# ORIGINAL ARTICLE

## Detection of Iron and Ferritin in Diabetes Mellitus Type 2 Patients

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## ABSTRACT

**Introduction:** Diabetes mellitus (DM) is a chronic metabolic sickness; the incidence of this disease has been increasing progressively all over the world. This study aimed to determine the serum iron and ferritin levels in Iraqi DM Type 2 patients. Materials and Methods: The study was carried out on 99 blood samples (79 DM Type 2 patients and 20 healthy people as control group) with age range (11-70) years from outpatient clinics. To identify ferritin, enzyme linked immunosorbent assay (ELISA) was used; iron levels were estimated using spectrophotometric method in the studied groups. **Methods:** The study was carried out on 99 blood samples (79 DM Type 2 patients and 20 healthy people as control group) with age range (11-70) years from outpatient clinics. To identify ferritin, enzyme linked immunosorbent assay (ELISA) was used; iron levels were estimated using spectrophotometric method in the studied groups. **Results:** The results of this study revealed that the majority age of studied subjects were between age range (31-50) years, which was 35/ 70 (44.3%) for DM patients. Ferritin levels in the serum was significantly increased (P<0.01) in DM patients in comparison with the healthy individuals; the mean of serum ferritin level was 150.35 ng/ mL in DM cases while it was 47.42 ng/mL in the control group. There were no significant differences in the iron level between DM patients and the healthy control group, which was (51.6  $\pm$  0.4 ng/mL, 60.12 $\pm$  0.1 ng/mL) respectively. **Conclusion:** These findings demonstrate that the ferritin serum levels should be taken into consideration of the DM patients.

Keywords: Diabetes Mellitus; Ferritin; Iron

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#### INTRODUCTION

Diabetes mellitus (DM) defines as a metabolic disorder; it describes by hyperglycemia due to the deficiencies in insulin secretion or action (1). There are several types of DM: Type 1 DM, gestational diabetes, and Type 2. Type 1 DM is an illness which is caused due to the autoimmune destruction of insulin producing by the pancreatic beta-cell (2) which is mostly identified in children and adolescents (3). Type 2 DM is the most common form of DM which characterized by hyperglycemia, insulin resistance, and relative insulin production shortage (4). This type of DM is a result of insulin resistance due to the pancreatic beta-cell disorder which causes a decrease in glucose carrying into the fat cells, liver, and muscle cells (5). There are increased morbidity and mortality seen in patients with type 2 DM (6).

Iron is a metal that can be oxidized and become an oxidant. The iron is able to transform from poorly reactive free radicals into highly reactive free radicals like hydroxyl radical. The insulin synthesis and secretion is affected by the increased accumulation of iron (7). Iron removal in muscle decreases glucose uptake because of muscle damage (8). On the other hand, insulin induces the uptake of cellular iron by improved the transferrin receptor externalization, hence this will lead to insulin resistance and diabetes (9). Ferritin, a cytosolic protein, is secreted in small amounts into the serum and act as an iron carrier. The ferritin in the plasma is an indirect marker of the iron overall amount that stored in the body; therefore ferritin levels in the serum is used to identify the iron-deficiency anemia (10). Ferritin is relative to the glucose concentration in the serum and insulin resistance (11); the higher the ferritin levels, the higher the prevalence of type 2 diabetes (12).

#### MATERIALS AND METHODS

#### Subject

From September, 2018 to the end of March, 2019; an overall of 99 blood samples were gathered (79 blood samples of DM Type 2 patients and 20 blood samples as healthy control group), their ages were between 11 – 70 years old. Samples were gathered from outpatient clinics in Baghdad province/ Iraq. The exclusion criteria were autoimmune dysfunction, inflammatory disease, and other systemic disorders. About 5 ml of venous blood were taken from redial vein from each subject. The samples were collected in plain tubes and allowed to colt at room temperature, centrifuged at 3000 round per minute for 10 minutes and then sera were dispensed into 5 eppendrof tubes and stored at -20OC to be used later.

### ETHICAL CLEARANCE

This study was approved by Research Ethics Committee, Faculty of University of Baghdad No. CSEC/0818/0009

### Iron and ferritin detection

The concentration of iron in DM patients was detected by human, German, Iron Assay kit using spectrophotometric method. The concentration of ferritin in DM patients was detected by ELISA method using Ferritin Human Enzyme Immunoassay Test Kit, Point scientific, INC, USA (Bleicher et al., 2018). The regular absorbance standards (A450) for each set of reference standards, control, and samples were measured to assess the ferritin levels. The level of ferritin in ng/ml was determined through the plotting of the mean absorbance value obtained from each reference against its concentration in ng/ml on linear graph paper. Statistical analysis system (SAS) program was used for data analysis. Mean ± SE and ANOVA Table by using computer program IBM SPSS version (SAS, 2004).

#### RESULTS

Demographical distribution of the studied groups according to the age is summarized in Table I. The results clarified that the age was ranged between 11-70 years and the mean age for DM patients was 42.8±2.0. The results recorded that most DM Type 2 patients (44.3 %) were within (31-50) year, while the lowest percentages were in age (51-70) year.

Classify the studied groups due to their gender showed that the majority of DM patients were females (65.82%) with males to females' ratio of (1.67:1) table II. It seems that female's preponderance among DM patients in comparison to males.

The mean of serum iron level in DM patients was  $51.6 \pm 0.4$  ng/mL while it was  $60.12 \pm 0.1$  ng/mL in the control group, there were no significant differences (p<0.05) compare with the healthy control group (Table III, Fig 1).

## Table I: The percentage distribution of the studied groups according to the age group.

		Age groups (years)				Mean age	
Groups					To- tal	(years)±SEM	
		11-30	31-50	51-70			
DM	Ν	26	35	18	79		
Type 2 patients	%	<b>32.91</b> %	44.31%	22.78%	100	42.8±2.0	
Healthy	Ν	6	9	5	20		
control	%	30%	45%	25%	100	36.6±2.2	

Table II: The percentage distribution of the studied groups according to the gender.

Gender					
Groups		Male Female		- Total	F/M
					Ratio
DM	Ν	27	52	79	
patients	%	34.17%	65.83%	100	1.9:1
Healthy control	Ν	8	12	20	
	%	40%	60%	100	1.5:1

Table III: The mean levels of Iron in DM patients in comparison with the healthy control group.

Iron test			
DM Patients	Control	P value	
51.6 ng/ml	60.12 ng/ml	2.6	

P< 0.05 significant differences



FIG 1: The mean levels of Iron in DM patients in comparison with the healthy control group.

The mean of serum ferritin level in DM patients was  $150.35\pm74.43$  ng/mL while it was  $47.42\pm21.71$  ng/mL in the control group. There was a statistically significant differences (p<0.01) compare with the healthy control group (Table IV, Fig. 2).

Table	IV: Mean	levels of	f ferritin	in DM	patients	in com	pari-
son w	ith the he	althy co	ntrol gro	up.	-	-	

Ferritin test					
DM Patients	Control	P value			
150.35 ng/ml	47.42 ng/ml	0.0009			
P< 0.01 significant differences					



FIG 2: The mean levels of Iron in DM patients in comparison with the healthy control group.

## DISCUSSION

The serum ferritin levels in the DM patients were elevated in compression with the control group. These findings agree with the result that obtained by Pourabbas et al., 2013 (13). A study indicated that the serum ferritin value was greater in the DM patients than the control group. They showed that the ferritin levels in serum could be used as an indicator for glucose homeostasis both in DM and control subjects (14). When iron rations are not improved, ferritin levels will effect on the diabetes development (15) and are related with the liver damage degree (16). Nutritional iron's impact on the liver and adipose tissue is an important element of metabolic disease danger (17). In the liver, the iron deposition can lead to insulin resistance by interfering with the insulin capability to reduce hepatic glucose assembly (18). Iron can be oxidized to produce an extremely reactive, lipid soluble iron-oxygen complex (19). The free radicals prompted oxidative stress and they are involved in several diseased conditions such as diabetes mellitus, through tissue damaging (20). Oxidative stress could lead to hyperglycemia by interfering with the metabolism of glucose. On the other hand, insulin induces the uptake of cellular iron via increasing the transferrin receptor externalization. Insulin resistance together with poor glycemic control can also raise the concentration of ferritin (21). Therefore, insulin and iron can commonly enhance their effects and cause the insulin resistance and diabetes (22).

## CONCLUSION

The results of this study indicated that the serum ferritin concentration was significantly raised in DM patients in comparison with the healthy individuals. Thus, this increase of serum ferritin in diabetes patients should be taken into consideration to prevent the consequences progress of the DM disease.

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