

Interleukin-8, Ferritin and Soluble Transferrin Receptors in Type II Diabetes Mellitus

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Type II diabetes mellitus (DM) is the most common form of diabetes that constitutes the majority of cases worldwide including Egypt. Chronic elevated glucose level in DM increases monocyte adhesion to aortic endothelial cells (ECs) which is mediated primarily through induction of interleukin-8 (IL-8). This study aimed to investigate the possible role of IL-8 as a potent chemoattractant, pro-inflammatory cytokine in the immuno-inflammatory response of type II diabetic patients in correlation to ferritin and sTFR as markers of glucose homeostasis that characterizes the disease. The current work was conducted on 20 diabetic females and 10 healthy age and sex matching subjects as a group of control. Serum levels of IL-8, ferritin and sTFR were measured in all study subjects under investigation. Results revealed that both serum levels of IL-8 and ferritin were significantly elevated in type II diabetic patients ($P = 0.0029$ and 0.03 respectively) compared with those of control group while no significant difference was detected between sTFR levels in diabetic patient and control groups. In addition, a significant positive correlation was detected ($P = 0.032$) between serum levels of IL-8 and sTFR of the studied diabetic patient group. In conclusion, quantitative determination of IL-8, ferritin and sTFR could help in predicting type II diabetes-associated immuno-inflammatory manifestations characterize the micro-and macrovascular disease complications, particularly for high risk populations.

Diabetes mellitus (DM) is a growing health problem both internationally and nationally (Commonwealth Dept, Health and Aged Care, 1999). It is a devastating metabolic disease caused by either insulin deficiency or resistance, and characterized by abnormal glucose, protein and lipid metabolism (Desir, 2005). Non insulin-dependent diabetes mellitus (type II DM) is the most common form of diabetes that constitutes the majority of cases worldwide including Egypt (Herman et al., 1997). It is resulting from interaction of obesity, inflammation and hyperglycemia (Moreno et al., 2004). Diabetic patients commonly have micro-and-macrovascular pathology that influences their perioperative course and critical illness and increases morbidity and mortality rates during hospitalization (Coursin et al., 2004). Gloser et al. (2003) reported that depression is associated with enhanced production of pro-inflammatory cytokines that influences a

spectrum of conditions associated with aging, including type II diabetes mellitus. Type II diabetes is a common manifestation of hemochromatosis, a disease of iron overload. Higher iron stores (reflected by an elevated ferritin concentration and a lower ratio of transferrin receptors to ferritin) are associated with an increased risk of type II diabetes (Jiang et al., 2004). Increased serum ferritin concentration was detected in type II diabetic patients in the absence of a reciprocal decrease of soluble transferrin receptors (sTFR) (Hernandez et al., 2005) and correlated to diabetic retinopathy (Canturk et al., 2003). It was found that iron depletion improves vascular dysfunction in type II diabetic patients with high ferritin concentration (Fernandez-Real et al., 2002) and that iron-related insulin-resistance is improved by iron depletion or treatment with iron chelators (Fernandez-Real et al., 2002). Uncontrolled hyperglycaemia in diabetic patients results in the formation of advanced

glycation end products (AGEs), which are detrimental to cell structure and function. Altered host resistance such as defective migration of polymorphonuclear (PMN) cells, impaired phagocytosis and an exaggerated inflammatory response to microbial products also compromises healing in uncontrolled diabetic patients (Soory, 2002). Chronic elevated glucose level in DM increases monocyte adhesion to human aortic endothelial cells (ECs) which is mediated primarily through induction of IL-8 (Srinivasan et al., 2004). Patients with type II DM had higher fasting IL-8 and IL-18 concentrations and lower adiponectin concentrations at baseline when compared with non-diabetic subjects (Esposito et al., 2003).

Altered immuno-inflammatory responses of patients with type II DM was found to be attributed as the cause of the underlying pathophysiology (Lo, 2005). So, we aimed in this study to investigate the possible role of IL-8 as a potent chemoattractant and pro-inflammatory cytokine in the immuno-inflammatory response in type II diabetic patients via measuring the IL-8 serum level in those patients in correlation to ferritin and sTFR serum levels as markers of glucose homeostasis characterize the disease.

Subjects and Methods

Subjects and exclusion criteria

Twenty type II diabetic females and 10 healthy subjects of matching age, sex, height and weight taken as a group of control were enrolled in this study. The mean age of the subjects under study was ranged between 37-62 years with mean value 48.52 ± 10.57 . The duration of diabetes ranged from newly diagnosed to 18 years (0-18 years) with a mean of 6.5 ± 3.8 years. Exclusion criteria: Patients were screened before participation with a medical history and physical examination. Individuals were excluded from the study if they had clinically significant concomitant medical illness such as cardiac, renal or hepatic disease. Individuals required medications that might affect glucose metabolism and patients who were treated by insulin at the time of the study were also excluded.

Methods

After an overnight fasting for at least twelve hours, about 10 ml of venous blood were withdrawn from each subject, centrifuged at 3000 rpm for 5 minutes rapidly after clotting. The separated serum samples were kept at -80°C till used for quantitative determination of IL-8, ferritin and sTFR serum levels.

- IL-8

IL-8 serum level was determined using solid phase ELISA kit (Bender Med-system GmbH, Austria) according to the manufacturer's procedures. Briefly anti-IL-8 monoclonal coating antibody was adsorbed onto microwells and binded to IL-8 present in the sample. Conjugated monoclonal anti-IL-8 was added and binded to IL-8 captured by the first antibody. After incubation of the microwells, nonbound enzyme conjugated anti-IL-8 was removed during a wash step. Substrate solution reactive with the conjugate was added to the wells. A coloured product was formed in proportion to amount of IL-8 present in the sample. The reaction was terminated by addition of phosphoric acid and colour absorbance was measured using microwell strip reader at 450 nm. A standard curve was prepared from seven standard concentrations (16-1000 pg/ml) to determine IL-8 concentration of each serum sample.

- Ferritin

Serum ferritin level was measured using the quantitative ELISA kit (Diamed Eruogen, 2300 Turnhout, Belgium). In this assay, the wells were coated with a monoclonal antibody directed against a unique antigenic site on the ferritin molecule, the microtiterstrips were incubated with patient samples then with a horseradish peroxidase-conjugated second monoclonal antibody direct against a different antigenic determinant of the ferritin molecule. A blue colour develops after a third incubation with a substrate solution containing a chromogen and hydrogen peroxide which is proportional to the amount of immune complexes bound to the wells. The absorbance values were determined at 450 nm, a standard curve was obtained by plotting each absorbance value versus the corresponding standard value. Ferritin concentration (ng/ml) in patient samples was determined by interpolation from the standard curve.

- Soluble transferring receptor (sTFR)

Serum soluble transferring receptor (sTFR) was measured using the DIAMED s-TFR ELISA kit (Diamed Eurogen, 2300 Turnhout, Belgium). In this assay, microtiterstrips coated with anti-sTFR monoclonal antibodies were incubated with standard and patients' samples. So, sTFR present in the samples was bound to the immobilized antibodies. In a second incubation step, this captured sTFR would react with a

second sTFR specific monoclonal antibody conjugated to horseradish-peroxidase (HRP). In a third incubation step, peroxidase in the resultant complex would react with a chromogen solution containing hydrogen peroxide and tetramethyl benzidine. A blue colour developed which was proportional to the amount of sTFR, the absorbance values were determined at 450 nm. The concentration of sTFR in patient samples was determined by interpolation from a standard curve obtained by plotting each absorbance value versus the corresponding standard value.

Results

The metabolic data of type II diabetic patients in the present study showed significantly higher serum levels ($P \leq 0.001$) of all parameters except in insulin level (insignificantly higher, $P > 0.05$) when compared with those of controls (Table 1).

Table 1. Metabolic data of type II diabetic patients and controls.

Metabolic data	Type II diabetics (mean \pm SD) n = 20	Control (mean \pm SD) n = 10	*p
LDL (mg/dl)	142.08 \pm 25.16	82.34 \pm 6.2	0.001
HDL (mg/dl)	38.5 \pm 6.81	61.5 \pm 8.41	0.001
TG (mg/dl)	151.11 \pm 42.38	78.67 \pm 13.6	0.001
Cholesterol (mg/dl)	226.55 \pm 47.38	130.27 \pm 5.12	0.001
FBG (mg/dl)	214.45 \pm 72.86	92.65 \pm 5.42	0.001
Insulin (μ l/ml)	12.18 \pm 5.9	10.8 \pm 4.5	N.S
IR	5.04 \pm 2.68	2.17 \pm 0.65	0.001

*Significant difference when compared to the corresponding values of controls at level of significant 0.001.

N.S. Not significant.

LDL: Low density lipoprotein cholesterol.

HDL: High density lipoprotein cholesterol.

TG: Triglycerides.

FBG: Fasting blood glucose.

IR: Insulin resistance as calculated by HOMA equation.

Both IL-8 and ferritin serum levels were significantly higher in type II diabetic patients ($P < 0.01$ and $P < 0.05$ respectively) when compared with those of control group while

sTFR serum level did not show any significant differences ($P > 0.05$) between the two studied groups (Table 2).

Table 2. Serum levels of interleukin-8 (IL-8), ferritin, and soluble transferrin receptors (sTFR) in type II diabetic patients and controls.

Variables	Type II diabetics (mean \pm SD) n=20	Controls (mean \pm SD) n=10	p
IL-8 (pg/ml)	184.27 \pm 75.29	127.06 \pm 28.714	0.01
Ferritin (ng/ml)	318.22 \pm 308.30	133.75 \pm 188.46	0.05
sTFR (mg/L)	1.16 \pm 0.643	1.57 \pm 1.076	N.S

Significant difference when compared to the corresponding values of controls at level of significant 0.05.

N.S. Not significant.

A significant positive correlation was detected between serum levels of IL-8 and sTFR in

type II diabetic patients of this study (Table 3).

Table 3. Correlation between serum levels of interleukin-8 (IL-8), ferritin and soluble transferrin receptors (sTFR) in type II diabetic patients.

	s-TFR	Ferritin
Ferritin		
r	0.151	
*p	N.S	
IL-8		
r	0.517	0.111
*p	0.05	N.S

r : Correlation coefficient

*Significant difference at $P < 0.05$.

Discussion

Type II diabetes mellitus is a manifestation of the inflammatory host response, orchestrated mainly by the production of pro- and anti-inflammatory cytokines under genetic control. In present study, IL-8 serum level was found to be significantly higher in type II diabetic patients than that of controls. This result is in concomitant with those obtained in many other studies (Lebovitz et al., 2004, Coursin et al., 2004; Srinivasan et al., 2004 and Fernandez-Real et al., 2002). Regarding the pleiotropic function of IL-8 as a potent chemoattractant / pro-inflammatory cytokine, this result denoted to the altered immunoinflammatory responses of these patients and consequent increase in their susceptibility to infectious complications following trauma and surgery (Lo, 2005) in addition to increased risk of micro- and macrovascular disease complications such as myocardial infarction or stroke from plaque rupture (Home, 2005 and Giacconi et al., 2005). Early study of Nawas et al. (1999) demonstrated that the elevated serum IL-8 level in type II DM after coronary artery bypass grafting (CABG) may contribute to later infiltration and associated oxidative damage. Srinivasan et al (2004) indicated that the pro-inflammatory cytokine

IL-6 production is increased in type II DM and contributed to early vascular inflammatory changes. They examined in previous study the mechanism by which glucose stimulates monocyte: endothelial interactions. Their data indicated that glucose regulates monocyte endothelial interactions through stimulation of IL-8 and reactive oxygen species (ROS) production and activation of the β_1 integrin complex on human aortic endothelial cells (HAECs). In their third study, Srinivasan et al. (2004) explained that glucose-stimulated monocyte adhesion, leads to IL-8 production.

Morino et al. (2004) and Lebovitz et al (2004) suggested that activated immunity and increased cytokine production lead to insulin resistance which is a cardinal feature of type II diabetes and other components of the metabolic syndrome, establishing the link between diabetes and atherosclerosis (Home, 2005 and Giacconi et al., 2005). Insulin resistance is increasingly recognized as a chronic, low level inflammatory state in which, the glycemic response to insulin is less than normal. Hyperinsulinemia has been proposed as the forerunner of hypertension, low high-density lipoprotein cholesterolemia, hypertriglyceridemia, abdominal obesity, and altered glucose tolerance, linking all these

abnormalities to the development of coronary vascular disease (CVD) (Fernandez-Real et al., 2003).

In addition, pro-inflammatory cytokines including IL-8 may have deleterious effects on both glucose homeostasis and beta-cell function and can disrupt insulin signaling pathways in both pancreatic beta-cells and liver and adipose tissue (Greenberg et al., 2002) suggesting a possible role of this cytokine in linking insulin resistance with beta-cell dysfunction which underlines most cases of type II diabetes. The role of IL-8 in the pathophysiology of type II DM could be similar to that played by IL-6 (both are Th2 pro-inflammatory cytokines) which is considered by Petersen et al. (2005) to be the first myokine that is produced and released by contracting skeletal muscle fibers, exerting its effects in other organs of the body, enhances lipid turnover, estimated lipolysis as well as fat oxidation. They also suggested that myokines may be involved in the protection against chronic diseases associated with low-grade inflammation such as DM.

Since all of type II diabetic patients in our study were obese middle age women, the elevated IL-8 serum level in these patients could be due to the increased adiposity that contributes to dyslipidemia, hyperglycemia, and hypertension. Adipose tissue not only secretes non-esterified fatty acids but this organ is also an active endocrine and paracrine system that can secrete pro-inflammatory factors, pro-insulin resistance factors, and other cytokines and hormones contributing to hypertension and fibrinolysis (Vega et al., 2004). This close association between obesity, inflammation and insulin resistance is well documented and establishes the leading risk factor for type II DM (Vega et al., 2004, Kelley et al., 2003, Greenberg et al., 2002, Hirosumi et al., 2002, Youssef et al., 2002 and Ibrahim et al., 2002). Recently, Lappas et al. (2005) demonstrated that the I Kappa 13 Kinase (IKK-beta)/NF-Kappa B

transcription pathway is a key regulator of IL-6, TNF- α and IL-8 from adipose tissue and skeletal muscle. They suggested that control of this pathway may therefore provide an alternative therapeutic strategy for regulating aberrant cytokine release and thereby alleviating insulin resistance in type II DM.

Our results also revealed that ferritin serum level was significantly higher in type II diabetic patients than that of controls. This result is in agreement with that obtained in previous studies (Jiang et al., 2004, Hernandez et al., 2005, Ren et al., 2004). The mean serum level of transferrin receptors in this study showed no significant difference between type II diabetic patients and controls resulting in lower TFR/ferritin ratio in diabetic patients than that of controls. This result also agrees with that obtained by Jaing et al. (2004) and Hernandez et al. (2005) reflecting the higher body iron stores in those patients. This result may referred to the well established finding that type II diabetes is a common manifestation of hemochromatosis, a disease of iron overload (Jiang et al., 2004). Elevated serum ferritin concentration was detected in study of Ren et al. (2004) in a glucose-impaired population and in normal glucose tolerant first-degree relative in familial type II diabetic pedigrees.

Elevated ferritin level in the type II diabetic patient group in present study may support the finding of Fernandez-Real et al. (2002) who suggested that iron depletion improves vascular dysfunction in type II diabetic patients with high ferritin concentrations, and their finding that iron related insulin-resistance is improved by iron depletion on treatment with iron chelators (Fernandez-Real et al., 2003). Independent positive association was found by Canturk et al. (2003) between serum ferritin concentration and markers of glucose homeostasis such as fasting and post-grandial glucose levels and glycated hemoglobin.

The insignificant positive correlation between ferritin and IL-8 serum levels and significant positive correlation between sTFR and IL-8 in present study denotes to IL-8 independent way of action in type II DM. In this aspect, Hernandez et al. (2005) suggested that elevated ferritin levels in type II diabetes are mainly a result of inflammatory mechanism rather than iron overload.

According to our results together with previous findings, we can suggest that quantitative determination of IL-8, ferritin and transferrin receptors may help in predicting type II DM-associated immuno-inflammatory manifestations characterize the micro-and macrovascular disease complications, particularly for high risk populations including those of over-weight, depressed and critical ill cases during hospitalization. In addition, meal modulation of inflammatory cytokines, regular exercises and effective administration of anti-inflammatory agents may offer protection against type II DM-associated complications.

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