Acute-phase proteins and diabetic indices in type 2 diabetes mellitus and their predictive values

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Context
Diabetes mellitus consists of a group of metabolic disorders that share a common phenotype of hyperglycemia. Chronic systemic subclinical inflammation has also been identified as a driving force for insulin resistance, metabolic syndrome, and type 2 diabetes mellitus (T2DM). Iron overload is a risk factor for diabetes. The link between iron and diabetes was first recognized in pathologic conditions such as hereditary hemochromatosis and thalassemia.

Aims
The research is approached with the idea of detecting any association between basal C-reactive protein (CRP) or serum ferritin levels with one of most valuable diabetic indices which is HbA1c as a clinical trial to establish an inflammatory role responsible to develop insulin resistance.

Settings and design
This comparative cross-sectional study was conducted on T2DM patients who attended the Outpatient Department at Assiut University Hospitals.

Patients and methods
Totally, 67 participants were enrolled for the study, out of which 47 were cases and 20 were age and sex controls. Fasting blood sugar, postprandial blood sugar, HbA1c, serum ferritin, and CRP were estimated.

Statistical analysis used
Statistical Package for the Social Sciences software version 19.0 was used for statistical analysis.

Results
There is statistically significant decrease in serum ferritin level after 3 months of follow-up and glucose control, which means that serum ferritin would decrease with reducing serum fasting blood sugar and postprandial BS ($P < 0.05$) compared with CRP. There was no significant difference between CRP before and after the study ($r = 0.251$; $P = 0.042$). There was a significant negative correlation between serum ferritin and duration of diabetes ($P = 0.034$).

Conclusion
From our study point of view, persistent elevated serum ferritin levels in patients with T2DM tend to exhibit a certain degree of inflammation that, in one way or another, is likely to increase their risk of developing cardiovascular complications or may be have a role in the development of insulin resistance.

Keywords:
acute-phase proteins, hemoglobin A1c, serum ferritin, type 2 diabetes mellitus

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Introduction

Diabetes mellitus (DM) has routinely been described as a metabolic disorder characterized by hyperglycemia that is developed as a consequence of defects in insulin release, insulin effect, or both. Type 2 diabetes encompasses individuals who have insulin resistance (IR) and usually relative (rather than absolute) insulin deficiency [1]. The hallmark of DM pathology involves the vasculature leading to both microvascular and macrovascular complications [2]. Long-term hyperglycemia is associated with failure of various organ systems mainly affecting the eyes, kidneys, heart, and nerves. These complications usually need more than 10 years of diabetes poor control to become apparent [1]. Ferritin has routinely been used as an index for body iron stores; in addition, it is an inflammatory marker for inflammatory process. In some researches, serum ferritin was the second strongest determinant of blood glucose (after BMI) in regression models and the third strongest determinant of serum insulin (after BMI and age). This supports a link between subclinical type 2 diabetes mellitus (T2DM) and hemochromatosis [3]. The process of inflammation induces hepatic synthesis of various acute phase proteins such as serum ferritin, which is believed to play a role in IR as well as atherosclerosis [4]. Elevated iron stores

have great risk for dyslipidemia, metabolic syndrome, cardiovascular disease, and hypertension [4]. When iron stores are raised it can cause diabetes through various ways including oxidative injury to beta cells located in the pancreas, impairment of hepatic insulin extraction by the liver, and interference with insulin's ability to suppress hepatic glucose production [5]. The serum levels of the iron storage protein ferritin are usually directly proportional to total iron stores in healthy individuals. However, because ferritin is an acute-phase protein, it may be elevated independently of the iron status in the presence of chronic or acute inflammation [6]. As DM is a chronic inflammatory state, an aberrant continuation of some aspects of the acute-phase response may lead to the underlying tissue damage that accompanies the disease and can contribute to further complications [7].

Patients and methods

As part of a quality assurance program, patients admitted to Assiut University Hospitals were recruited if they met the inclusion criteria.

Inclusion criteria

T2DM patients diagnosed on the basis of WHO criteria irrespective of duration and treatment with glomerular filtration rate greater than 60 ml/min were selected for the study. Age-matched and sex-matched controls were selected for the study. Exclusion criteria: include consumption of steroids or immunosuppressive drugs, presence of infection or autoimmune diseases, and patients using statins within 1 month were excluded from the analysis due to the possible effects of the drugs on C-reactive protein (CRP) levels, patients having anemia (Hb < 10 g/dl), any other disease or drugs, those on anemia therapy that could affect ferritin levels, and patients suffering from hemochromatosis were excluded from the study.

Sample size calculation: This quasiexperimental study was performed on 67 persons, out of which 20 served as the control group: this group consisted of age-matched and sex-matched healthy subjects coming to the hospital for fitness purpose and also from medical or paramedical staff. Diabetic patients enrolled in this study (n = 47) were divided into three groups based on the values of serum HbA1c: good control with HbA1c of less than 7% (14 cases, 31%), moderate control group with HbA1c of 7–9% (14 cases, 31%), and poor control group with HbA1c values greater than 9% (17 cases, 38%) (n = 20).

Study tool, history, physical examination, demographic information including age, gender, body weight, length, BMI duration of diabetes and blood pressure. Laboratory findings include fasting blood sugar (FBS), 2-hour postprandial blood sugar, ferritin, CRP, serum creatinine, HbA1c, high-density lipoprotein-cholesterol (HDL-c), low-density lipoprotein-cholesterol (LDL-c), and triglycerides. FBG, 2HPPBG, Cr will be checked by Biotechnica (BT3000, Rome, Italy). Ferritin was checked with ELEXIS. CRP as a quantitative factor was measured with the immunoturbidimetric method using BT3000. The HbA1c was estimated by the NycoCard Reader method. Patient's blood glucose was controlled with insulin or oral hypoglycemic drugs. Laboratory tests will be checked at the beginning of the study and 3 months later.

Ethics: informed oral consent was obtained from all patients; approval was gained by the local ethics committee to conduct and to publicate the study. There is no risk during application of the research, tools to assess the patients psychologically. Confidentiality was maintained during all stages of the assessment.

Results

As the study started, the selected cases have a mean age of 56.5 ± 9.7 (30–82) years. Forty-five (67.2%) patients were women; the mean BMI (weight/height^2) was 28.5 ± 4 (22–42); the mean glomerular filtration rate estimated using Cockcroft Gault formula (140 – age × body weight/72 × serum creatinine) was 77.9 ± 12.9 (99–60) ml/min, and the mean period of diabetes was 6.3 ± 4.1 (1–15) years (Table 1). Despite HDL and LDL cholesterol, which did not change during the study, the factors of FBS, 2hppBS, HbA1C, and triglycerides decreased significantly during the study (P < 0.05) (Tables 2, 3).

Mean serum CRP before and after the study were 1.43 ± 0.09 and 1.42 ± 0.08 mg/l, respectively (normal range: 1–3 mg/l). There was a significant positive correlation between serum ferritin and duration of diabetes (P = 0.034). There was a weak positive correlation between CRP and HbA1c at the beginning of study (P = 0.042). But one patient showed a CRP level of 115 and 111, respectively, before and after the study; therefore, this patient was excluded from analysis. Results show an insignificant difference between CRP before and after the study; however, serum ferritin levels decreased in response to control of blood glucose (Figs. 1–4).

Mean values of ferritin, before and after the hyperglycemia control were 115 ± 109.4 and 91.4 ± 61.9 ng/ml, respectively (normal range: men:

23–336 ng/ml and women: 11–306 ng/ml). There was no significant alteration between CRP at the beginning and at the end of study; however, after 3 months follow-up levels of ferritin significantly decreased which means that serum ferritin would decrease with reducing serum FBS and postprandial BS. The ferritin levels were not significantly different in male and female diabetics 123 ± 97 vs 94.4 ± 64 μg/ml, respectively (Table 4).

Diabetic patients enrolled in this study were divided into three groups based on the values of serum HbA1c: good control with HbA1c of less than 7% (14 cases, 31%), moderate control group with an HbA1c of 7–9% (14 cases, 31%), and poor control group with HbA1c values greater than 9% (17 cases, 38%). The mean ferritin level was 91.4 ± 61.9 μg/ml in the first group, 211 ± 97.4 μg/ml in the second, and 295 ± 101.2 μg/ml in the third. There were significant differences between these three groups regarding ferritin level (Table 5).

Table 1 Descriptive data

	n (%)
Sex	
Male	20 (32.8)
Female	46 (67.2)
Age	
Range	30-82
Mean±SD	56.5±9.7
BMI (kg/m^2)	
Range	22-42
Mean±SD	28.5±4
Glomerular filtration rate (ml/min)	(140–age×body weight/72×serum creatinine)
Range	99-33
Mean±SD	77.9±12.9
Duration of diabetes	
Range	1-15
Mean±SD	6.3±4.1
Comorbidities	
HTN	21
IHD	20
None	25

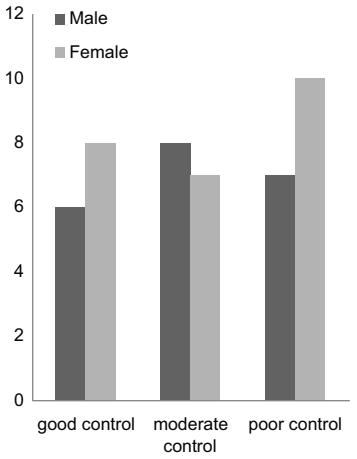
n=18.5-25 (kg/m^2). Glomerular filtration rate greater than 60 ml/min calculated by Cockcroft-Gault formula. HTN, hypertension; IHD, ischemic heart disease.

Discussion

Our study is atrial to detect the inflammatory role in the development of T2DM by comparing two of acute-phase proteins (inflammatory markers) with one of the most valuable diabetic indices (HbA1c). In this study, FBS concentrations were elevated at high serum ferritin concentrations.

In the last few years, facts about pathogenesis of DM has changed; this suggests that inflammatory pathways precedes the progression of diabetic complications. The new pathogenic theories may open approach to new therapeutic treatment [8]. Ferritin is the major intracellular iron storage protein. Recently, it has been suggested that when markers of the iron metabolism are elevated, the incidence of metabolic syndrome is increased [8]. Metabolic disorders are common among patients with high ferritin without genetic hemochromatosis, than among patients

Figure 1



compare between studied grouos according to their HbA1c, poor control group have high HbA1c hhile good control patents have less reading of HbA1c

Table 2 Variables of investigation parameters before and after the study

Inflammation indices	Before study			After study			*P*
	Minimum-maximum		Mean±SD	Minimum-maximum		Mean±SD	
FBG (mg/dl)	87	294	155.6±48	86	285	129.4±33.9	<0.001
2hppBS	124	457	238.3±73.1	123	356	206.3±48.4	<0.001
HbA1C (%)	4	11	7±1.2	4	8	6.3±0.8	<0.001
TG (mg/dl)	63	800	196.9±125.3	70	449	175.6±91.8	0.03
LDL (mg/dl)	4	197	99.5±28.9	40	197	97.3±32.4	0.477
HDL (mg/dl)	30	105	47.7±11.5	30	70	47.3±8	0.747
CRP (mg/l)	1.25	1.67	1.43±0.07	1.25	1.55	1.42±0.08	0.192
Ferritin (ng/ml)	5.7	610.6	115±109.4	5.7	327	91.4±61.9	0.004

2hppBG, 2-h postprandial blood sugar; CRP, C-reactive protein; FBG, fasting blood sugar; HbA1C, hemoglobin A1C; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TG, triglycerides.

Table 3 Comparing between groups' demographic data

Demographic data	Groups [*n* (%)]			*P*
	Good control	Moderate control	Poor control	
Sex				
Male	6 (13.2)	8 (17.3)	7 (15)	0.249
Female	8 (17.3)	7 (15)	10 (19.2)	
Age				
Mean±SD	49.56±7.83	48.70±8.67	57.52±9.68	0.020
Range	48-64	43-60	53-61	

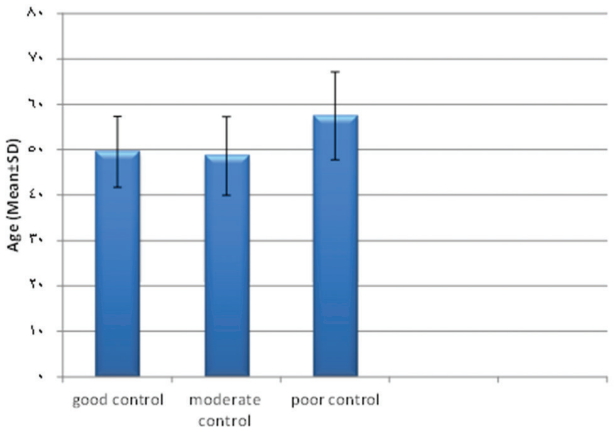
Statistically significant difference ($P<0.05$).

Table 4 Comparing between groups

Demographic data	Groups [*n* (%)]			*P*
	Good control	Moderate control	Poor control	
Sex				
Male	6 (13.2)	8 (17.3)	7 (15)	0.249
Female	8 (17.3)	7 (15)	10 (19.2)	
Age				
Mean±SD	49.56±7.83	48.70±8.67	57.52±9.68	0.020*
Range	48-64	43-60	53-61	
Hemoglobin A1C %				
Mean±SD	6.4-7	7-8.9	9-10.5	<0.001
Range	6.7.59±0.39	7±0.9	7.7±7.4	
Serum ferritin (ng/ml)				
Mean±SD	91.4±61.9	211±97.4	295±101.2	0.003
Range	5.7-331.2	44.7-421.7	99.7-610.1	
C-reactive protein				
Mean±SD	1.42±0.04	1.43±0.02	1.43±0.03	0.177
Range	1.25-1.52	1.29-1.54	1.24-1.62	
Low-density lipoprotein				
Mean±SD	97.3±32.4	97.9±29.2	99.5±28.9	0.486
Range	4-197	32-197	40-197	
High-density lipoprotein				
Mean±SD	47.3±8	47.5±7.1	47.7±11.5	0.774
Range	30-70	30-91	30-105	
Triglycerides				
Mean±SD	175.6±91.8	191.9±122.1	196.9±125.3	0.03
Range	70-449	63-710	63-800	

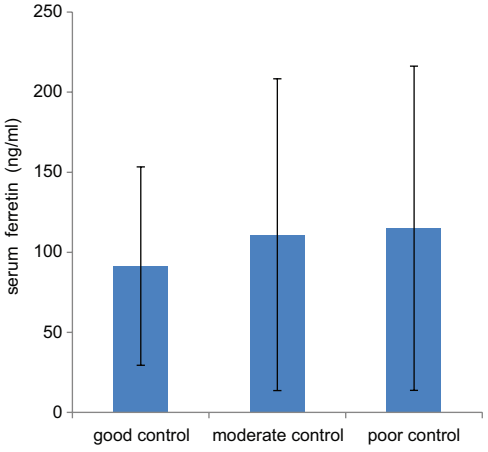
2hppBg, 2-h postprandial blood glucose; CRP, C-reactive protein; FBG, fasting blood glucose; HbA1C, hemoglobin A1C; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TG, triglycerides.

Figure 2



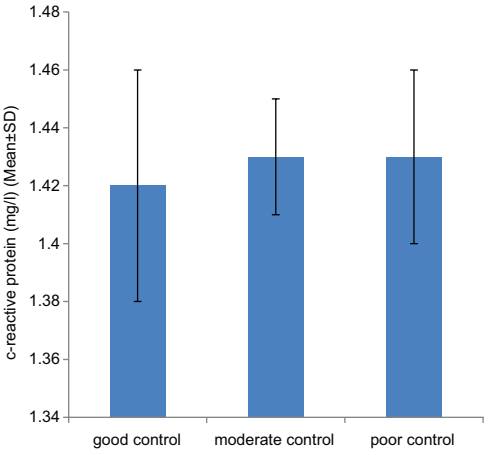
Age (years).

Figure 3



Serum ferritin (ng/ml).

Figure 4



C-reactive protein.

with genetic hemochromatosis. Iron deposition in various tissues affects insulin sensitivity and function, thereby leading to IR and inflammation. A few studies have demonstrated a link between markers of IR (HOMA-IR, fasting insulin) and ferritin [9]. Fumeron *et al.* [10] have also found that plasma ferritin concentrations positively correlate with fasting insulin and fasting glucose.

Oxidative stress has been implicated in the pathogenesis of complications seen in T2DM [11]. Iron in moderate quantities is essential for all cell metabolism and growth, but it is toxic when unleashed by protein [12]. A variety of mechanisms implicated in impaired glycemic control by elevated iron stores, including impairment of hepatic insulin extraction by the liver, interference with insulin's ability to suppress hepatic glucose production, and oxidative damage to pancreatic beta cells may possibly be related to the occurrence of long-term complications of diabetes, both microvascular and macrovascular, and may be

Table 5 Mean±SD of studied groups as regards studied parameters

	P_1	P_2	P_3	P_4
Hemoglobin A1C %	<0.001	<0.001	<0.001	0.007*
Serum ferritin (ng/ml)	0.003	0.004	0.005	0.005
C-reactive protein	0.177	0.225	0.217	0.196
Low-density lipoprotein	0.003	0.009	0.003	0.007
High-density lipoprotein	0.086	0.075	0.094	0.058
Triglycerides	0.843	0.826	0.673	0.374

CRP, C-reactive protein; HbA_1C, hemoglobin A_1C; HDL, high-density lipoprotein; LDL, low-density lipoprotein; P_1, comparison between all studied groups; P_2, comparison between good control and moderate control; P_3, comparison between good control and poor control; P_4, comparison between moderate control poor control; TG, triglycerides. Statistically significant difference (P<0.01).

secondary to elevated serum ferritin. This possibly reflects the subclinical hemochromatosis developing in a long-standing diabetic patient [13].

One study suggested that the use of deferoxamine can lower ferritin concentrations and help in control of diabetes, but these findings were not confirmed in subsequent studies. Ferritin level in this study is found to be higher in newly diagnosed cases and low in those with more than 10 years of established diagnosed diabetes. The results agrees with the previous studies. Elevated serum ferritin could be a marker of IR. So, more researches are needed to confirm this relation [14].

CRP is one of the best laboratory evidence of systemic subclinical inflammation, and may have a prognostic value for risk of cardiovascular events [15]. There are different and sometimes controversial results in the other studies. In cross-sectional studies, highly sensitive CRP has been found to correlate with increased fasting plasma glucose concentrations, increased blood pressure, increased triglycerides, and decreased HDL, suggesting its link to the elevated risk to metabolic syndrome associated with IR [16–18]. Few studies have established the association of CRP with IR independent of obesity [19]. Also, in a recent study, CRP was found to correlate with several surrogate measures of IR like quantitative insulin sensitivity, fasting insulin, and the Raynaud index. Because of the simplicity of measurement, stability, and improved high-sensitivity method, CRP may be useful as a clinical measure for identifying individuals at risk for IR [20]. Poor glycaemic control is the root cause of escalated protein glycation—especially haemoglobin, which restores the free state of iron. This amplified free iron pool revitalizes oxidant generation, conferring damage to biomolecules and leading to complications [21].

However, the study had some limitations including comparing a small group; therefore, the associations between studied parameters cannot be clearly established. Also, it is difficult to define serum level of ferritin asossiated with risk of developping type 2 diabetes is increased. Although ferritin is considered a good measure of body iron stores, it is not a 'gold standard.' Ferritin also has acute-phase proteins that may be raised in response to inflammation. Further studies are needed to confirm the implications of serum ferritin as a marker for T2DM and its role in T2DM pathogenesis.

Conclusion

From our study point of view, persistent elevated serum ferritin levels in patients with T2DM exhibit a certain degree of inflammation that, in one way or another, is likely to increase their risk of developing cardiovascular complications or may have a role in the development of insulin resistance.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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