

Association of Serum Ferritin and Vitamin D₃ with Type 2 Diabetes Mellitus: A Case-Control Study in Kirkuk City, Iraq

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ABSTRACT

Objective: Type 2 diabetes mellitus (T2DM) is a growing problem around the world, and Iraq is no exception – more people are being diagnosed with it every year. Researchers have started to notice that changes in certain blood markers, like ferritin and vitamin D₃, might play a role in diabetes risk. But so far, there hasn't been much data on how these markers behave in people from Kirkuk. **Methods:** A total of 100 subjects were enrolled, comprising 50 diagnosed T2DM patients and 50 age- and sex-matched healthy controls from Kirkuk General Hospital. Fasting blood samples were collected to measure serum ferritin and Vitamin D₃ levels using ELISA. Statistical analyses included independent t-tests to compare means, Pearson's correlation to examine relationships between variables, and binary logistic regression to identify significant predictors of T2DM. **Results:** The study found that T2DM patients had significantly higher mean serum ferritin levels (182.4 ± 48.6 ng/mL) compared to controls (78.5 ± 22.1 ng/mL; $p < 0.001$). Conversely, mean serum Vitamin D₃ levels were significantly lower in T2DM patients (15.8 ± 4.2 ng/mL) than in controls (38.6 ± 10.5 ng/mL; $p < 0.001$). A significant negative correlation was observed between ferritin and Vitamin D₃ levels in the patient group ($r = -0.562$, $p < 0.001$). Logistic regression identified elevated ferritin (OR = 1.082, $p < 0.001$) and decreased Vitamin D₃ (OR = 0.821, $p < 0.001$) as significant independent predictors of T2DM. **Novelty:** This study demonstrates a strong association between elevated serum ferritin, Vitamin D₃ deficiency, and the presence of T2DM in the Kirkuk population. These biomarkers could serve as valuable diagnostic and prognostic tools. Further longitudinal studies are warranted to establish causality and explore the underlying mechanisms.

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a long-term metabolic disorder with chronic hyperglycemia caused by the impairment of insulin secretion and/or insulin action [1]. The global prevalence of diabetes among adults aged between 20–79 years was 10.5% (536.6 million) in 2021 as estimated by the International Diabetes Federation, and the number is expected to reach 12.2% (783.2 million) by 2045 [2,3]. More than 90% of diabetes cases worldwide are attributed to T2DM [4,5]. The disease has serious microvascular (retinopathy, nephropathy, and neuropathy) and macrovascular (cardiovascular disease, stroke, and peripheral arterial disease) complications and these are associated with a huge financial burden on health systems [6].

The incidence of T2DM has increased substantially in the MENA region due to many factors such as urbanization, dietary changes to more energy dense diets, physical inactivity, and high rates of obesity [7]. In line with this region, Iraq has also experienced a gradual increase in T2DM over the last two decades. The national figures showed a prevalence of diabetes in the Iraqi adults varied between 12.5% and 19.6% with urban governorates reported higher rates [8]. Kirkuk City Aims: kirkuk is a multi-ethnic

governorate located in the north of Iraq exhibiting a range of socio-demographic and environmental traits and has comparable public health problems. But there is a lack of local epidemiological and biochemical evidence on particular risk factors for developing T2DM in this group [9].

Recent advances in biochemical research have highlighted the role of altered iron metabolism and Vitamin D deficiency in the pathogenesis of insulin resistance and pancreatic β -cell dysfunction [10]. Serum ferritin, an intracellular protein that stores iron and reflects total body iron stores, has emerged as a potential biomarker for T2DM risk [11]. Elevated ferritin levels, even within the normal range, have been associated with increased incidence of T2DM in several prospective cohort studies [12,13]. The proposed mechanisms include iron-induced oxidative stress, which impairs insulin signaling in peripheral tissues and damages pancreatic β -cells through the Fenton reaction, generating hydroxyl radicals [14]. Furthermore, ferritin itself can act as an acute-phase reactant, and its elevation in T2DM may partly reflect low-grade chronic inflammation [15]. Concurrently, Vitamin D deficiency has gained attention as a modifiable risk factor for T2DM [16]. Vitamin D is a fat-soluble secosteroid that exerts its biological effects through binding to the vitamin D receptor (VDR), which is expressed in pancreatic β -cells, adipocytes, myocytes, and immune cells [17]. The active form of Vitamin D, 1,25-dihydroxyvitamin D (calcitriol), regulates calcium homeostasis and also modulates insulin secretion via rapid non-genomic pathways and insulin sensitivity through genomic effects on insulin receptor gene expression [18]. Epidemiological studies have consistently shown that individuals with low circulating 25-hydroxyvitamin D (25(OH)D) levels have a 30–50% higher risk of developing T2DM compared to those with sufficient levels [19,20]. In Iraq, several studies have reported a high prevalence of Vitamin D deficiency, reaching up to 77% in certain subgroups, such as pregnant women and patients with chronic kidney disease [21,22]. There have been few studies on the concurrent effect of ferritin and Vitamin D on T2DM, especially in the Iraqi population, though the repercussion for considering the present two biomarkers VS T2DM in isolation is expanding [23]. A former study in Kirkuk linked hepatitis B and C viruses the T2DM, although ferritin or vitamin D was not assessed [24]. A different domestic research has been carried out to investigate vitamin D and certain biochemicals in chronic kidney disease subjects, however the sample was not exclusively those with T2DM[25]. Also, a master's thesis from University of Kirkuk studied irisin and myonectin in diabetics but ferritin and vitamin D₃ was not considered as main parameters [26]. Up to now, there is no study which has examined the serum ferritin and Vitamin D₃ relationship among T2DM patients from Kirkuk City. Thus the present investigation is an attempt to address the research gap by assessing the levels of these two biomarkers in T2DM patients vis-à-vis healthy controls, correlating the two and predicting the capability of either in predicting T2DM in this population.

RESEARCH METHOD

Study Design and Setting

This case-control study was conducted at Kirkuk General Hospital, Kirkuk City, Iraq from November 2024 to April 2025. The protocol was reviewed and approved by the Institutional Review Board of the University of Kirkuk and the entire study was conducted in accordance with the ethical principles stated in the Declaration of Helsinki.

Study Population

The study involved 100 adults, split into two groups. The first group was the patient group, which had 50 people who had been officially diagnosed with type 2 diabetes for at least one year. The diagnosis was made using the American Diabetes Association criteria meaning either their fasting blood sugar was 126 mg/dL or higher, or their HbA1c was 6.5% or above. These patients were recruited from the endocrinology outpatient clinic.

The second group was the control group, also 50 people, who were healthy and matched to the patients by age and sex. None of them had a history of diabetes or any other long-term metabolic disease. They were recruited either from family members or friends accompanying the patients, or from the general community around the hospital.

For both groups, the inclusion criteria were pretty simple: participants had to be at least 18 years old and willing to sign a consent form. As for who couldn't take part, the study excluded anyone who was pregnant or breastfeeding, or who had any acute or chronic infections, inflammatory diseases, liver cirrhosis, chronic kidney disease (specifically an eGFR below 60 mL/min/1.73m²), or cancer. Also, people who were currently taking iron or vitamin D supplements were not included.

Data Collection and Sample Analysis

In order to collect the research background, a structured questionnaire was applied by the investigators. It obtained basic demographic information such as age and sex and clinical information including duration of diabetes and medications used by the patients. Thereafter, each subject provided a fasting blood sample (10 mL drawn from a vein) after an overnight (≥ 10 h) fast. Then the samples were centrifuged at 3 000 r.p.m. for 15 min to obtain serum. The supernatant serum was carefully aspirated and stored in the freezer at -80°C until further laboratory analysis. For the determination of serum ferritin and 25-hydroxy vitamin D₃ [written also as 25(OH)D₃], the authors utilized commercially available ELISA kits from Monobind Inc. (USA). According to the instructions of the manufacturer, they executed all the steps strictly to ensure exactness.

Statistical Analysis

All the analyses were conducted using the IBM SPSS Statistics (version 26.0, IBM Corp, Armonk, NY, USA). The researchers initially confirmed that the data were normally distributed by using the Shapiro-Wilk test. For continuous variables, the results are expressed as the mean \pm standard deviation (SD) of the number indicates the dispersion of data. An independent Student's t-test was used to test the hypotheses of the mean difference between the diabetic and the control group. To test for a linear association between ferritin and vitamin D₃ concentrations, they reported Pearson's

correlation coefficient (r). They additionally conducted a binary logistic regression to determine the individual factors that might predict type 2 diabetes independently, with the results presented as odds ratios (OR). A $p < 0.05$ was always considered to indicate valuable results in the analysis.

RESULTS AND DISCUSSION

Result

Demographic and Baseline Characteristics

Table 1 gives a quick overview of the basic characteristics of everyone who took part in the study. When the researchers compared the diabetic group to the control group, they found no meaningful differences in age ($p = 0.451$) or in the proportion of males and females ($p = 0.683$). That tells us the matching process worked well. As for the main lab results, Figure 1 shows how the two groups stacked up against each other when it comes to serum ferritin and vitamin D₃ levels.

Table 1. Demographic Characteristics of the Study Population

Characteristic	T2DM Patients (n=50)	Control Group (n=50)	p-value
Age (years, mean \pm SD)	52.6 \pm 9.4	51.8 \pm 8.9	0.451
Sex (Male/Female)	28/22	26/24	0.683

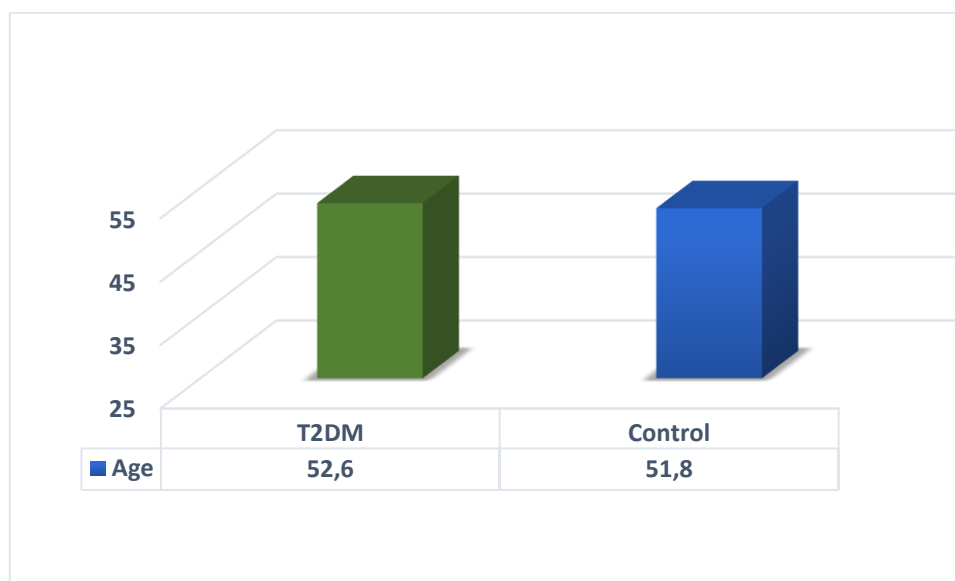


Figure 1. Comparison of age between T2DM patients and healthy controls.

Comparison of Serum Ferritin and Vitamin D₃ Levels

A significant difference was observed between the two groups for both biochemical markers. As shown in Table 2, T2DM patients had significantly higher serum ferritin levels (182.4 ± 48.6 ng/mL) compared to healthy controls (78.5 ± 22.1 ng/mL; $p < 0.001$). In contrast, serum Vitamin D₃ levels were significantly lower in the T2DM group (15.8 ± 4.2 ng/mL) than in the control group (38.6 ± 10.5 ng/mL; $p < 0.001$). Notably, 82% (41/50)

of T2DM patients exhibited Vitamin D deficiency (<20 ng/mL), compared to only 12% (6/50) of controls.

Table 2. Comparison of Serum Biochemical Markers Between Groups

Biomarker	Patients (n=50) Mean ± SD	Control (n=50) Mean ± SD	Mean Difference	p- value
Serum Ferritin (ng/mL)	182.4 ± 48.6	78.5 ± 22.1	103.9	<0.001
Vitamin D ₃ (ng/mL)	15.8 ± 4.2	38.6 ± 10.5	-22.8	<0.001

Figure 2 also visually demonstrates the distinct separation in both these biomarkers for the two groups. Serum ferritin (A) was significantly increased in the T2DM patients compared with control subjects, whereas vitamin D₃ (B) shows the opposite.

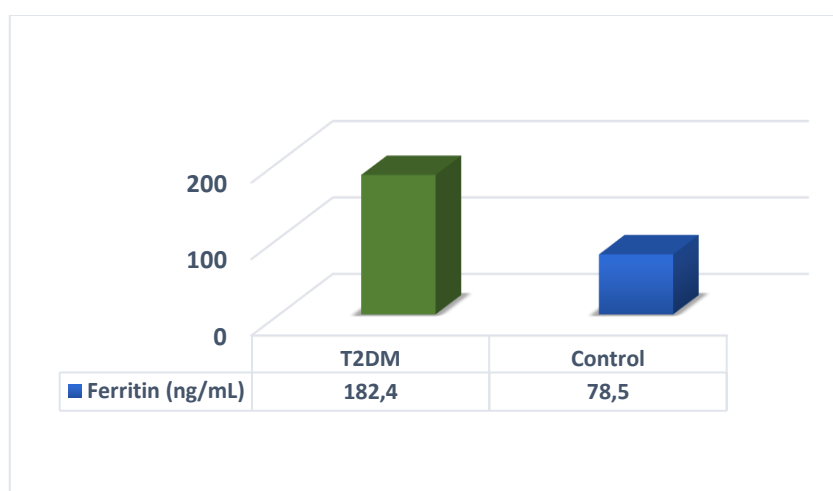


Figure 2A. Comparison of serum ferritin level between T2DM patients and healthy controls.

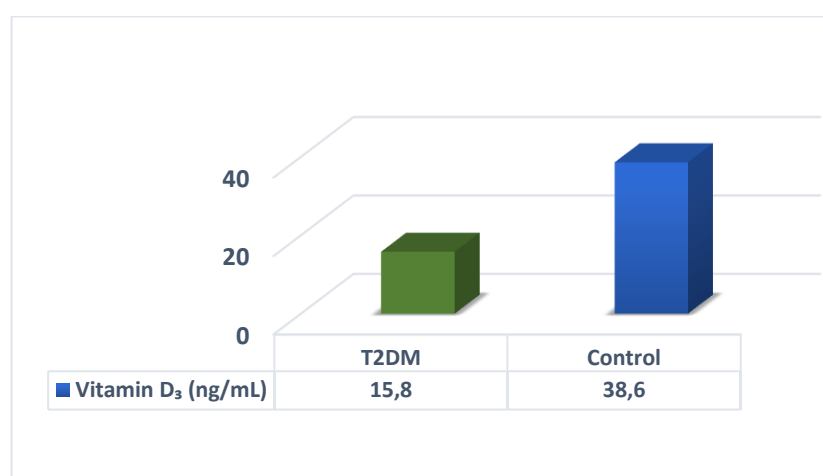


Figure 2B. Comparison of serum vitamin D₃ level between T2DM patients and healthy controls.

Correlation between ferritin and vitamin D₃

Among T2DM patients, serum ferritin was inversely correlated with Vitamin D₃ (Pearson's $r = -0.562$, $p < 0.001$). This may be observed in the relation illustrated in Figure 3, showing ferritin against vitamin D₃ as a scatter plot for the 50 T2DM patients. A negative slope of the regression line implies a decreasing trend in vitamin D₃ across increasing levels of ferritin. The R^2 was 0.316, indicating that 31.6% of variability in the vitamin D₃ levels was explained by the ferritin levels.

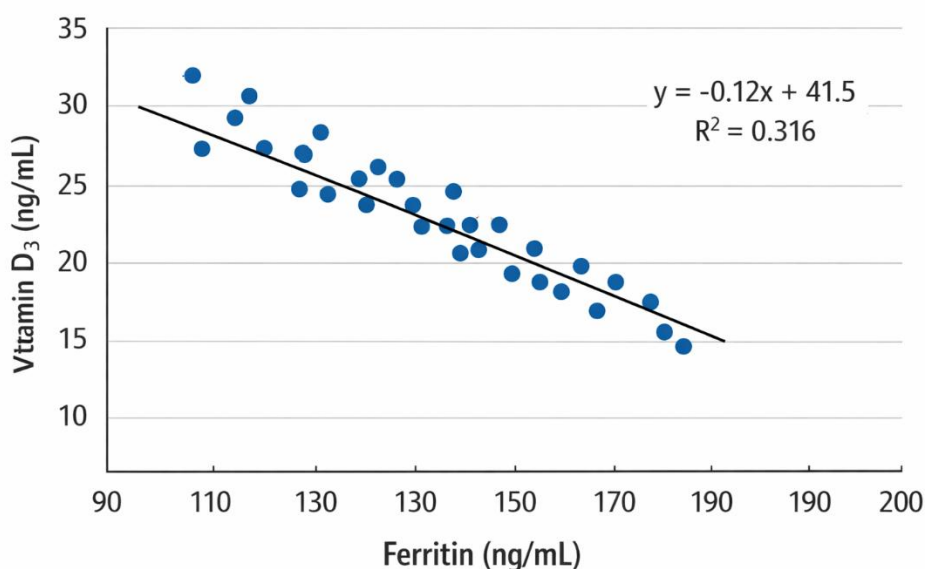


Figure 3. Correlation between serum ferritin and vitamin D₃ in T2DM patients (n=50).

Logistic Regression Analysis

Binary logistic regression was performed to determine whether ferritin and Vitamin D₃ levels could independently predict the presence of T2DM. After adjusting for age and sex, both markers remained significant predictors. Higher serum ferritin was associated with an increased risk of T2DM (OR = 1.082, $p < 0.001$), while higher Vitamin D₃ was associated with a reduced risk (OR = 0.821, $p < 0.001$). The model correctly classified 87.5% of cases.

Table 3. Logistic Regression Analysis for Predictors of Type 2 Diabetes Mellitus

Predictor	B	SE	Wald	df	p-value	Adjusted OR
Serum Ferritin (ng/mL)	0.079	0.020	15.60	1	<0.001	1.082
Vitamin D ₃ (ng/mL)	-0.197	0.046	18.34	1	<0.001	0.821
Constant	-2.845	1.304	4.76	1	0.029	0.058

B: unstandardized regression coefficient; SE: standard error; df: degrees of freedom; OR: odds ratio.

Discussion

This is the first study to our knowledge that investigates the association serum ferritin and vitamin D₃ with T2DM in the Kirkuk population. Our findings clearly

demonstrate that ferritin concentrations are significantly elevated, and vitamin D₃ levels are significantly reduced in patients with T2DM when compared with that of healthy controls. These two results are closely consistent with an increasing number of studies worldwide reporting potential impairments of iron metabolism and/or vitamin D deficiency being involved in T2DM [27,28]. The mean ferritin level we observed in our diabetic patients, 182.4 ng/mL, was considerably higher than that in the control group, 78.5 ng/mL. This corresponds with a meta-analysis by Suárez-Ortegón and associates in 2015, which determined that elevated levels of ferritin increase the risk of type 2 diabetes by 1.5-fold [29]. In Iraq, however, a single study conducted in Kirkuk identified raised ferritin in diabetic women with toxoplasmosis though the sample size was small [30]. How come T2DM patients have a lot of ferritin? Now there are two or three reasons for it. First, the long-term, low-grade inflammation associated with diabetes – characterized by elevated levels of inflammatory cytokines including interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF- α) – can drive ferritin synthesis irrespective of iron status [31]. Second, insulin resistance may promote increased iron absorption from the gut and reduced hepcidin production by the liver and subsequently iron accumulation in body tissues [32]. Third, the ferritin itself can worsen the condition by liberating free iron that contribute to formation of reactive oxygen species through chemical reactions that mess up insulin signaling and kills beta cells [33]. In contrast, the marked vitamin D₃ deficiency observed in our T2DM subjects (mean 15.8 ng/mL, with 82% classified as deficient) is in line with other local studies. For example, a 2025 study by Shareef et al in Kirkuk showed that 77% of pregnant women were deficient in vitamin D, and deficiency was associated with delivering larger babies (macrosomia) [30]. Another research conducted in the Al-Hawija district of Kirkuk governorate also showed that vitamin D levels were found to be deficient in patients with chronic kidney disease [25]. So, what is the link between vitamin D deficiency and type 2 diabetes. There's layers to it. Vitamin D also makes the body more responsive to insulin by upregulating the expression of the insulin receptor and by activating peroxisome proliferator-activated receptor δ (PPAR- δ) [34]. It also impacts pancreatic β -cell function directly through facilitation of calcium influx via voltage-dependent Ca²⁺ channels, a critical event for insulin secretion [35]. Plus, vitamin D keeps the renin-angiotensin system in check and lowers overall inflammation, both of which tend to go haywire in diabetes. Among the novel results of this study is a significant inverse correlation was observed between ferritin and vitamin D₃ ($r = -0.562$, $R^2 = 0.316$). This type of association has not been previously described among populations of Kirkuk. The inflammatory condition in T2DM could have been a bias for inducing the high ferritin level (because it is an acute-phase reactant) and for reducing the serum vitamin D level. Inflammatory cytokines such as IL-6 or TNF- α , are able to inhibit the 1- α -hydroxylase (CYP27B1) enzymatic activity within renal cells, leading to a decreased conversion of stored vitamin D into its active compound [36,37]. In addition, the oxidative stress associated with iron overload may also directly affect vitamin D metabolism by causing injury to hepatocytes which is the site for the first step of vitamin D activation (25-hydroxylation) [38]. Low vitamin D levels might also

promote hepcidin production, which could then affect iron distribution and result in a secondary increase in ferritin [39]. After logistic regression analysis (age, sex adjustment), both ferritin (OR = 1.082) and vitamin D₃ (OR = 0.821) were found to be significant, independent determinants of type 2 diabetes. This could potentially be clinically relevant. In a city such as Kirkuk where specialized screening for diabetes is rudimentary at best, testing for ferritin and vitamin D₃ on a regular basis may indeed be the most useful way for some people to get examined. Also, these tests are very cheap and can be done in many Iraqi labs. That being said, studies on whether vitamin D supplementation leads to better glycemic control has been mixed [40]. Similarly, decreasing iron burden through phlebotomy or iron chelation has been shown to improve insulin sensitivity in patients with iron excess, where additional studies in this regard are desirable [41].

CONCLUSION

Fundamental Finding : This case-control study demonstrates a clear association between elevated serum ferritin, low vitamin D₃ levels, and type 2 diabetes in adults from Kirkuk, Iraq, with both markers acting as independent predictors of the disease. Additionally, 82% of diabetic participants were found to have vitamin D deficiency. **Implication :** The high prevalence of vitamin D deficiency highlights the importance of implementing public health strategies such as food fortification, supplementation programs, and promoting safe sunlight exposure. Incorporating ferritin and vitamin D₃ testing into routine screening could also enhance early detection and disease management. **Limitation :** The study does not establish a causal relationship between elevated ferritin, vitamin D deficiency, and type 2 diabetes. **Future Research :** Further research should investigate the causal role of these biomarkers and assess whether reducing iron levels or correcting vitamin D deficiency can influence disease progression.

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