IL 18 in diabetic patients with and without coronary atherosclerosis

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Abstract: Objective: To assess interlukein 18 in diabetic patients with and without coronary atherosclerosis and to detect its relation to lipid profile and blood glucose. Also to investigate the hypothesis that the serum level of IL-18 is a predictor of coronary atherosclerosis in patients with type 2 diabetes. **Patients and Methods:** The study included 45 diabetic patients (15 with no complication and 30 with coronary atherosclerosis) and 15 age and sex matched as a control group. **Results:** Total cholesterol, HbA1c and IL 18 is significantly different in the 3 groups and the highest level is in the diabetic patients with atherosclerosis. On the contrary HDL-c is significantly lower in diabetic patients with atherosclerosis than the other 2 groups. IL8 shows a strong significant positive correlation with blood glucose in diabetic patients with no complication and in diabetics with atherosclerosis. **Conclusion:** that, IL8 is a useful inflammatory marker in diabetic patients and it is higher in those with atherosclerosis. IL-18 might serve as a marker of future cardiovascular risk in diabetic patients.
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1. Introduction

Inflammation plays a key role in atherosclerosis, whose complications have drawn the attention of researchers and health entities. IL-6 is a cytokine that acts both in the innate and in the adaptive immune response. It is synthesized by monocytes, endothelial cells, fibroblasts and other cells, in response to microorganisms and also to the stimulation by other cytokines, mainly interleukin-1 (IL-1) and TNF. IL- 18, a recently described cytokine from the IL-1 family, has a pleiotropic action, and participates in the innate as well as in the acquired immune response. Among its main functions is the stimulation of Interferon (INF) production by T lymphocytes, Natural Killer (NK) cells and macrophages (1).

Inflammatory mediators are intimately associated with the cascade of events leading to atherosclerotic plaque initiation, development, and rupture. This recognition has stimulated the evaluation of several markers of inflammation as a potential tools for cardiovascular risk prediction (2,3).

Atherosclerosis is the major cause of premature death in patients with type 2 DM, and nearly 80% of all deaths and 75% of all hospital admissions result from complications of atherosclerosis (4).

Interleukin (IL)-18, originally identified as an interferon inducing factor, stimulating interferon production in T lymphocytes and natural killer cells, which is believed to play a crucial role in atherosclerotic plaque rupture. Furthermore, IL-18 acts in synergy with IL-12 to promote the development of T helper 1 (TH1) responses (5, 6).

Interleukin (IL)-18 is a pleiotropic proinflammatory cytokine and plays a central role in the inflammatory cascade (7). Recently, evidence from experimental studies has shown that expression of IL-18 is intimately related to atherosclerotic plaque progression and vulnerability (8, 9). These results could be translated into the clinical setting, as shown in the AtheroGene Study, which suggested that the concentration of circulating IL-18 was one of the strongest predictors of future cardiovascular events in patients with stable and unstable Angina (10).

In this study, we are aiming to assess interlukein 18 in diabetic patients with and without coronary atherosclerosis and to detect its relation to lipid profile and blood glucose.

2. Materials and Methods

Patients:

The study included 45 diabetic patients, 15 with no complication (normal ECG, echocardiography and negative stress ECG.) and 30 with coronary atherosclerosis (admitted by ACS in the form of chest pain, ST depression and T wave changes and significant coronary artery lesion). 15 age and sex matched were included as a control group.

Methods:

The study was done after obtaining approval from the local institutional review board and human

subject's protection. Written informed consent was obtained from patients.

All patients were subjected to the following: Full history taking including chest pain and presence of risk factors of IHD, physical examination, ECG, Echocardiography and coronary angiography.

Venous blood samples (10 ml) were drawn in the morning after an overnight fasting from each subject. The venous blood sample was divided into three test tubes. 1 ml was added to a mixture of potassium oxalate and sodium fluoride (for plasma glucose estimation by oxidase/peroxidase kit (11), 2 ml was added to EDTA powder (whole blood to estimate HbA1c by cationic exchange resin(12) and the remaining 7 ml were allowed to clot at room temperature then centrifuged at 1000 rpm for 15 minutes. Serum was separated and divided into aliquots then frozen at -70 ^{0}C till the time of assay. The serum samples were used to estimate the Interleukin 18: by a solid phase enzyme linked immunosorbent assay (ELISA) Kit (13).

Plasma concentrations of cholesterol and triglycerides, LDL and HDL were determined by quantitative colorimetric kit.

Statistical Methods:

Statistical Package for social science (SPSS) version 9.0 was used for analysis of data (Chicago,Illinois, USA). One way ANOVA was used for comparison of the 3 groups followed by post hocc test (LSD) for detection of significance. Pearson's correlation was also done.

3. Results:

The study included 45 diabetic patients, their mean age were 65.3 ± 3.3 yrs (60 – 72 yrs). Fifteen diabetic patients with no complication ,their mean age was 64.5 ± 2.6 yrs (61.0 – 70.0 yrs), 30 diabetic patients with coronary atherosclerosis, their mean age was 65.8 ± 3.6 yrs (60 – 72 yrs) and 15 age and sex matched as a control group, their mean age was 64.2 ± 3.1 yrs(55 – 68 yrs).

Table 1 : Comparison between laboratory data of diabetic patients and controls included in the study

Variables	Diabetic Patients Mean ± SD N = 45	Controls Mean ± SD N = 15	P –value
Fasting blood glucose (mg \ dl)	226.6 ± 64.5 (115.0 – 308.0)	89.7 ± 9.4 (70.0 – 105.0)	0.0001*
Total cholesterol (mg\dl)	234.2 ± 31.7 (178.0 – 296.0)	185.6 ± 9.8 (166.0 – 200.0)	0.0001*
Triglyceride (mg\dl)	178.1 ± 21.7 (122.0 – 230.0)	169.6 ± 4.9 (159.0 – 177.0)	0.02*
HDL-c (mg\dl)	39.0 ± 6.7 (28.0 – 58.0)	47.4 ± 5.4 (40.0 – 58.0)	0.0001*
LDL –c (mg\dl)	155.0 ± 20.9 (120.0 – 199.0)	148.1 ± 7.3 (136.0 – 159.0)	0.1
IL18	141.2 ± 34.6 (80.6 – 194.2)	69.2 ± 7.8 (55.6 – 80.5)	0.0001*

Table 2: Correlation between IL 18 of diabetic patients with other laboratory data

Variables	Diabetic patients	
	R	P- value
Fasting blood glucose (mg \ dl)	0.9	0.0001*
HbA1(%)	0.6	0.0001*
Total cholesterol (mg\dl)	0.7	0.0001*
Triglyceride (mg\dl)	0.1	0.7
HDL-c (mg\dl)	- 0.6	0.0001*
LDL –c (mg\dl)	- 0.2	0.3

Table 3 : Comparison between laboratory data of diabetic patients (with no complication and with coronary atherosclerosis) and controls included in the study

Variables	Diabetics with no complication Mean ± SD (Range) N =15	Diabetics with atherosclerosis Mean ± SD (Range) N = 30	Controls Mean ± SD (Range) N = 15	P -value
Fasting blood glucose (mg \ dl)	142.9 ± 17.5[a] (115.0 – 164.0)	268.5 ± 27.1[b] (222.0 – 308.0)	89.7 ± 9.4[c] (70.0 – 105.0)	0.0001*
Total cholesterol (mg\dl)	199.9 ± 12.7[a] (178.0 – 220.0)	251.4 ± 27.1[b] (220.0 – 296.0)	185.6 ± 9.8[c] (166.0 – 200.0)	0.0001*
Triglyceride (mg\dl)	177.3 ± 9.9 (166.0 – 198.0)	178.5 ± 25.9 (122.0 – 230.0)	169.6 ± 4.9 (159.0 – 177.0)	0.3
HDL-c (mg\dl)	45.4 ± 6,2[a] (36.0 – 58.0)	35.8 ± 4.3[b] (28.0 – 42.0)	47.4 ± 5.4[a] (40.0 – 58.0)	0.001*
LDL –c (mg\dl)	148.1 ± 7.3 (136.0 – 159.0)	158.5 ± 24.5 (120.0 – 199.0)	148.1 ± 7.3 (136.0 – 159.0)	0.09
IL18	100.4 ± 11.4[a] (80.6 – 115.5)	161.7 ± 21.6[b] (125.5 – 194.2)	69.2 ± 7.8[c] (55.6 – 80.5)	0.0001*
HbA1 (%)	8.8 ± 0.9[a] (7.4 – 10.2)	10.6 ± 1.2[b] (8.5 – 12.8)	5.4 ± 0.5[c] (4.4 – 6.2)	0.0001*

Different symbol indicate significance.

Table 4 : Correlation between IL 18 of diabetics (with no complication and with coronary atherosclerosis) and controls included in the study with other laboratory data

Variables	Diabetics with no complication		Diabetics with atherosclerosis		Controls	
	R	P- value	R	P- value	R	P- value
Fasting blood glucose (mg\dl)	0.9	0.0001*	0.8	0.0001*	- 0.02	0.9
Total cholesterol (mg\dl)	0.2	0.4	0.05	0.8	0.5	0.07
Triglyceride (mg\dl)	0.4	0.2	0.03	0.9	0.2	0.4
HDL-c (mg\dl)	- 0.01	1.0	0.1	0.8	0.01	1.0
LDL –c (mg\dl)	- 0.3	0.3	0.1	0.6	0.5	0.07
HbA1 (%)	- 0.1	0.7	0.1	0.5	- 0.1	0.8

4. Discussion:

The prevalence of diabetes worldwide is increasing rapidly in association with the increase in obesity. Complications are a major fear of patients with diabetes. There is increasing evidence that an ongoing cytokine-induced inflammatory response is related closely to the pathogenesis of type 2 DM and the associated complications such as retinopathy, nephropathy, neuropathy, cardiovascular diseases, and peripheral vascular diseases (14).

The present study aimed to assess interleukin 18 in diabetic patients with and without coronary atherosclerosis and to detect its relation to lipid profile and blood glucose.

Our study showed that the mean serum level of Fasting blood glucose, HbA1c, total cholesterol, TG and IL8 is significantly higher, while HDL-c is significantly lower in diabetic patients than that of the control group (table 1). Also, Fasting blood glucose, HbA1c and total cholesterol have a significant positive correlation, while HDL-c has a significantly negative correlation with IL18 (table 2).

In accordance to these results Esposito et al. (15) revealed that circulating levels of IL-18 concentrations were higher in type 2 diabetic patients than in control subjects. Also, they concluded that there was a significant positive correlation between fasting glucose and IL-18 levels in the diabetic patients. Also, a study done by Fischer et al. (16) demonstrated a higher plasma level of IL-18 in type 2 diabetic patients compared to control subjects.

Additionally, Hivert et al. (17) reported an elevated IL-18 levels that are associated with higher risk of diabetes. This association was independent of usual risk factors.

Atherosclerosis is an inflammatory disease and serum levels of inflammatory markers such as interleukin 18 are used to evaluate patients with coronary artery disease. By sharing classic risk factors with atherosclerosis, type 2 DM may also share a common etiologic pathway which can be the inflammatory pathway. In patients with type 2 diabetes, atherosclerosis is related to a larger number of events such as myocardial infarction and death, when compared with patients without diabetes (1).

In the current study, total cholesterol, HbA1c and IL8 is significantly different in the 3 groups and the highest level in the diabetic patients with atherosclerosis. On the contrary HDL-c is significantly lower in diabetic patients with atherosclerosis than the other 2 groups (table 3). IL8 shows a strong significant positive correlation with fasting blood glucose in diabetic patients with no complication and diabetics with atherosclerosis. No significant correlation was found between IL18 and total cholesterol, triglyceride, HDL or LDL (table 4).

Consistent with these results Suchanek et al. (18) concluded that the type 2 DM patients with coronary artery disease (CAD) had a higher concentration of IL-18 compared to the non diabetic CAD group. Also, the diabetic patients with triple vessel disease were characterized by a higher concentration of IL-18 than the non diabetic patients with the same grade of CAD. Therefore, these results may help to explain the vulnerability of those patients to fatal secondary cardiovascular events.

Additionally, Aso et al. (19) reported an elevated plasma concentrations of IL-18 that might be associated with acceleration of atherosclerosis in type 2 diabetic patients. Furthermore, numbers of carotid plaques were higher in diabetic patients with high IL-18 than in those with normal IL-18. Also, they found a significant positive correlation between plasma IL-18 and FPG in those patients.

IL-18 is thought to exert its main proatherogenic effects by inducing IFN-γ production, which potentiates the inflammatory process and may lead to thinning or inhibition of the fibrous cap formation, resulting in vulnerable, rupture-prone plaques. In addition, IL-18 seems to increase the expression of matrix metalloproteinases in vascular cells and macrophages, which might also contribute to plaque destabilization (20).

The distribution of several cardiovascular risk factors in the Irish PRIME population was different from the French cohort and thus might account, at least in part, for the excess risk of coronary heart disease (CHD) seen in Belfast. Indeed, several studies demonstrated that the Belfast participants, as compared with the French, had much higher levels of triglycerides and LDL-C and significantly lower concentrations of HDL-C (21).

In the present study, IL8 shows a strong significant positive correlation with blood glucose in diabetic patients with no complication and diabetics with atherosclerosis. This coincide with Thorand et al (22) and Koenig et al (23) who reported that IL-18 is strongly and independently predicted incident type 2 diabetes mellitus over 10.8 years.

The plasma IL-18 concentration was associated with a range of traditional cardiovascular risk factors such as BMI, LDL- and HDL-cholesterol, triglyceride, insulin and proinsulin (24).Other study revealed that different features of the metabolic syndrome such as BMI, triglycerides, HDL-cholesterol, blood pressure and insulin are related to IL-18 levels (24).

Other studies have also investigated the relationship between IL-18 and atherosclerosis. For example, over expression of IL-18 was found in carotid plaques retrieved from symptomatic patients at endarterectomy, as compared to carotid Plaques from non-symptomatic patients (25).

Furthermore, in Japanese patients with type-2 diabetes it was shown that greater intima-media thickness, as measured by ultrasound, was associated with high IL-18 levels (19). So far, however, the relationship between the plasma IL-18 concentration and coronary atherosclerosis has not been investigated.

5. Conclusion:

IL8 is a useful inflammatory marker in diabetic patients and it is higher in those with complication as atherosclerosis.

IL-18 might serve as a marker of future cardiovascular events in human with manifest CHD and/or in areas of high absolute risk of CHD. Thus, further studies are warranted to establish the role of IL-18 in the prediction of CHD risk in diabetic patients.

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