Original Article

Inflammatory markers and control of type 2 diabetes mellitus

Hanan Elimam [a, *], Azza M. Abdulla [b], Inas Mohamed Taha [c]

[a] Biochemistry Department, Faculty of Pharmacy, University of Sadat City, Sadat City, Egypt
[b] Medical Biochemistry and Molecular Biology Department, Faculty of Medicine, Menoufia University, Egypt
[c] Internal Medicine Department, Faculty of Medicine, Taibah University, Saudi Arabia

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ABSTRACT

Background: Subclinical inflammation and presence of almost all indicators of systemic inflammation are found in type 2 diabetic patients. Such a systemic and subclinical inflammatory process can be characterized by elevated circulating levels of inflammatory markers.

Aim: To study the state of subclinical inflammation in patients with type 2 diabetes mellitus and establish a correlation between glycemic control and inflammatory markers.

Methods: This research included 90 subjects divided into 2 groups; Group A: 70 patients with type 2 diabetes and Group B: 20 Age and sex matched people as the control group. All patients were clinically examined, had laboratory investigations including; fasting and 2 h postprandial blood sugar, HbA1c, serum ferritin., high sensitivity C-reactive protein hs-CRP, kidney functions tests, liver function tests, complete blood count and erythrocyte sedimentation rate and antinuclear antibody.

Results: The estimated levels of ESR, FBS, serum ferritin, hs-CRP and HbA1c in T2DM were 10.69 ± 3.05, 186.01 ± 92.21, 6005.2 ± 2639.83, 155.75 ± 73.95, 7.5 ± 3.23, respectively. In a similar way, in control subject, the estimated levels for respective parameters were 12.4 ± 3.41, 83.25 ± 6.25, 45.088 ± 39.35, 19.97 ± 18.51, 4.555 ± 0.58, respectively. Mean values of all parameters, except ESR, were found to be significantly augmented in T2DM subjects when compared to control group. There is significant positive correlation between HbA1c and hs-CRP ($r=0.761$, $p < 0.001$). Moreover, serum ferritin has shown significant positive correlation with HbA1c ($r = 0.853$, $p < 0.001$).

Conclusion: Strong correlation between inflammation and glycemic control in patient with type 2 diabetes mellitus suggests that inflammation plays an important role in the pathogenesis of diabetes.

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1. Introduction

Type 2 diabetes (T2D) is a major global health problem affecting approximately 415 million people. By 2040, this number is expected to increase to 642 million. Approximately 90–95% of these cases are type 2 diabetes mellitus (T2DM) [1]. Diabetes mellitus (DM) is a devastating disease. The estimated 5-year mortality rate is calculated as 18.9% of patients with T2DM. Diabetic complications, namely, cardiovascular diseases, neuropathy, and nephropathy with subsequent amputation, usually lead to death [2].

Subclinical inflammation and the presence of almost all indicators of systemic inflammation have been observed in patients with T2DM. Inflammation has recently been suggested to be a crucial factor contributing to the development of this disease. This systemic and subclinical inflammatory process is characterized by elevated circulating levels of inflammatory parameters, including C-reactive protein (CRP) or high-sensitivity CRP (hs-CRP) and inflammatory cytokines [3]. Hs-CRP is a CRP measured using a highly sensitive assay. CRP represents the classical acute phase protein. CRP is a pentameric protein that is produced in the liver in response to inflammatory stimuli, and plasma levels of hs-CRP provide a sensitive marker of increased inflammatory activity in the arterial wall [4].

Chronic systemic subclinical inflammation has also been identified as a driver of insulin resistance, metabolic syndrome, and T2DM. The process of inflammation induces the hepatic synthesis of various acute phase proteins, such as ferritin, which is believed to play roles in insulin resistance and atherosclerosis. Scientific evidence has predicted effects of elevated serum ferritin levels on insulin resistance and T2DM, either because of increased body iron stores or influences from several inflammatory diseases [5].

* Corresponding author. Biochemistry Department, Faculty of Pharmacy, University of Sadat City, Sadat City, Egypt.
E-mail address: Hanan.elimam@fop.usc.edu.eg (H. Elimam).

The development of complications is linked to the accumulation of glycation adducts in tissue proteins. Optimal monitoring of glycemic control involves plasma glucose measurements (fasting (FBS) and postprandial blood sugar (PPBS)) and measurements of glycated haemoglobin (HbA1c) levels. These measurements are complementary; the patient's glucose measurements provide a picture of short-term glycemic control, whereas the HbA1c level reflects the average glycemic control over the previous 3 months [6].

The goal of the present study was to analyse the levels of the inflammatory markers hs-CRP and ferritin, leukocyte counts and the sedimentation rate in patients with T2DM and a group of healthy controls and to correlate their values with the parameters of glycemic regulation, such as HbA1c levels.

2. Subjects and methods

This study was approved by the Healthcare Ethics Committee of the Faculty of Medicine, Menoufia University. All participants were given and completed a written informed consent form justifying the purpose, methods, results, and difficulties of the study.

Ninety subjects were enrolled in the study, including 70 patients with poorly controlled T2DM who were recruited from the Internal Medicine Department of El Menshawy Hospital and Menoufia University Hospital. Patients were selected on the basis of poor glycemic control (HbA1c = 7.5%). As a control group, we selected 20 normoglycemic (non-diabetic), apparently healthy subjects. The control subjects were matched to the T2DM group by age and sex.

Subjects included in this study did not have a history of iron deficiency or anaemia, active liver or kidney diseases, chronic pancreatitis, gastrointestinal diseases, recent infectious diseases, endocrine disorders, autoimmune diseases and were not using hormone therapy. Non-diabetic control subjects were of approximately the same age (40–60 years old) and exhibited normal glucose tolerance. These subjects also did not display abdominal obesity, a criterion of insulin resistance.

2.1. Laboratory analysis

After providing written informed consent, all subjects were clinically examined, and arterial blood pressure and body parameters, including height, weight and waist circumference, were measured. Seven millilitres of venous blood were collected from all subjects after an overnight fast of at least 8 h. Two millilitres were placed in vacutainer tubes containing EDTA for routine CBC analysis and use in measuring the HbA1c level (using quantitative colorimetric kits) (Teco Diagnostics, USA) [7]. The other 5 ml of blood

were placed in plain tubes and centrifuged to obtain serum, which was used to measure the levels of ferritin, hs-CRP, kidney function parameters, liver function parameters and anti-nuclear antibody (ANA). Serum hs-CRP levels were measured using an ELISA (Calbiotech Inc. (CBI)), based on the principle of a solid phase enzyme-linked immunosorbent assay, according to a previously described method [8]. Human serum ferritin [9] and ANA levels [10] were measured using ELISAs (Calbiotech Inc. (CBI)). FBS and 2 h PPBS levels were determined by the glucose oxidase method using a Spinreact diagnostics kit (Spinreact, Spain). Biochemical tests designed to analyse serum aspartate aminotransferase (SGOT) and serum alanine aminotransferase (SGPT) levels were performed using the kinetic UV-optimized method of the International Federation of Clinical Chemistry (LTEC Kit, England) [11].

2.2. Statistical analysis

All data were collected, tabulated and statistically analysed using SPSS software (SPSS Inc., Chicago, IL, USA). Data are presented as the means±standard deviations. Student's t-test was used to determine significant differences in analysed variables between the two groups. The Mann-Whitney U test was used to assess the null hypothesis that a randomly selected value from one sample is equally likely to be less than or greater than a randomly selected value from a second sample. The frequencies are presented as %. Correlations between parameters were analysed using the Pearson R test for variables with a normal distribution and the Spearman test for variables with a non-normal distribution. The probability value (p) was considered significant if it was less than 0.05 and highly significant if was <0.001.

3. Results

The demographic data did not reveal differences in age between the group (70 patients) with T2DM (50.83 ± 8.26) and non-diabetic healthy controls (50.3 ± 5.6) (Table 1). Moreover, the chi-square (X^2) test did not show a significant difference in sex between the two groups (p = 0.342) (Table 1).

The criteria used to select the subjects were based on FBS and HbA1c levels. Subjects with T2DM had significantly higher FBS and HbA1c levels than non-diabetic subjects (mean values 186.01 mg/dL versus 83.25 mg/dL and 7.50% versus 4.55%, respectively) (Table 2). A higher postprandial blood sugar level was also observed in the T2DM group (289.7 ± 125.54 mg/dL) than in the control group (120.9 ± 8.76 mg/dL). Notably, subjects with T2DM and controls showed no differences in the white blood cell count.

Table 1
Comparison of the sex and age distributions between the two groups.

		Non-diabetic (n = 20)	T2DM (n = 70)	Test of sig.	p
Age (years) Mean ± SD		50.3 ± 5.6	50.83 ± 8.26	t-test=0.331	0.742
Sex	Male	6	12	X^2=0.904	0.342
	Female	14	58		

Table 2
Comparison of laboratory parameters between the two groups.

	Non-diabetic (n = 20)	T2DM (n = 70)	Unpaired (t) test	P value
ESR(mm/h)	12.4 ± 3.41	10.69 ± 3.05	2.158	0.034[a]
FBS(mg/dL)	83.25 ± 6.25	186.01 ± 92.21	11.155	0.000[a]
PPBS(mg/dL)	120.9 ± 8.76	289.7 ± 125.54	9.251	0.000[a]
hs-CRP (ng/mL)	45.088 ± 39.35	6005.2 ± 2639.83	13.697	0.000[a]
Ferritin (ng/mL)	19.97 ± 18.51	155.75 ± 73.95	13.913	0.000[a]
HbA1c (%)	4.555 ± 0.58	7.5 ± 3.23	9.95	0.000[a]

[a] Significant difference.

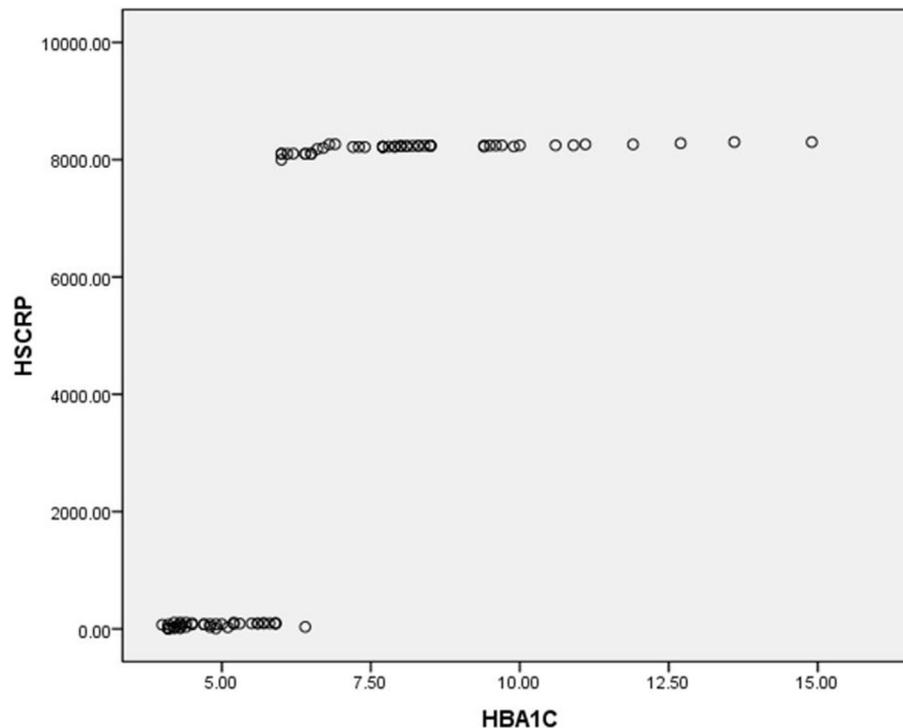**Fig. 1.** Correlations between HbA1c and hs-CRP levels (r = 0.761, p = 0.000*).

However, control subjects showed a greater statistically significant difference in the ESR than the patient group (Table 2).

In addition, after investigating the serum levels of the inflammatory biomarkers ferritin and hs-CRP in both patients with T2DM and controls, highly significant differences in the serum hs-CRP and ferritin levels were observed in the T2DM group compared to the control group (Table 2).

HbA1c levels were positively correlated with hs-CRP levels, with a highly significant p value (r = 0.761, p < 0.001) (Fig. 1). Similarly, HbA1c levels were positively and significantly correlated with ferritin levels (r = 0.853, p < 0.00) (Fig. 2). Another correlation study revealed significant positive correlations between hs-CRP levels and other parameters, such as body mass index (BMI), FBS and PPBS (Table 3). Furthermore, significant positive correlations were observed between serum ferritin levels and BMI, FBS and PPBS (Table 4).

Based on these results, HbA1c and FBS levels were significantly correlated with hs-CRP and serum ferritin levels in patients with T2DM.

4. Discussion

The current study confirms the presence of a low level of systemic inflammation in patients with T2DM, which is mostly related to features of metabolic syndrome. However, the main new findings are related to the observations of positive correlations between HbA1c and hs-CRP levels and between ferritin and HbA1c levels.

In the present study, the ESR was significantly elevated in the control group compared with the patients with T2DM. Nadeem and her colleagues found higher ESRs in patients with diabetes than in healthy subjects, indicating a role for inflammation in the pathogenesis of the disease. The levels of inflammatory markers were increased in older patients [12].

Studies conducted in participants of both sexes have revealed the importance of inflammatory markers in T2DM pathogenesis.

The hs-CRP level was significantly elevated in patients with T2DM compared with healthy controls. These findings are consistent with the findings from a study conducted to assess the hs-CRP levels in patients with T2DM. Significantly higher hs-CRP levels were observed in patients with T2DM compared to the healthy population [13]. However, this result contradicts the findings reported by Lima and colleagues, as the authors did not observe a significant difference between patients with T2DM and healthy controls [14]. The discrepancy may be explained by the use of median values in the published study and means ± SD in the present study.

Serum ferritin is an acute phase reactant and a marker of iron stores in the body [15]. Patients with T2DM exhibited significantly elevated serum ferritin levels compared with those in healthy individuals. This finding is supported by results from a prospective case control study conducted by Kumar et al. in which patients with T2DM exhibited significantly higher serum ferritin levels than healthy controls [16].

In 2008, Markis et al. were the first to establish a strong correlation between the mean blood glucose and HbA1c levels in patients with T2DM, which supported the idea of expressing HbA1c levels as MBG [17]. In the present study, we observed a significant positive correlation between HbA1c and hs-CRP levels. This finding is consistent with the findings reported by Sarinnapakorn et al., who observed a correlation between hs-CRP levels and HbA1c levels. Significantly higher mean HbA1c levels were observed in patients with hs-CRP levels ≥1 mg/L. Other factors, such as age, blood pressure, BMI, LDL cholesterol, and serum creatinine levels, were not correlated with the hs-CRP level [18].

The serum ferritin level was significantly correlated with HbA1c, FBS and PPBS levels. These results are consistent with the findings from a recent study conducted by Arora, in which the serum ferritin level was proportional to the blood glucose level [19]. However, our finding contradicts the results reported by Sharifi and Sazandeh, who did not observe a correlation between serum ferritin and HbA1c levels in patients with T2DM and normal controls (r = 0.23).
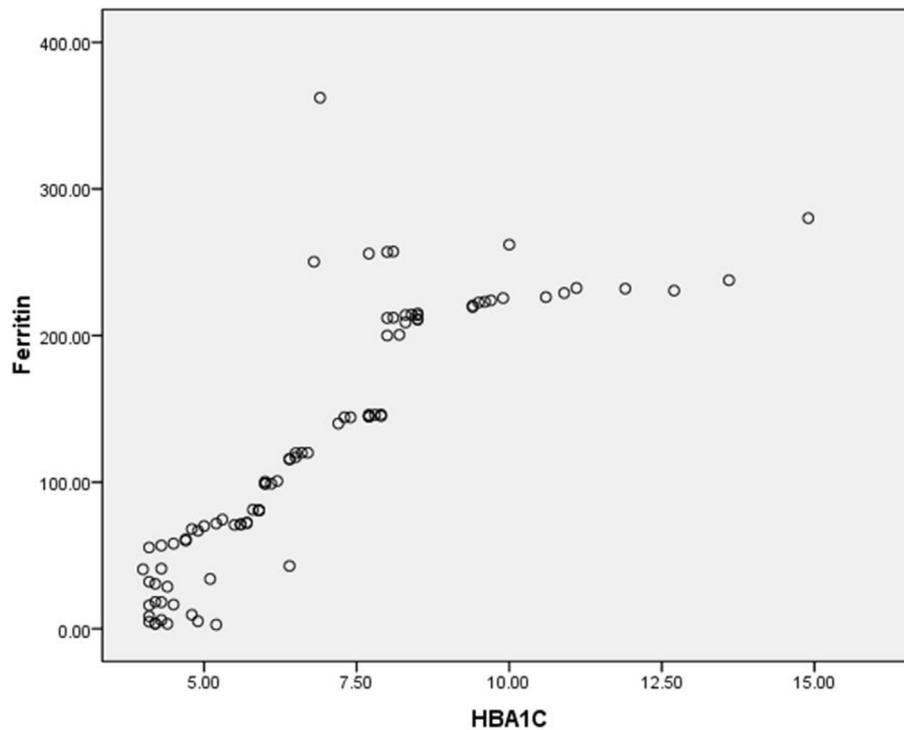
Fig. 2. Correlations between HbA1c and serum ferritin levels (r = 0.853, p < 0.001*).

Table 3
Correlations between hs-CRP levels with BMI, FBS and PPBS.

	R	P value
BMI	0.272	0.01[a]
FBS	0.288	0.006[a]
PPBS	0.417	0.000[a]

[a] Significant difference.

Table 4
Correlation and regression analysis of ferritin levels with BMI, FBS and PPBS.

	R	P value
BMI	0.319	0.002[a]
FBS	0.352	0.001[a]
PPBS	0.484	0.000[a]

[a] Significant difference.

The authors suggested that the use of bloodletting may affect the total haemoglobin and HbA1c levels; therefore, the authors postulated that HbA1c is not an appropriate marker of blood glucose control [20].

Positive correlations between ferritin, HbA1c, and FBS levels indicated that hyperglycaemia increased the glycation of haemoglobin and increased the release of free iron from glycated proteins such as haemoglobin. These processes form a vicious cycle of hyperglycaemia, haemoglobin glycation and increased levels of free iron and ferritin. This increased iron pool will enhance oxidant generation, leading to biomolecule damage [21].

In the present study, we did not observe a correlation between serum ferritin levels and age (r = 0.037, p = 0.726). This result is consistent with the findings reported by Fernandez-Real and colleagues, who did not observe a correlation between serum ferritin levels and age among patients with diabetes [22]. However, our results are not consistent with the findings reported by Padmaja

and colleagues, who observed a low but significant positive correlation between serum ferritin levels and age in patients with diabetes [23].

The elevated serum ferritin level recorded in our study might be explained by the possible correlation with the occurrence of long-term complications of diabetes, including both microvascular and macrovascular disorders [24].

The present study reported correlations between elevated glycated haemoglobin levels, which reflect poor glycemic control, and both hs-CRP and serum ferritin levels, which are inflammatory markers that reflect the role of glycemic control and subclinical inflammation in patients with T2DM. Consistent with our findings, elevated hs-CRP levels were postulated to represent subclinical inflammation in a previous study [25]. The higher positive correlation between serum ferritin and HbA1c levels indicates that hyperglycaemia affects ferritin levels, probably due to inflammation and/or oxidative stress [23]. Further studies including a larger number of subjects are needed before these parameters can be used effectively as biomarkers of the primary prevention of T2DM in this population.

5. Conclusions

Increased inflammation was observed in patients with T2DM, as manifested by elevated ESR, serum ferritin and hs-CRP levels. Furthermore, we observed significant positive correlations between HbA1c and hs-CRP levels and between HbA1c and serum ferritin levels. All these findings suggest a link between inflammation and glycemic control in patients with T2DM.

Abbreviations

T2DM   type 2 diabetes mellitus
hs-CRP   high-sensitivity C-reactive protein
FBS   fasting blood sugar

PPBS	postprandial blood sugar
HbA1c	glycated haemoglobin
ESR	erythrocyte sedimentation rate
BMI	body mass index
SGOT	serum aspartate aminotransferase
SGPT	serum alanine aminotransferase

References

[1] Dall TM, Narayan KM, Gillespie KB, Gallo PD, Blanchard TD, Solcan M, et al. Detecting type 2 diabetes and prediabetes among asymptomatic adults in the United States: modeling American Diabetes Association versus US Preventive Services Task Force diabetes screening guidelines. Popul Health Metrics 2014;12:12.

[2] Cusick M, Meleth AD, Agron E, Fisher MR, Reed GF, Knatterud GL, et al. Associations of mortality and diabetes complications in patients with type 1 and type 2 diabetes: early treatment diabetic retinopathy study report no. 27. Diabetes Care 2005;28:617–25.

[3] Herder C, Carstensen M, Ouwens DM. Anti-inflammatory cytokines and risk of type 2 diabetes. Diabetes Obes Metabol 2013;15(Suppl 3):39–50.

[4] Pfutzner A, Forst T. High-sensitivity C-reactive protein as cardiovascular risk marker in patients with diabetes mellitus. Diabetes Technol Therapeut 2006;8:28–36.

[5] Fernandez-Real JM, Moreno JM, Lopez-Bermejo A, Chico B, Vendrell J, Ricart W. Circulating soluble transferrin receptor according to glucose tolerance status and insulin sensitivity. Diabetes Care 2007;30:604–8.

[6] Alvin C. Powers: diabetes mellitus. sixteenth ed. ed. New York: McGraw-Hill; 2005.

[7] Gonen G, Rubenstein AH. Haemoglobin A1 and diabetes mellitus. Diabetologia 1978;15:1–8.

[8] Schultz DR, Arnold PI. Properties of four acute phase proteins: C-reactive protein, serum amyloid A protein, alpha 1-acid glycoprotein, and fibrinogen. Semin Arthritis Rheum 1990;20:129–47.

[9] Stacy DL, Han P. Serum ferritin measurement and the degree of agreement using four techniques. Am J Clin Pathol 1992;98:511–5.

[10] Emlen W, O'Neill L. Clinical significance of antinuclear antibodies: comparison of detection with immunofluorescence and enzyme-linked immunosorbent assays. Arthritis Rheum 1997;40:1612–8.

[11] Bergmeyer HU, Horder M, Rej R. International Federation of Clinical Chemistry (IFCC) Scientific Committee, Analytical Section: approved recommendation (1985) on IFCC methods for the measurement of catalytic concentration of enzymes. Part 3. IFCC method for alanine aminotransferase (L-alanine: 2-oxoglutarate aminotransferase, EC 2.6.1.2). J Clin Chem Clin Biochem 1986;24:481–95.

[12] Nadeem A, Naveed AK, Hussain MM, Raza SI. Correlation of inflammatory markers with type 2 Diabetes Mellitus in Pakistani patients. J Postgrad Med Inst 2013;27:267–73.

[13] Malenica M, Silar M, Dujic T, Bego T, Semiz S, Skrbo S, et al. Importance of inflammatory markers and IL-6 for diagnosis and follow up of patients with type 2 diabetes mellitus. Med Glas 2017;14:169–75.

[14] Lima LM, Carvalho M, Soares AL, Sabino Ade P, Fernandes AP, Novelli BA, et al. High-sensitivity C-reactive protein in subjects with type 2 diabetes mellitus and/or high blood pressure. Arq Bras Endocrinol Metabol 2007;51:956–60.

[15] Koorts AM, Viljoen M. Ferritin and ferritin isoforms II: protection against uncontrolled cellular proliferation, oxidative damage and inflammatory processes. Arch Physiol Biochem 2007;113:55–64.

[16] Kumar GT, Saravanan A, Ramachandran C, JN A. Mean blood glucose level and glycated haemoglobin level in patients of non-insulin dependent diabetes mellitus and its correlation with serum ferritin level. Int J Med Sci 2011;4:13–7.

[17] Makris K, Spanou L, Rambaouni-Antoneli A, Koniari K, Drakopoulos I, Rizos D, et al. Relationship between mean blood glucose and glycated haemoglobin in Type 2 diabetic patients. Diabet Med 2008;25:174–8.

[18] Sarinnapakorn V, Wanicagool W. Association between hs-CRP and HbA1c in overweight type 2 diabetic female patients. J Med Assoc Thai 2013;96(Suppl 3):S54–8.

[19] Arora P. Correlation between serum ferritin and glycated hemoglobin level in patients of type 2 diabetes mellitus. Int J Cur Res Rev 2017;9:30–4.

[20] Sharifi F, Sazandeh S. Serum ferritin in type 2 diabetes mellitus and its relationship with HbA1c. Acta Med Iran 2004;42:142–5.

[21] Shetty JK, Prakash M, Ibrahim MS. Relationship between free iron and glycated hemoglobin in uncontrolled type 2 diabetes patients associated with complications. Indian J Clin Biochem 2008;23:67–70.

[22] Fernandez-Real JM, Ricart-Engel W, Arroyo E, Balanca R, Casamitjana-Abella R, Cabrero D, et al. Serum ferritin as a component of the insulin resistance syndrome. Diabetes Care 1998;21:62–8.

[23] Padmaja P, Shabana S, Shariq M. Serum ferritin and HbA1c levels in type 2 diabetes mellitus. Int J Clin Biochem Res 2015;1:30–7.

[24] Eshed I, Elis A, Lishner M. Plasma ferritin and type 2 diabetes mellitus: a critical review. Endocr Res 2001;27:91–7.

[25] Gohel MG, Chacko AN. Serum GGT activity and hsCRP level in patients with type 2 diabetes mellitus with good and poor glycemic control: an evidence linking oxidative stress, inflammation and glycemic control. J Diabetes Metab Disord 2013;12:56.