Serum levels of Interleukin 6 and Homocysteine in Type 2 Diabetic Patients with renal failure complication

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Abstract

Background: Cardiovascular diseases are more common among type 2 diabetic patients than healthy subjects without a family history of diabetes. Serum interleukin-6 (IL-6) and homocysteine (Hcy) levels are markers of endothelial dysfunction and cardiovascular disease.
Aim: this study demonstrated to evaluate levels of IL-6, Hcy and their association with cardiovascular risk in type 2 diabetic patients with renal failure complication.
Material & Methods: The circulating IL-6 and Hcy levels were measured in 45 type 2 diabetic patients and 25 control subjects without a known family history of diabetes. The BIOSOURCE IL-6 ELISA kit used to measure IL- 6, high performance liquid chromatography HPLC technique used to measure serum Homocystein, and enzymatic methods are used for blood suger, urea, creatinine, and lipid profile measurements.
Results: A significant differences were found in serum levels of IL6 and Hcy between the two groups diabetic and healthy control ($p>0.05$). IL6 levels correlated significantly with Hcy ($p=0.02$), hemoglobin A_1C ($p=0.03$), and serum Hcy correlated significantly with HbA_1C ($p=0.03$) levels in patients with type 2diabetes mellitus.
Conclusion: These results suggest that serum IL-6 and Hcy levels do not directly contribute to the development of endothelial dysfunction and cardiovascular risk factors in type 2 diabetic patients with renal failure complication.

الخلاصة

الخلفية: ان الامراض القلبية هي من بين الامراض المرتبطة بداء السكري النوع الثاني عنه في الاصحاء الذين ليس لديهم تاريخ عائلي مع داء السكري. ان مستويات كل من الهوموسيستين والانترلوكين 6 تعتمد كمقياس لعجز الخلايا الطلائية والامراض القلبية .
الهدف: تعيين مستوى الانترلوكين 6 والهوموسيستين وارتباطهما بالمخاطر القلبية لدى المصابين بداء السكري النوع الثاني ممن لديهم مضاعفات العجز الكلوي.
النماذج وطرق القياس: ان مستويات الهوموسيستين و الانترلوكين 6 تم قياسها لدى 45 مريضا من المصابين بداء السكري النوع الثاني ممن لديهم عجز كلوي و25 من الاصحاء الذين يخلو تاريخهم الاسري من داء السكري. تقنية الايلايزة استخدمت لقياس مستوى الانترلوكين 6 كما واستخدمت تقنية كروماتوغرافيا السائل العالي الاداء لقياس مستويات الهوموسيستين. اما قياس مستوى كل من سكر الدم,اليوريا,الكرياتنين والدهون الفوقية فتم باستخدام الطرق الانزيمية.
النتائج: اثبتت التحليلات الاحصائية وجود فروقات ملحوظة في مستويات الانترلوكين 6 و الهوموسيستين بين المجموعتين المرضى والاصحاء($p<0.001$). ولوحظ وجود علاقة احصائية بين كل من الانترلوكين6 .الهوموسيستين مع خضاب الدم المسكر لدى المرضى المصابين بداء السكري.
الاستنتاج: نستنتج مما تقدم ان للالتهاب دورا في التسبب بداء السكري النوع الثاني و أرتفاع السايتوكاين-6 يعتبر مؤشر لذلك. كما ان زيادة الهوموسيستين اصبح منتشرا" لدى مرضى السكري ويعتبر عامل خطوره

ومؤشر لامراض القلب. وان هذين العاملين يعتبران كواصمين حيويين ينبئان بخطورة هذا الداء وخصوصا ممن لديهم عجز كلوي.

Introduction

Patients with type 2 diabetes have a high incidence of atherosclerosis, which leads to increased morbidity and mortality from coronary artery disease (CAD), cerebrovascular disease, and peripheral vascular disease (PVD) [(1)]. Atherosclerosis is a chronic low-grade inflammatory disease [(2)]. Plasma concentrations of several inflammatory markers such as interleukin (IL)-6 have been linked with future cardiovascular disease (CVD) in a variety of clinical settings [(3,4)]. A recent study [(5)] identified high serum IL-6 concentrations as a strong predictor of death from cardiovascular causes in patients with CAD. Hyperhomocysteinemia has been associated with atherothrombotic vascular diseases such as CAD, stroke, and PVD [(6)]. A previous study [(7)] demonstrated that moderate hyperhomocysteinemia is a stronger risk factor for CVD in patients with type 2 diabetes than in nondiabetic subjects, suggesting a synergistic effect of diabetes with hyperhomocysteinemia that accelerates the development of atherosclerosis. Although homocysteine can exert vascular toxicity via several mechanisms [(8)]. Moreover, no reports have examined the associations of plasma IL-6 with CVD or homocysteine (Hcy) concentrations in patients with type 2 diabetes. The present study was therefore undertaken to compare serum concentrations of IL-6 in patients with type 2 diabetes with those in age-matched control subjects and to investigate whether serum IL-6 is associated with Hcy concentration, in patients with type 2 diabetes with renal failure complication.

Subjects and Methods

- **Subjects**

Forty five patients with type 2 diabetes mellitus diagnosed with renal failure aged between (40-60) years, their (mean±SD) age were (56.12± 9.32) attended the National Diabetes Center, University of AL-Mustansiriya for treatment and research. And twenty fife healthy controls with match age and sex without a history family of diabetes mellitus, all patients and control subjects had been a fill question air. The study conducted in Augest-2009.

All patients were on oral-hypoglycemic agents (i.e. metformin and/or glibenclamide). While type 2 diabetic patients who received insulin injection were excluded from this study.

- **Blood sample**

Twelve milliliters (ml) of venous blood sample were taken, using plastic disposable syringes. Two milliliters were added to an ethylene diamine tetra acetic acid (EDTA) tube for Hemoglobin A_1C measuring, and ten milliliters were separated by centrifugation at (3000 rpm) for 15 min. the sera were stored frozen at (-20 ºC) until assayed. Each serum sample was anayzed for lipid profile, glucose, createinine, urea.homocysteine, and IL-6,

Method

-Determination of serum Homocystein (Hcy):

Serum Homocystein (Hcy) levels were measured by reversed phase liquid chromatography (HPLC) after pre-column derivetization with ortho-phthalaldehyde (OPA) as described previously by Zeiger et al. 1992 (9).

-Determination of Interlukine-6 (IL-6):
The BIOSOURCE IL-6 ELISA, is a solid phase Enzyme Linked Immuno Sorbent Assay (ELISA) performed on micro titer plate. The assay is based on an oligoclonal system in which a blend of monoclonal antibodies (MAbs) directed against distinct epitopes of IL-6 are used. Antibody-producing cells are immortilized using the myeloma cell fusion method of (kohler and Milstein)(10).

-Haemoglubin A_1C:
Haemoglubin A_1C (HbA_1C) was measured by variant ^{TU} HbA_1C program, which intended for determination of HbA_1C in human whole blood using Ion Exchange High Performance Liquid Chromatography (HPLC)(11).

-Enzymatic methods were used for measuring fasting blood sugar, lipid profile, urea and creatinine.

Statistical analysis

The data throughout this work was reported in the form of (mean value ± standard deviation). Quantitative differences between groups were determined by student T-test, where differences considered as highly significant when ($p<0.001$). The data were processed with Microsoft excel XP version.

Results

The results of Fasting blood sugar, HbA_1C for type 2 diabetes mellitus showed a significantly ($p<0.001$) higher difference when compared to the healthy controls also, there was highly significant differences in the concentration of blood urea and creatinine between diabetic group and healthy control subjects as in table (1).

Serum triglyceride (TG), total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), Atherogenic index (expressed as LDL-C/ HDL-C) & LDL size index (expressed as TG/ HDL-C) are shown in Table 2.

So Homocysteine (Hcy) level and Interlukin-6 in these diabetic groups are show highly significant difference when compared with control group as shown in table (3). Also we found a strong positive correlation between Hcy concentration with HbA_1C and IL-6 with HbA_1C for diabetic and healthy control. as shown in figure 1, 2,3 and 4 respectively. But there was weak positive correlation between IL6 levels and Homocystein concentration in healthy control and diabetic patient.

Table 1.The biochemical parameters of studied groups.

Biochemical parameters	Diabetic Mean±SD	Control Mean±SD	p-value
HbA1C	9.88±0.82	3.73±0.51	0.001
FBS mg/dl	190.34±39.01	84.54±7.45	0.001
S. urea mg/dl	67.3±12.45	39.2±6.4	0.001
S. creatinine mg/dl	3.1±0.34	0.98±0.25	0.001

Table 2. Serum lipid profile (mean±SD) in diabetic and healthy control subjects

Groups	HDL-C mg/dl	LDL-C mg/dl	VLDL-C mg/dl	Cholesterol mg/dl	Triglyceride mg/dl	Atherogenic index (LDLC/HDL-C)	LDL size Index (TG/HDL-C)
Diabetic	41.4±4.5	135.4±12.4	27.3±3.4	228.5±27.6	144.8±28.4	3.27±0.8	3.49±0.7
Control	48.4±3.6	126.5±24.0	20.1±4.1	173.3±19.6	103.6±34.2	2.6±0.7	2.14±0.6
t-test p-value	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*

Table 3. Hcy and IL-6 concentrations in all diabetic groups and healthy control.

Groups	n	Hcy µmol/l	IL-6 pg/ml
Diabetic	45	28.14±5.12	148.83±44.36
Control	25	12.63±2.2	58.75±41.72
t-test p-value		0.001	0.001

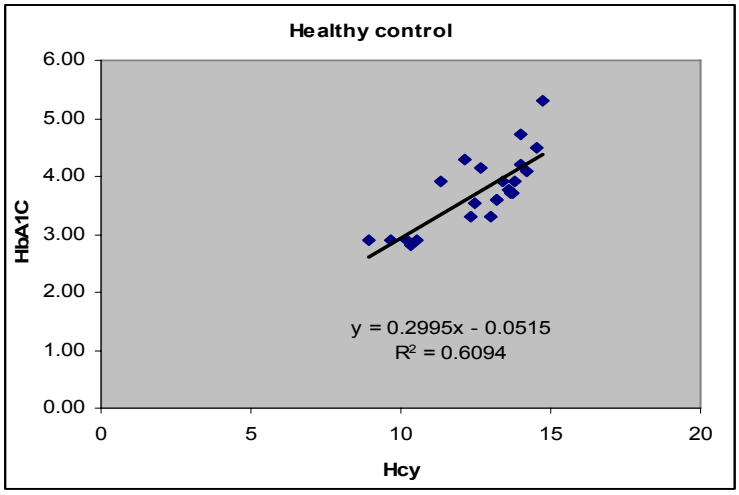

Figure 1. Correlation between HCY level and HbA1C in healthy controls

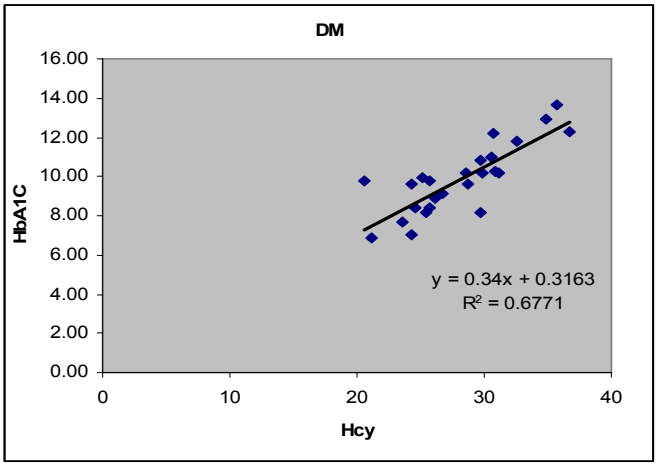

Figure 2. Correlation between HCY level and HbA_1C in diabetic patients

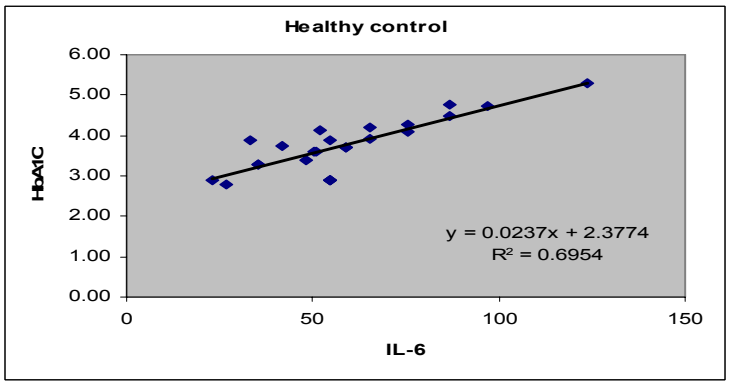

Figure 3. Correlation between IL6 level and HbA_1C in healthy control.

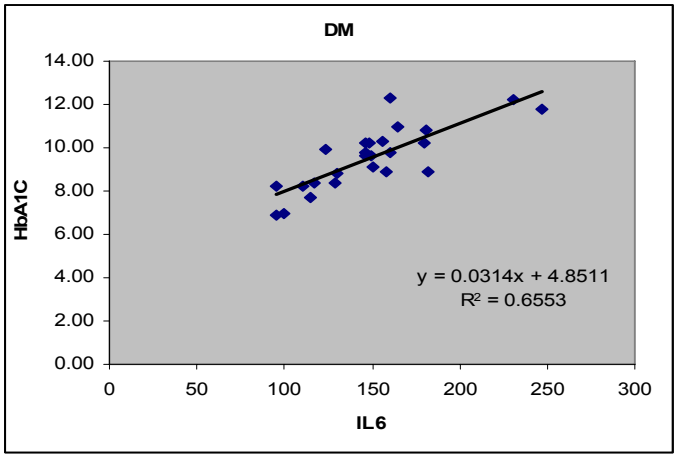

Figure 4. Correlation between IL6 and HbA_1C in diabetic patients

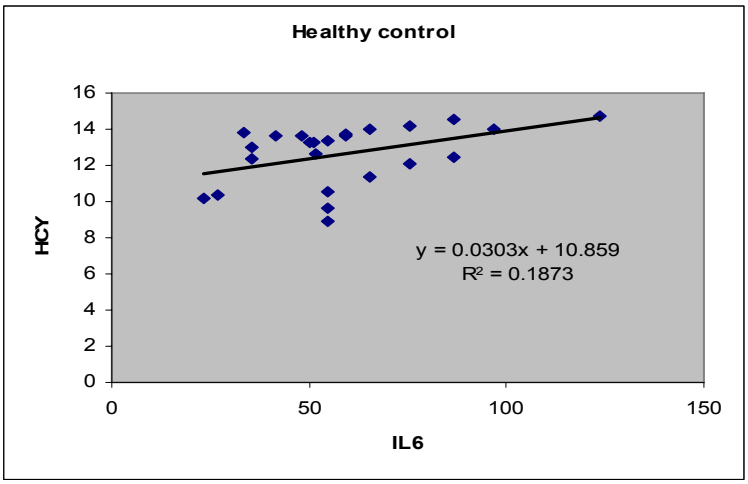

Figure 5. Correlation between IL6 level and HCY in healthy control.

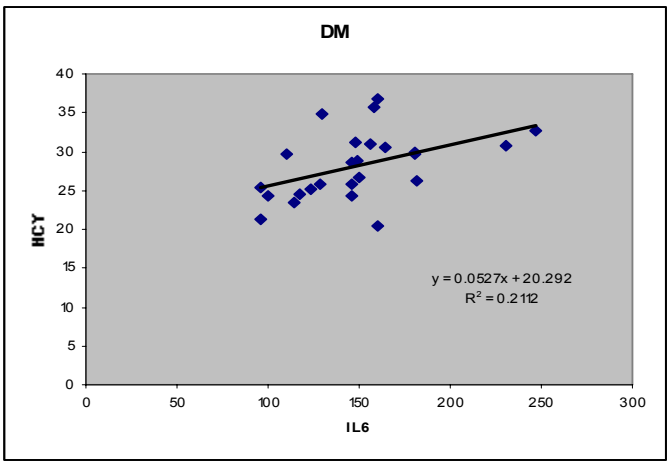

Figure 6. Correlation between IL6 level and HCY in diabetic patients.

Discussion

The number of patient with type 2 diabetes mellitus is growing world wide: the focus is how optimizing care in patients with permanent kidney failure. Elevated serum levels of creatinine and urea are pathogenetic of renal insufficiency. The creatinine levels is a more reliable parameter than urea level for identification of renal dysfunction, since the serum level of creatinine rises earlier than that of urea and the formation of creatinine is largely

independent of protein metabolism, in contrast to the formation of urea [12] and because creatinine has lower back diffusion from tubules lumen to poritubular blood [13]. This study revealed highly significant increase in urea and creatinine in all patients compared to control which represent a pathologic renal insufficiation. However, consensus on how type 2 diabetes affects plasma homocysteine concentrations has not been achieved.

Homocysteinemia has been established as a risk factor for cardiovascular disease and occurs with high prevalence in patients with type 2 diabetes: 31% of type 2 diabetic patients have homocysteine concentrations above >15 mmol/L [14]. Epidemiological research suggests an association between elevated total homocysteine (tHcy) levels and cardiovascular disease (CVD), which is the most common cause of mortality in patients with type 2 diabetes mellitus [15]. The association between homocysteinemia and atherosclerotic vascular disease is especially strong in patients with type 2 diabetes, compared to nondiabetic subjects. Increased plasma tHcy levels are reported to be associated with hypertension, hyperlipidemia, smoking, hyperuricemia, and impaired adrenal function [16]. Plasma tHcy concentration is strongly related to renal function. A study in rats identified the kidney as a major site for removal and metabolism of Hcy [17]. Two mechanisms appear to be involved. The main source of Hcy is adenosylmethionine-dependent methylation of guanidoacetate to form creatine and its anhydride creatinine. Second, renal function plays a central role for clearance of both creatinine and Hcy [18]. A large amount of evidence supports increased plasma tHcy levels in type 2 diabetes [19]. Buysschaert et al [20] reported increased plasma tHcy levels in type 2 diabetics with advanced nephropathy, compared to control subjects and diabetics without nephropathy. Lipid abnormalities are common in patients with renal disease, probably contributing to the high incidence of CVD in this population [21]. Patients with CRF, their mean serum abnormalities are type IV hyperlipidaemia (increase VLDL and TG) this results from delayed VLDL catabolism in peripheral tissues and impaired TG removal due to functional defect in lipoprotein lipase [22,23]. inspite of restoration of renal function there appear to be a change from predominant TG problem in dialysis to Cholesterol abnormality in the transplanted population. The prevalence of hyperlipidaemia in other reports had vaired from (16-78) %. The variation in the results depends on two major factors : first: the time between the transplantation date and lipid profile estimation, secondly : the method of estimation of serum lipid profile . Studies done in seventies were depends mainly on ultracentrifugation and lipoprotein electrophoresis while in ours we depend on kit enzymatic method [24].

Patients with PD exposed to large quantities of dextrose and tend to have rising TG, VLDL, and total Ch, where as HDL and LDL remain constant. This compound is concerned in the intracellular transport of fatty acids to the oxidative site in the mitochondria. During dialysis treatment plasma levels of carnitine fall. However, there is still controversy as to the contribution that any deficiency may make to the changes in plasma lipids seen in CRF patients, thought it is likely that the uraemic process plays a part in producing the altered lipids, nutrition also seems to be a factor since decreasing the calories derived from carbohydrate and

increasing the polyunsaturated to saturated fatty acid ratio to 1:1 has been reported to result in a lowering TG to normal value [25].

The study revealed a highly significant increased in lipid profile LDL. VLDL, Cholesterol and Triglyceride in patients of type 2 diabetes mellitus with renal disfunction and an increased in etherogenic index in those patient which give indication for increased risks of cardio vascular disease among them.

Liver is the target of adipose and muscle-derived IL-6, which has been shown to increase blood glucose through elevated hepatic glucose output and increased IL-6 level have been linked to inhibition of hepatic glycogen synthase, activation of glycogen phosphorylase, lipolysis and increased TG production, indeed IL-6 plays a role as a glucoregulatory hormone [26]. Preclinical studies indicate that interleukin 6 (IL-6) may interact with vitamin B-6 metabolism and compromise cystathionine ß-synthase activity, thereby rising plasma homocysteine concentrations [22]. Interestingly, high circulating concentrations of proinflammatory cytokines are associated with a high risk of medical conditions that have also been associated with hyperhomocysteinemia, such as acute ischemic stroke, myocardial infarction, and, more recently, osteoporosis [24-26]. Thus, it may be hypothesized that hyperhomocysteinemia and cardiovascular disease risk may be both mediated, in whole or in part, by a proinflammatory state.

In summary, the present study shows that plasma homocysteine concentrations are elevated in type 2 diabetic patients with renal disfunction, type 2 diabetic patients have higher plasma homocysteine levels than controls. Elevated plasma homocysteine concentrations in type 2 diabetic patients suggest an association between homocysteinemia and impaired renal function, as evidenced by increased serum creatinine and urea levels. These findings suggest that homocysteinemia may partly explain the link between diabetic nephropathy and cardiovascular complications of diabetes and the biochemical inflammation marker IL6 was elevated in serum of patient with type 2 diabetes with renal failure and this indicate the cell injury complication because of diabetes mellitus.

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