invasive tool recommended for fibrosis risk stratification [(54)]. Second, NAFLD diagnosis was based on ultrasonography combined with the NAFLD liver fat score, rather than histopathological confirmation. While liver biopsy remains definitive, ultrasonography is a widely accepted method, and the NAFLD liver fat score has demonstrated strong diagnostic accuracy [(55)]. Third, the study's single-center, cross-sectional design limits the generalizability of the results and precludes causal inference, with the possibility of residual confounding from unmeasured variables.

Conclusion

The study findings indicated that

- Serum Zn levels inversely correlate with hepatic fibrosis severity in T2DM patients with NAFLD, independent of age, BMI, and metabolic confounders. This supports zinc's potential role as a biomarker for fibrosis risk stratification.
- Zinc deficiency was more prevalent in advanced fibrosis and independently predicted fibrosis. The dose-response relationship across Zn quartiles further underscores its protective association.
- Advanced fibrosis was associated with older age, higher BMI, elevated AST, and hypoalbuminemia. However, Zn remained a modifiable risk factor after adjusting for these confounders.

Recommendations

- Serum Zn levels may serve as a cost-effective and non-invasive biomarker for fibrosis risk stratification.
- Routine assessment of Zn status in diabetic patients with NAFLD may aid in identifying individuals at higher risk for fibrosis progression.
- Longitudinal studies are needed to establish causality, and intervention trials should assess whether Zn supplementation improves fibrosis outcomes in T2DM patients with NAFLD.

Conflict of Interest

The authors declare no conflict of interest.

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