Interleukin-18 can predict pre-clinical atherosclerosis and poor glycemic control in type 2 diabetes mellitus

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Abstract

Background: Contradictory reports about the role of cytokines, particularly interleukins (IL) in atherosclerosis are found in the literature. Objectives: This study was aimed to investigate the association between the levels of cytokines notably IL-4, IL-12, IL-18 and, the atherogenicity and glycemic control in patients with type 2 diabetes mellitus. Materials and Methods: Seventy five patients with type 2 diabetes mellitus (25 males and 50 females) attending diabetic clinic during 1st August 2008 to 30th December 2009 as well as seventy healthy subjects (38 males and 32 female) were enrolled in the study. Fasting serum lipid profile and IL-4, IL-12 and IL-18 levels were determined. Results: The serum lipid profile of diabetic patients was significantly different from healthy subjects, favoring atherogenicity. IL 4, 12, and 18 were significantly higher in diabetic patients compared with healthy subjects. Significant association of high serum IL-18 with poor glycemic control \( (P < 0.001) \) assessed by HbA1c, long duration of diabetes and atherogenic index were observed. Conclusions: IL-18 can serve as a predictor for pre-clinical atherosclerosis and poor glycemic control in type 2 diabetes mellitus.

Keywords: Atherogenicity, HbA1c, IL-18, type 2 diabetes

Introduction

The role of interleukins (ILs) in atherogenesis remains controversial. Some studies have shown that certain ILs exert atherogenic effect under favorable circumstances; like IL-17 is considered pro-atherogenic and is reported to be involved in the development of atherosclerosis in the presence of high fat diet and accelerating the atherosclerosis following Chlamydia pneumoniae infection. [1] The high IL-6 level in type 1 diabetes mellitus is associated with atherogenic lipid profile and is reported to contribute to accelerated atherosclerosis in young; independent of adiposity and glycemic control. [2] IL-6 has also been reported to play a role in the development of atherosclerosis complications in patients with metabolic syndrome. [3] According to gene expression analysis of atherosclerotic tissue-samples obtained from live patients, hypertension interacted significantly with IL-18 genotype; affecting the risk of sudden cardiac death and coronary atherosclerosis. [4] Serum levels of tumor necrosis factor-alpha (TNF-\( \alpha \)) of > 6 pg/mL was found in patients with confirmed atherosclerosis and is considered as an independent risk factor of cardiovascular death and myocardial infarction. [5] On the other hand IL-10 is reported to exert anti-atherogenic effect. It mediates the uptake of cholesterol from modified lipoproteins and the efflux of stored cholesterol. [6] Goswami et al, demonstrated the role of TNF-\( \alpha \) and IL-10 during unstable atherosclerosis in patients with coronary artery disease. [7] On the other hand, no relationship was observed between the rate of decrease of serum IL-18 or the rate of increase of serum IL-10 and serum lipids levels after using lipid lower agent in patients with coronary artery syndrome in another study. [8]

IL-4 has important role in immune cell chemotaxis, formation of endothelial cell adhesion molecules and has numerous anti-inflammatory effects which prevent the complications of atherosclerosis, the primary cause of coronary heart
disease. In type 2 diabetes the frequency of invariant natural killer T cells expressing IL-18 did not significantly differ from that of healthy subjects.[10] IL-12 has been identified as a pro-inflammatory cytokine which is thought to contribute to the development of atherosclerosis. The serum level of IL-18 in type 2 diabetes mellitus is higher than corresponding healthy subjects or patients with coronary artery disease.[10] Keeping this background in mind, and the contradictory reports of association of levels of IL and type 2 diabetes, the present study was aimed to investigate the association between the levels of cytokines, notably IL-4, IL-12, IL-18, and the atherogenicity and the glycemic control in patients with type 2 diabetes mellitus.

Materials and Methods

This study was conducted in a center for diabetes mellitus in Erbil, Iraq during the period of 1st of August 2008 to 30th December 2009. Known cases of type 2 diabetes of both genders, referred to the diabetic center for clinical follow-up were enrolled in the study. The criteria of exclusion include history of familial hyperlipidemia, ischemic heart disease, chronic renal failure and patients on the lipid lowering agents. All the patients were on the oral hypoglycemic agents including glibenclamide and metformin and some of them during the period of illness had short course of insulin therapy. None of the patients admitted in the study received drugs that interfere with IL levels like monoclonal antibodies, immuno-modulators or immunosuppressive agents.

The study was approved by the Institutional scientific committee. An informed written consent was obtained from each participant prior to the study. Seventy five patients with history of type 2 diabetes and 70 healthy subjects serving as control were enrolled in the study. The body mass index (BMI) was (kg/m²) calculated according to the Quetlet’s equation: BMI = weight (kg) / height²(m). Fasting venous blood samples were obtained from participants and the sera were separated for determination of glucose, glycosylated hemoglobin (HbA1c) and lipid profile including serum total cholesterol (TC), triglycerides (TGs) and high density lipoprotein-cholesterol (HDL-C). Very low density lipoprotein-cholesterol (VLDL-C) was calculated as equal to 1/5 of the TGs level and the low density lipoprotein-cholesterol (LDL-C) was calculated according to the Friedewald equation [LDL=TC-(HDL+VLDL)]. The atherogenic index (AI) was calculated using the ratio of TGs and HDL-C (TGs/HDL-C) and it represented small dense LDL-C particles.

Cytokines including IL-4, IL-12 and IL-18 were determined in serum using enzyme linked immunosorbent assay (ELISA) using the quantitative sandwich enzyme immunoassay technique. Standards and samples were pipetted into the wells and any IL present was bound by the monoclonal antibody (specific for IL) pre-coated onto a microplate. A biotinylated polyclonal antibody specific for IL was added to the wells, followed by a wash to remove any unbound reagent, and then an enzyme complex was added to the well. Then after incubation and washing, a substrate solution was added to the wells. The intensity of the color was measured at λ 450nm.

Statistical analysis

The results were expressed as percentage and mean ± SD. The data were analyzed using two tailed unpaired student’s t test, difference between percentage test and simple correlation test, taking p ≤ 0.05 as the lowest limit of significance.

Results

Of the 75 diabetic patients, 50 were females and 25 males, compared to 38 males and 32 females in control group. Mean duration of diabetes was 8.21 ± 1.42 years. Diabetic patients were significantly over-weight and 8 of the 75 were obese (BMI > 30) compared to 2 obese of the 70 controls. The HbA1c levels of 8.64 ± 0.73 shows that diabetic patients were significantly in a state of poor glycemic control [Table 1].

The serum lipid profile in patients with type 2 diabetes differed significantly from the healthy subjects. The serum TC, TGs, LDL-C and VLDL-C levels were significantly higher and serum HDL-C levels were significantly less in type 2 diabetic patients compared to healthy subjects. Only eight female diabetic patients had a serum level of HDL-C < 40 mg/dL and no male diabetic patient had a serum level of HDL-C ≤ 35 mg/dL. The AI that represented small dense LDL-C particles was 4.28 ± 0.65 in diabetic patients compared to 2.53 ± 0.07 in healthy subjects (P < 0.001) [Table 2].

Serum levels of IL-4, IL-12 and IL-18 in diabetic patients were significantly higher than those of healthy subjects (P < 0.001) [Table 3]. There was significant correlation between the levels of IL-18 and the variables related to diabetes viz BMI (r=0.152, P < 0.01), duration of disease (r=0.406, P < 0.001), fasting serum glucose level (r=0.464, P < 0.001), HbA1c (r=0.562, P < 0.001) and atherogenic index (r=0.468, P < 0.001). No such correlation existed between IL-4 and IL-12 levels and BMI, duration of diabetes, fasting serum glucose level, HbA1c and atherogenic index [Table 4]. Thus, IL-18 shows significant correlation with atherogenicity as depicted by AI (TGs/HDL-C) and with poor glycemic control as depicted by HbA1c levels [Figure 1].

Discussion

The present study demonstrated that the higher IL-18 level is significantly associated with poor glycemic control
Interleukin 18 in type 2 diabetes mellitus (assessed by HbA$_1c$) and atherosclerosis (assessed by atherogenic index) in type 2 diabetes mellitus. Recent studies demonstrated the significant high level of IL-18 as pro-inflammatory marker in patients with hypercholesterolemia and any pharmacological intervention to reduce the atherogenic lipids were associated with decline in IL-18 levels.[8,11] Also serum IL-18 level were found to be increased in the stage 3 diabetic nephropathy presented with proteinuria.[12,13] Fujita et al reported that IL-18 has another effect on the glomeruli of diabetic patient with nephropathy, not related to its pro-inflammatory effect.[14] Moreover, IL-18 is not only a predictor of cardiovascular disease, but it improves the prediction of risk of all cause and non-cardiovascular mortality also.[15] Another study showed that elevated levels of IL-18 were associated with the presence of subclinical atherosclerosis evaluated with intima media thickness of the carotid artery.[16] IL-18 has been shown to be highly expressed in atherosclerotic plaques, mainly in plaque macrophages, and in particular in unstable plaques,[17] and the circulating IL-18 level is a useful biomarker for atherosclerosis prone patients with metabolic syndrome.[18]

The present study shows that significant increase in serum IL-18 levels is accompanied with significant abnormal and atherogenic lipid profile, and thus can be a predictor of pre-clinical atherosclerosis. This study adds another finding that the significant high level of IL-18 is associated with significant poor glycemic control. Though the difference between mean age of control and diabetic patients was significant in this study, but it is unlikely to attribute to the significant differences in the IL-18 levels observed, as there is no evidence that the IL-18 levels vary with age. This is the first report that highlights that the significant high level of IL-18 in type 2 diabetes mellitus presented with poor glycemic control and pre-clinical atherosclerosis in type 2 diabetic patients.
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