Original article

Evaluation of Ferritin Levels in Saudi Female Patients with Type 2 Diabetes

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Abstract

Background and Aims:
The main aim of the present work was to establish the relationship between ferritin level (FERR) and glucose status in diabetic Saudi females and to validate serum FERR as a marker of insulin resistance (IR).

Materials and Methods:
case- control study was done on 180 subjects including 100 female patients with type 2 diabetes and 80 healthy female (control) of the same age were enrolled. Fasting blood samples were procured to measure concentrations of FERR by an electrochemiluminescence immunoassay using the Cobas e 411 immunoassay analyzer. Fasting serum glucose, glycated hemoglobin, lipid profile, kidney function tests and minerals were automatically measured by Siemens Dimension X P and auto analyzer. The association of FERR with parameters of adiposity, glucose tolerance, and lipid profile was examined.

Results:
In the present study, unpaired student's t test detect-

الملخص

الخلفية والهدف :
أشارت بعض الدراسات الحديثة إلى أن زيادة مخزون الحديد (الفرتنين) في الجسم يلعب دور هام في تنظيم الجلوكوز في مرضى داء السكري من النوع الثاني. ولقد أجرينا دراسة مستعرضة لتحقق و إثبات العلاقة بين مستوى الفيريتين و داء السكري من النوع الثاني .و التأكد من ان ارتفاع مخزون الحديد (الفرتينين) في الجسم يؤدي الى ارتفاع خطر الاصابة بإمراض القلب لدى مرضى السكري من النمط الثاني .

طريقة البحث :
قد شمل البحث على ١٨٠ عينة ، منها ١٠٠ إناث مصابات بداء السكري من النوع الثاني و ٨٠ شخصا سليمين كمجموعة ضابطة. تم جمع العينات لقياس تركيز كلاً من الفيريتين بجهاز (Cobas e٤١١) و الجلوكوز و الهيموجلوبين و الدهون و وظائف الكلى بشكل آلي بواسطة جهاز (Siemens Dimension XP) ، و كذلك تم فحص علاقة الفيريتين مع الجلوكوز والدهون و ارتفاع خطر الاصابة بإمراض القلب و الاوعية الدموية .(AIP)

النتائج :
أظهرت النتائج (باستخدام T-test) على أن هنالك اختلاف في مستوى الفيريتين بين الإناث المصابات بداء السكري من النوع الثاني و الإناث السليمات في المجموعة الضابطة . (p< ٠،٠٥) ولوحظ

ed significant increase in the FERR levels in diabetic female patients compared to the control ($p< 0.05$). A noteworthy positive correlation of FERR with IR ($r=0.632$) and fasting insulin levels ($r=0.497$) was observed in female patients. Furthermore, the Atherogenic Index of Plasma (AIP=Log TG/HDL) conjectured cardiovascular risk showing that 65% of these patients with diabetes had increased risk while only 20% had intermediary risk. The AIP predicts that the cardiovascular risk showed statistically significant correlation with FERR levels and hyperglycemia.

Conclusion:
High serum FERR level was associated with impaired glucose metabolism and increased cardiovascular risk in type 2 diabetes female patients.

Keywords:
Cardiovascular Risk, Metabolic Syndrome, Type 2 Diabetes Mellitus, Serum Ferritin

وجود ارتباط إيجابي بين الفرتينين و معامل مقاومة الانسلوين ((IR r) = ٠,٦٣٢) و ايضا مع مستوى الأنسولين (r = ٠,٤٩٧) في المرضى الإناث . (p< ٠,٠٥) و مؤشر تصلب الشرايين من البلازما (AIP=Log TG/HDL) ينبأ باحتمال ظهور مخاطر أمراض القلب والأوعية الدموية بنسبة ٦٥٪ للخطر العالي و نسبة ٢٠٪ للخطر المتوسط لمرضى الإناث. مؤشر تصلب الشرايين في البلازما ينبأ بظهور مخاطر أمراض القلب و الأوعية الدموية مرتبطاً بدلالة احصائية مع ارتفاع مستوى الجلوكوز وارتفاع الفرتينين في الدم .

الخلاصة :
إن ارتفاع مستوى الفيريتين مرتبطاً مع ارتفاع مستوى الجلوكوز و ارتفاع مقاومة الانسلوين وزيادة خطر الإصابة بأمراض القلب والأوعية الدموية في مرضى السكري من النوع الثاني.

Introduction

The term diabetes mellitus (DM) describes a group of abnormal carbohydrate metabolic disorders that lead to hyperglycemia. Individual metabolic lesions in diabetics lead to their relative or absolute weakness in insulin secretion and/or varying degrees of peripheral resistance to insulin actions [1, 2]. According to American Diabetes Association (2010) about 85-95% of all diabetics have a latent, asymptomatic period of subclinical stages that often remain undiagnosed for several years. Signs and symptoms of diabetes are ignored by many because of the chronic development of the disease [3, 4]. People do not consider this a serious problem because unlike many other diseases, the symptoms of hyperglycemia do not appear immediately. People do not realize that damage may begin several years before symptoms are observed. This is unfortunate because identifying early symptoms can help control the disease immediately and prevent vascular complications. The dramatic increase in diabetes is a major health concern in communities of both developing and developed nations. Unregulated blood glucose levels in this metabolic and endocrine disorder can lead

to many debilitating conditions such as nephropathy, neuropathy, retinopathy, cardiovascular disease, stroke and amputation[5].

Iron prevents tissue damage from free radicals by binding to proteins, and the iron cellular state regulates the iron storage protein, FERR at the post-transcription level. In an individual's health, serum FERR is associated with iron storage; High levels of FERR can exist in acute or chronic inflammation. In T2DM, high FERR levels have been reported which may be due to the underlying inflammatory state [6, 7]. Current studies have shown that shortage in the body's iron supplies have been related to the onset of glucose intolerance, T2 DM, and metabolic disorders. It has also been linked to the progression of diabetes related retinopathy, nephropathy and vascular diseases [6, 7]. High levels of iron can induce diabetes through various mechanisms like oxidation of beta-pancreatic cells, weak extraction of insulin by the liver, and hindrance in the ability of insulin to inhibit glucose production by the liver [8, 9]. FERR serum can be used in healthy people as a sign of iron overload but does not give a reliable result for obese patients with diabetes [10]. The synthesis of FERR is stimulated by insulin which increases iron absorption. FERR is also considered a sign of pancreatitis. Its level is 2.5 times higher in T2DM patients without any evidence of iron overload [11-13]. A high level of FERR can disturb the glucose balance causing IR and inflammation with a high level of reactive protein [11-13]. Excess iron production produced a high level of serum FERR; IR may increase in females with T2DM but not in males [11-13]. High FERR level in deformed glucose metabolism is associated with increased fasting blood glucose, blood pressure, triglycerides, and hemoglobin A1C and assessment of the equilibrium model - insulin resistance (HOMA-IR) [12].

There is a strong connection between clinically linked FERR and the development of diabetes. The diabetic risk increased seven-fold in the clinically excited FERR groups. This striking association is potentially important in understanding the T2DM [12, 13]. Serum FERR can be considered as one of the routine biomarkers for diabetics, and measures should be taken to reduce iron load in diabetics to improve glycemic control and prevent the development of cardiovascular disease (CVD) because increased iron storage causes organ damage [14].

Objective of the stud: The main objective was to establish a relation between FERR levels and glucose status in diabetic females and validate serum FERR as a marker of insulin resistance (IR).

Materials and Methods

Study design and Subjects:

A case-control study was conducted in Al Madinah from January 2018 to January 2019 among adult females participants with type 2 diabetes and without aged between 20– 50 years.

This study was performed with a group of 100 patients with diabetes from Prince Abdul-Aziz Ben Maged Ben Abdul-Aziz diabetic center and different hospitals in Al Madinah, KSA. The control group consisted of 80 healthy female who had been referred to the laboratory center for routine checkup with no history of any medical disorder. Exclusions were made on the basis of complete blood count (CBC) showing iron deficiency or anaemia in patients above 65 years of age and/or with clinically significant simultaneously occurring conditions (like anaemia, CVD, acute infectious diseases or chronic inflammatory or debilitating diseases), and patients who smoke or are on medication (multivitamins and iron supplements). This study was approved by the Medical Ethics Committee of College of Applied Medical Sciences, Taibah University (CLS 201801). All participants signed the written informed consent.

Blood Sample Collection:

After obtaining written informed consent from all subjects, blood samples were (3 ml) collected in tubes containing heparin and EDTA by the lab technician at the Applied Medical Sciences collage at the main female campus of Taibah University and Prince Abdul-Aziz Ben Maged Ben Abdul-Aziz diabetic center – Al Madinah, KSA . The blood sample in the EDTA tubes was used for the estimation of glycated hemoglobin % (HbA1c %). The serum was separated from each sample by centrifuging at 3000 rpm for 5 min to estimate the lipid profile i.e. the concentration of Low-density lipoprotein cholesterol (LDL-c), High-density lipoprotein cholesterol (HDL), Triglycerides (TG) and total cholesterol. Also, fasting serum glucose was estimated immediately after the sample was collected. The collected serum was stored at -20oC until tested for FERR and Insulin hormone. All the assays were conducted using the automated Dimension XP machine and, Siemens Healthcare Diagnostics Ltd. (Frimley, Camberley, UK), with the help of kits as per the manufacturer's directions. The IR level was estimated using the homeostasis model assessment of insulin resistance (HOMA-IR) index [fasting insulin Uμ/mL x fasting glucose mg/dl /405]. The range considered healthy is lies between the values 0.5–1.4. The

values found less than 1.0 imply that the patient is insulin-sensitive and this is optimal. A value above 1.9 indicates early IR while above 2.9 indicates significant IR as described earlier.

Other Measurements

Each participant was physically examined for the measurement of height, weight and Body mass index (BMI) which is weight divided by square of the person's height (kg/m2). When high plasma triglycerides are present along with low HDL-concentrations the condition is defined as Atherogenic dyslipidemia. It was calculated as Atherogenic Index of Plasma = log (TG/HDL-C). An AIP value of less than 0.11 depicts low risk of CVD; the values between the range 0.11-0.21 is of intermediate risk while the values above 0.21 are associated with high risk CVD.

Statistical Analysis:

Statistical analysis was performed using Graph pad Prism 7 (GraphPad Software, CA, USA). Quantitative data was normally distributed and expressed as mean ± SD. Differences between groups were evaluated by Student's t-test and Two Away Anova test. Simple correlations were used to investigate the involvement among different parameters. $p<0.05$ indicated statistical significance. Calculation of Pearson correlation coefficients was performed to assess relationships between key variables.

Statement of ethics:

• The volunteers have given their written or verbal informed consent prior to the start of the study. They were assured that the information obtained will only be shared between the researchers and that they were free to withdraw from the study at any time without any pressure on them. Participants were identified by codes and never by their real names.

• The study protocol has been approved by the Faculty of Applied Medical Sciences' ethical committee on human research.

Results:

A total of 100 diabetic female patients with a range of age between 36-60 years against 80 healthy control females were investigated. The mean age of the patients with type-2 diabetes was 60±10.38 years while that of the control group was 40±7.54 years. The mean ± SD level for BMI in diabetics was 27.84 ±5.38 kg/m2 while for the control group it was 25.26±4.63kg/m2. The two groups differed in their body mass index (P=0.04) significantly. The haemoglobin A1clevels in patients with diabetes (8.95%±1.79) was significantly higher than those found in normal controls

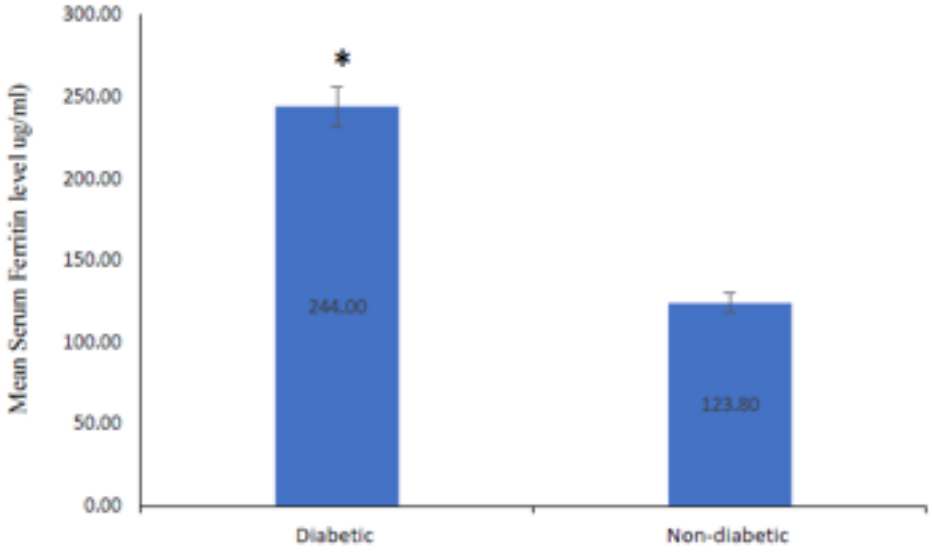
(5% ±0.49, P<0.001). The mean serum FERR in diabetics was also significantly higher than that found in the normal individuals (244.4±6.49 vs. 123.8±1.29 µg/ml, P<0.001, Figure 1). The results further show that the triglyceride (TG) concentration was higher in comparison to the control group, whereas, serum High-density lipoprotein (HDL) levels were significantly lower in type 2 patients with diabetes in comparison to non-diabetic healthy control group (p<0.001) (Table 1). The chief characteristics of the study population under consideration are presented in Table 1.

Table 1: Characteristics of the study population

Parameter	Non-diabetic N= 80	Diabetic N= 100	P- value
Age (years)	40.50±7.54	60.00±10.38	>0.05
Duration of diabetes (years)		11.2±9.7	
FBG (mmol/L)	5.2±0.49	11±4.21	<0.001**
HbA1c (%)	3.3±0.82	8.95±1.79	<0.001**
LDL-cholesterol (mmol/L)	3.59±1.10	2.45±1.23	0.03*
HDL-cholesterol (mmol/L)	1.67±0.46	0.8±0.23	0.09
Total cholesterol (mmol/L)	4.7±0.94	4.01±1.52	0.006*
Triglycerides (mmol/L)	0.88±0.49	2.01±1.41	0.002*
Weight (kg)	70.9±9.38	77±12.3	0.03*
Length (m)	1.66±0.09	1.66±0.09	0.070
Serum Ferritin µg/mL	123.8±1.29	244.40±83.49	<0.001**
BMI (kg/m2)	25.26±4.63	27.84±5.38	0.04**
Fasting insulin µ/Ml	5.8±1.45	11.77±3.03	0.03**
IR	1.36±0.32	5.065±2.52	0.02**
AIP	0.11±0.03	0.26±0.23	0.002**

The values represent the mean concentrations ± SD of biochemical parameters and general characteristics of patients and controls. BMI (body mass index), FBG (fasting serum glucose), HbA 1C (glycated hemoglobin), lipid profile (LDL-C (low-density lipoprotein cholesterol), HDLc (high-density lipoprotein cholesterol), total cholesterol and TG (triglyceride)), Serum Ferritin, Fasting Insulin, Insulin resistance (IR) and AIP (Atherogenic Index of Plasma) for control group and T2DM patients. *p<0.05, **p<0.01.

Fig. 1. Comparison between diabetic and normal cases regarding their ferritin



Statistical analysis indicated that there was no significant correlation between serum FERR and HbA1c (r= 0.15), BMI (r= 0.071) and FBS (r= 0.035) in patients with diabetes . However, a significant positive correlation of FERR was found with IR (r=0.632), and fasting insulin levels (r=0.497) in patients with diabetes (Table 2).

Table 2: Correlations of Serum Ferritin levels with other parameters

Correlations of Ferritin with	Pearson correlation coefficients (r)	P- value
FBG	0.035	**0.31**
HbA1c (%)	0.159	**0.22**
HDL	0.078	**0.34**
TG	0.027	**0.33**
CHOL	0.057	**0.21**
LDL	0.095	**0.31**
Fasting insulin	0.497	**0.03***
IR	0.632	**0.01***
BMI	0.071	**0.23**

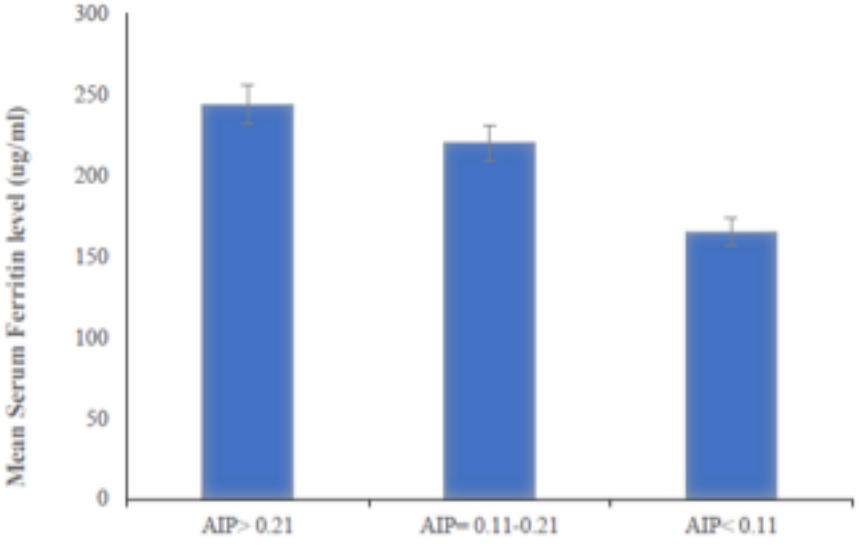
The values represent correlation between Serum Ferritin levels with different variables in diabetic female patients. *Correlation is significant at the 0.05 level (2-tailed)

Our results interesting showed that there was a positive correlation of IR with FBG (r=0.72), HbA1c (r=0.61), BMI (r=0.65) and Fasting Insulin levels (r=0.60, Table 3).

Table 3: Correlations of IR and Fasting Insulin levels with other parameters

	IR		Fasting insulin	
	r	P	R	**p**
FBG	0.720	0.02*	0.074	0.08
HbA1c (%)	0.619	0.04*	0.048	0.06
HDL	0.110	0.060	0.033	0.07
TG	0.092	0.076	0.126	0.08
CHOL	0.101	0.075	0.078	0.06
LDL	0.095	0.073	0.058	0.07
Fasting insulin	0.604	0.025*		
IR			0.604	0.02*
BMI	0.659	0.01*	0.546	0.01*
The values represent correlation between IR and Fasting insulin levels with different variables in diabetic female patients. *Correlation is significant at the 0.05 level (2-tailed)				

Fig. 2. The values represent the mean of serum ferritin levels in low, moderate, and high cardiovascular risk groups of diabetic patients; *P*= 0.03, n=100. 65% of diabetic patients had high risk and 20% had intermediate risk, whereas, 15% had low risk.



Atherogenic dyslipidemia or TG/HDL ratio increased significantly in patients with diabetes (p<001). Atherogenic Index of Plasma (AIP=Log TG/HDL) predicts cardiovascular risk. An AIP less than 0.11 denotes low risk while that between0.11 – 0.21denotes intermediate risk. An AIP greater than 0.21 denotes high cardiovascular risk. In the present study, 65% of the patients with diabetes had high risk while only 20% had intermediate risk (Figure 2). The Two Away Anova test detected a large difference between patients with low, moderate and high cardiovascular risk with respect to high levels of FERR (p= 0.03, Figure 2). Using the same test, significant differences were observed between patients with low, moderate and high cardiovascular risk with respect to the levels of FBG, HbA1C, IR, LDL, HDL, BMI and Cholesterol (Table 4). However, no significant differences were seen in the levels of TG (Table 4) in the three groups of patients with diabetes studied here.

Table 4: Represents the difference in the means between the three studied groups

Parameter	High risk (AIP>0.21) Mean ± SD, N=65, 65%	Intermediate risk (AIP= 0.11 – 0.21) Mean ± SD, N=20, 20%	Low risk (AIP<0.11) Mean ± SD, N= 15, 15%	P-value
FBG (mmol/L)	14.7 ±3.36	8.45 ±0.52	7.1 ±0.76	**0.04***
HbA1c (%)	9.6 ±1.47	7.9 ±0.27	6.8 ±0.36	**<0.001****
LDL-cholesterol (mmol/L)	3 ±0.98	1.8 ±0.11	0.9 ±0.36	**<0.001****
HDL-cholesterol (mmol/L)	1.8 ±0.07	1.5 ±0.09	1 ±0.08	**0.002***
Total cholesterol (mmol/L)	5.01±1.12	3.01 ± 0.30	2.01 ±0.48	**<0.001****
Triglycerides (mmol/L)	2.01 ±1.45	1.01 ±0.01	1 ±0.08	**0.06**
Serum Ferritin µg/mL	261.42 ±64.4	174.33 ±14.64	131.01 ±15.48	**0.03***
BMI (kg/m2)	31.23 ±3.93	24.9 ±0.75	21.04 ±1.84	**0.01***
IR (Insulin resistance)	6.64 ±2.15	3.97 ±0.33	2.69 ±0.43	**0.02***

The table shows the difference in the means between the three studied groups of diabetic patients (according to cardiovascular risk (AIP)) of diabetic patients using an ANOVA test: 15% of our patients with low risk (AIP<0.11), 20% of our patients with AIP intermediate risk (AIP= 0.11 – 0.21) and 65% of our patients with high risk (AIP>0.21), which displays statistically significant difference in serum Ferritin, FBG (Fasting Blood Glucose),HbA1C%, IR (Insulin resistance), LDL, HDL, BMI and Cholesterol levels. Whereas, non-significant difference in TG. *=p<0.05, **=p<0.001.

Discussion

Excess Iron is related to IR in case of T2DM patients. Deposition of this metal in the liver can cause IR as it can interfere with the capacity of insulin to reduce production of glucose by the liver. Free iron leads to the congregation of reactive oxygen species (ROS) which perpetually disrupts the body's homeostasis and causes oxidative stress-mediated diabetic complications. Recent studies have suggested that FERR level is higher in T2DM patients. As high FERR level can be present in acute or chronic inflammation, its elevation in T2DM could be due to the underlying inflammatory conditions [11,14]. The fact that excess iron in the system contributes to anomalous glucose metabolism was first confirmed from the observation that the incidence of diabetes increases in typical genetic hemochromatosis (HH). Improved insulin sensitivity and secretion was found blood was donated frequently and iron stores depleted [14, 15]. However, FERR's role as a marker of iron overload in pancreatic damage and peripheral resistance or its role as an inflammatory marker is not clear and hence this study was aimed to examine the relationship between serum FERR and glycemic status and markers in type 2 diabetes.

In type 2 diabetes, FERR is increased by 2.5-fold and there was no evidence of any excess iron load. However the high level leads to disturbance in the glucose homeostasis by causing IR as iron is auto-oxidized to form free radicals that are extremely reactive. . These iron–oxygen complexes are lipid soluble pro-oxidants which can cause tissue damage by changing membrane properties [16, 17].

In the present study, it was revealed that FERR level was statistically significantly augmented in diabetic female patients in comparison to the control group. The increase in FERR levels in patients with diabetes has a direct relation with IR. Previous studies suggest that metabolism of iron in the body could possibly induce IR by inhibiting adiponectin and Osteocalcin. This may have led to the assumption that iron metabolism can contribute to the development of T2DM [18]. Higher FERR levels have been reported by some studies to be associated with obesity, metabolic syndrome and cardiovascular diseases. It is under consideration that serum FERR, a marker of body iron status, maybe considered as a constituent of insulin-resistance disorder [19, 20]. There is also a recognized association between FERR levels and the presence and severity of fatty liver disease. This was in partial agreement with our results regarding increased ALT levels in diabetic female patients with micro vas-

cular and macro vascular complications. This differs significantly from female patients without complications. Also, LDL differed significantly between these two groups[21,23].

At present it is believed that IR is the main factor in inducing T2DM pathogenesis. The well-established IR markers are glucose, insulin, and C-peptide concentrations [24]. In our study we found a significant correlation between FERR level and IR in T2DM.The IR level was calculated using the homeostasis model assessment of HOMA-IR index. This finding is consistent with previous studies conducted on Chinese and Korean populations [11, 19]. Elevated levels of FERR have been recognized as an attribute of T2DM but its association with the iron levels is multifaceted. Insulin stimulates the synthesis of FERR and activates the uptake of iron. On the contrary, iron influences the insulin inhibition of glucose production by the liver [25]. On the other hand, FERR acts as an indicator of pancreatic inflammation and is thus referred to as marker for IR [26, 27].

In the present study, 65% of diabetic subjects had increased risk of CVD while only 20% had intermediate risk. American diabetes association studies have shown that there is an apparent risk of getting CVD in type 2 patients with diabetes especially in conditions of hypertension and dyslipidemia. It has been shown that CVD is the major cause of morbidity and mortality in people living with diabetes. Besides, the efficiency with which cardiovascular risk is controlled is also dependent on factors that can prevent or slow CVD in patients with diabetes [28].

Conclusion and recommendation

In conclusion, our studies showed that FERR levels are higher in people who have diabetics than those who do not (controls). No correlation was observed between serum FERR and blood glucose control in diabetics implying that that FERR may be playing a significant role in the pathogenesis of T2DM. A decrease in insulin production and induction of IR may be caused by hyper ferritinemia in T2DM. Nevertheless, it seems important to consider the question as to whether serum FERR levels should be routinely estimated in all type 2 diabetes patients and to whether cutoff threshold value be maintained in order to achieve good glycemic control. Further studies should be done to understand this relationship in patients with diabetes and to lay down specific guidelines so that this chronic disease can be controlled.

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Declaration

Author(s) declare that We have no conflicts of interest to disclose.

References

[1] Elhadd AT. The rising menace of diabetes in the Middle East: Time for action. Saudi J Health Sci 2012;1: 44-45.

[2] Abdulfatai O, Lateef. Type 2 Diabetes Mellitus: A Review of Current Trends. Oman Med J. 2012; 27: 269–273.

[3] World Health Organization. https://www.who.int/news-room/fact-sheets/detail/diabetes. Diabetes in Saudi World Health Organization (diabetes) Online Resources (updated 2019). Available from: https://www.who.int/countries/sau/en/.

[4] American Diabetes Association. https://www.diabetes.org/. Online Resources (updated 2019). Available from: https://www.diabetes.org/diabetes/type-2.

[5] Aastha, Rajeev, Shalini. Microvasular and Macrovascular complications in diabetes mellitus: Distinct or continuum. Indian J Endocrinol Metab 2016; 20: 546–551.

[6] Anjana, Joseraj, Geetha. Evaluation of serum ferritin in type 2 diabetes mellitus. Journal of evaluation of research in medical biochemistry 2016; 1: 4-6.

[7] Shim, Kang, Oh, Baek, Yang, et al. Association of serum ferritin with insulin resistance, abdominal obesity, and metabolic syndrome in Korean adolescent and adults, Medicine 2017; 96:8.

[8] Bao W, Chavarro JE, Tobias DK, Bowers K, Li S, et al. Long term risk of type 2 diabetes in relation to habitual iron intake in women with a history of gestational diabetes: a prospective cohort study. Am J Clin Nutr 2016;103:375-81.

[9] Wrede E, Buettner R, Bollheimer C, Scholmerich J, Palitzsch D, Hellerbrand C. Association between serum ferritin and the

insulin resistance syndrome in a representative population. Eur J Endocrinol 2006; 154:333-40.

[10] Faiza, Fasiha, Shehryar, Najeeha, Syeda. Elevated levels of Ferritin and hs-CRP in type 2 diabetes, Journal of Pakistan medical Association 2014; 64:89-91.

[11] Sun L, Franco H, Hu B, Cai L, Yu Z, Li H. Ferritin concentrations, metabolic syndrome and type 2 diabetes in middle-aged and elderly Chinese. J Clin Endocrinol Metab 2008; 93:90-6.

[12] Bo-wei, Xu-min, Jun-ru, Fang-ning, Fu-Zai. The Relationship between Serum Ferritin and Insulin Resistance in Different Glucose Metabolism in Non-obese an Adults. International Journal of Endocrinology 2015; 1: 34-36.

[13] Barton, Barton, Acton. Insulin Resistance and Metabolic Syndrome: Clinical and Laboratory Associations in African Americans Without Diabetes in the Hemochromatosis and Iron Overload Screening Study. Metab Syndr Relat Disord 2018;16:267-273.

[14] Suárez , Arbeláez, Mosquera, Méndez, Aguilar. C-reactive protein, waist circumference, and family history of heart attack are independent predictors of body iron stores in apparently healthy premenopausal women. Biol Trace Elem Res 2012;148:135-8.

[15] Tang, Liu, Tang , Tan, Lu Z, et al. High serum ferritin level is an independent risk factor for metabolic syndrome in a Chinese male cohort population. Diabetol Metab Syndr 2015; 7:11.

[16] Cantur KZ, Cetinarslay B, Tarkun I, Canturk NZ. Serum ferritin levels in poorly- and well-controlled diabetes mellitus. Endocr Res 2003; 29:299-306.

[17] Carrera, Simó, Lecube, Hernández. Soluble transferrin receptors and ferritin in Type 2 patients with diabetes . Diabetic Medicine 2004; 1:97–101.

[18] Doyard M, Fatih N, Monnier A, Island ML, Aubry M. Iron excess limits HHIPL-2 gene expression and decreases osteoblastic activity in human MG-63 cells Osteoporos Int 2012; 23:2435–2445.

[19] Kim CH, Kim HK, Bae SL, Park JY, Lee KU. Association of elevated serum ferritin concentration with insulin resistance and impaired glucose metabolism in Korean men and women. Metabolism 2011; 60:214-20.

[20] Hernandez A, Lecube A, Carrera RS. Soluble transferrin receptors and ferritin in type 2 patients with diabetes . Diabetic Med 2005; 22:97.

[21] Aclan Ozder. Lipid profile abnormalities seen in T2DM patients in primary healthcare in Turkey: a cross-sectional study. Lipids Health Dis 2014; 13:183.

[22] Xifra G, Moreno, Ricart W, Fernández. Obesity status influences the relationship among serum osteocalcin, iron stores and insulin sensitivity. Clin Nutr 2018;37:2091-2096.

[23] Colagiuri S, Craig J. Assessment of kidney function in type 2 diabetes. Nephrology 2010; 15:146–16.

[24] Olga G, Evgenya U, Yulia D, Ekaterina B, Ekaterina S, Olga B. Insulin resistance and inflammation markers in myocardial infarction. J Inflamm Res
2013; 6:83-90.

[25] Pravenec M, Kajiya T, Zídek V, Landa V, Mlejnek P, Simáková M. Effects of human C-reactive protein on pathogenesis of features of the metabolic syndrome. Hypertension 2011; 57:731-7.

[26] Rajpathak S, Ma J, Manson J, Willett WC, Hu FB. Iron intake and the risk of type 2 diabetes in women: a prospective cohort study. Diabetes Care 2006; 29:1370-6.

[27] Buse JB, Ginsberg HN, Bakris GL. Primary prevention of cardiovascular diseases in people with diabetes mellitus: a scientific statement from the American Heart Association and the American Diabetes Association. Diabetes Care 2018; 30:162–172. https://www.diabetes.org/.

[28] American Diabetes Association. Cardiovascular disease and risk management. In Standards of Medical Care in Diabetes. Diabetes Care 2015; 38:49–57. https://www.diabetes.org/.