Neopterin, interleukin-6, and non HDL-C as predictors for cardiac disease among type 2 diabetic women with and without renal complications

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Objective To estimate serum concentration of pro-inflammatory marker interleukin-6 (IL-6) and cell-mediated immunity marker neopterin and their relation with non-HDL-C as predictors for cardiovascular risks in type 2 diabetes mellitus patients with and without renal complications. To estimate the serum levels of IL-6 and neopterin in patients groups and compare their levels with healthy control group and study their correlations with other biochemical parameters.

Methods The circulating neopterin and IL-6 levels were measured in 45 type 2 diabetic women with renal failure (ESRD), 50 women in T2DM without renal failure and 55 healthy control women without a known family history of diabetes with matched age and body mass index (age range 46–70 years). They were attending at Al-Kadhmia Teaching Hospital in Baghdad during the period from March to September 2014.

The BIOSOURCE ELISA kit was used to measure S. IL-6 and neopterin, and enzymatic methods are used for blood sugar, urea, creatinine, and lipid profile measurements.

Results A significant increase was found in serum levels of IL-6 and neopterin between the two diabetic groups when compared with healthy control group ($P < 0.05$). IL-6 levels were positively correlated with neopterin, triglyceride and non-HDL-C ($P < 0.05$), and serum neopterin was correlated significantly with non-HDL-C, eGFR ($P < 0.001$) levels in ESRD and type 2 diabetic women.

Conclusion Serum neopterin, IL-6 and non-HDL-C levels directly contribute to the development of cardiac risk factors in type 2 diabetic women with renal failure complication.

Keywords neopterin, IL-6, non-HDL-C, T2DM, ESRD

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] Neopterin is a 2-amino-4-hydroxy-(1,2,3-trihydroxypropyl)-pteridine with a low molecular mass (253 Da), it is produced by activated monocytes/macrophages in humans from guanosine triphosphate (GTP) via GTP cyclo hydrolase I. The activity of this enzyme is greatly increased by interferon-γ (INF-γ) and, to a lesser degree, by interferon-α (INF-α), endotoxins and other cytokines.[3] Neopterin has been associated with both immune activation and coronary artery disease (CAD) activity. It enhances inflammatory processes and, together with the pro-inflammatory cytokine TNF-α and IL-6 inducible for nitric oxide synthesis (iNOS) by stimulating gene transcription, which cause NO- free radicals and cytotoxic production.[4] An increase in active T cells number and higher levels of serum neopterin have been noted in metabolic syndrome, suggesting chronic stimulation of immune system.

Studies show a strong association of neopterin with cardiovascular disease.[5,6] Study by Sasaki et al. showed relation between high neopterin concentration and increased the rate of cardiac events.[7] The concentration of neopterin in cross-sectional study correlated significantly with the extent of peripheral vascular and coronary disease.[8] Non-HDL-C is the whole cholesterol in all lipoprotein fractions, except HDL.[9] The role of elevated TG levels in the calculation of LDL-C with the Friedewald formula propose that non-HDL-C is important marker in determining the risk of atherosclerosis and cardiovascular disease in patients with hyperlipidemia.[10] Safari and the Copenhagen City Heart Study proposed that non-HDL-C correlated with apo lipoprotein B100 better than LDL-C and its diagnostic value as a risk factor is the same or as high as apoB.[11,12] In a meta-analysis of lipid-lowering therapies observed a 1:1 correlation between the 1% non-HDL-C lowering and coronary heart disease risk reduced by lipid-modifying drugs.[13] The lowering of non-HDL-C is an important aim of protection and treatment of cardiovascular diseases.

This study was therefore undertaken to compare serum concentrations of neopterin in persons with type 2 diabetes with those in age-matched control subjects and to investigate whether serum neopterin is associated with IL-6 concentration, in persons with type 2 diabetes with renal failure complication, and their relations with lipid profile and increase the risk of cardiac complications.

Subjects and Methods

A total of 150 postmenopausal women with age ranged between (46–70 years) were enrolled in this study. Fifty of them with type 2 diabetes mellitus, 45 with end stage renal failure and 55 with matched age apparently healthy control group. They were conducted at Al-Kadhmia Teaching Hospital during the period from March to September 2014.

Methods

Nine milliliters (9 ml) of fasting venous blood was undertaken from controls and patients groups. The sample was

transferred to gel tube and was allowed to clot at room temperature for 20 min, and then centrifuged at 3000 rpm for 15 min. The serum was separated into aliquots in eppendorf tubes for immediately fasting blood sugar, urea, creatinine, lipid profile measured by colorimetric methods and the rest stored at −20°C to be used later for interleukin-6 (IL-6) and neopterin determination by Enzyme-Linked Immune Sorbent Assay (ELISA). Body mass index was calculated by dividing weight (kg) to the square of height (m^2). Glomerular filtration rate was calculated by CKD EPI Calculator – four variables using standardized serum creatinine, age, race, gender. MDRD CKD EPI equation with SI units. The concentration of non-HDL-C is calculated by subtraction of HDL-C from total cholesterol.

Statistical Analysis

Data were analyzed using SPSS. Continuous data were expressed as mean ± SD and difference of mean of groups *P* value derived from ANOVA test. Level of significance was set at 0.05.

Results

The results of this study showed significant increase in serum level of IL-6, neopterin, non HDL-C, total cholesterol and LDL-C in type 2 diabetic women when compared with healthy control group ($P < 0.01$) and significantly decrease in HDL-C Levels ($P < 0.01$) (Table 1). As well as the levels of IL-6 and neopterin showed highly significant increase in T2DM with ESRD when compared with healthy control group ($P < 0.0001$). TC, triglyceride and non-HDL-C was highly significant increase in T2DM with ESRD when compared with healthy control group ($P < 0.001$). IL-6, neopterin and non-HDL-C showed highly significant increase in their levels in T2DM with ESRD when compared with T2DM without renal complications ($P < 0.0001$). These findings predict that the levels of these inflammatory markers and the level of non-HDL-C considered as risk factors for cardiac disease in diabetic women with ESRD. IL-6 levels positively correlated with neopterin ($r = 0.68$, $P = 0.008$) in women with T2DM without renal complications as shown in Fig. 1, and it is correlated positively with non-HDL-C ($r = 0.28$, $P = 0.033$) in T2DM with ESRD Fig. 2. Serum neopterin was negatively correlated with eGFR and non-HDL-C ($r = −0.314$, $P = 0.016$) and ($r = 0.275$, $P = 0.037$), respectively, in women with ESRD and type 2 diabetes mellitus Figs. 3 and 4, respectively.

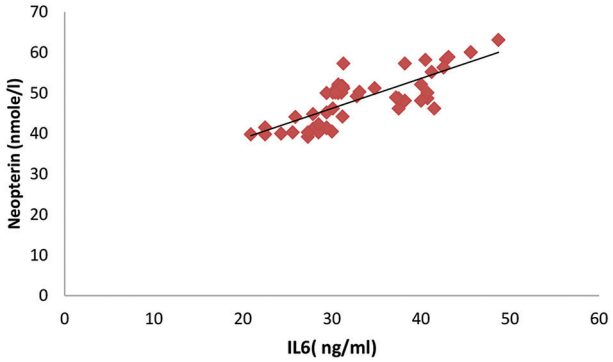

Fig. 1 **The correlation between interleukin 6 and neopterin in type 2 diabetic women ($r = 0.68$, $P = 0.008$).**

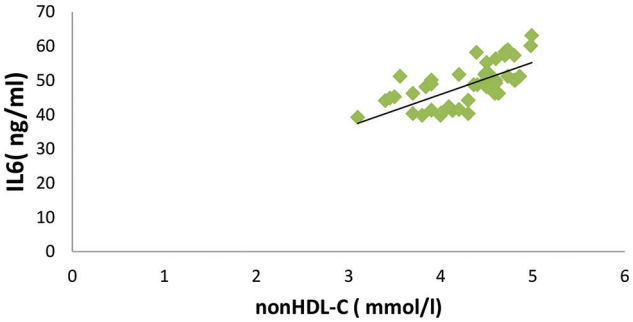

Fig. 2 **Correlation between serum IL-6 and non-HDL-C in type 2 diabetes mellitus with ESRD ($r = 0.28$, $P = 0.033$).**

Table 1. **Inflammatory and biochemical markers in all studied groups**

Parameters	T2DM (mean ± SD) ($n = 50$)	T2DM with ESRD (mean ± SD) ($n = 45$)	Control (mean ± SD) ($n = 55$)
IL-6	53.03 ± 10.81[a,b*a]	137.3 ± 14.35[a,b**]	17.37 ± 4.21
Neopterin	30.73 ± 12.3 *	89.4 ± 9.3[a,b**]	15.46 ± 1.59
Glucose	7.23 ± 1.2 **	8.12 ± 1.84**	4.45 ± 1.53
TG	2.6 ± 0.52[b*]	2.02 ± 0.43 [b*]	1.33 ± 0.32
TC	5.18 ± 0.93	6.24 ± 0.51	4.03 ± 0.54
HDL –C	0.88 ± 0.34	0.81 ± 0.16 [b**]	1.38 ± 0.11
LDL –C	3.41 ± 0.81	4.01 ± 0.24 [a,b**]	2.41 ± 0.53
Non HDL-C	4.19 ± 0.57 [a,b**]	5.43 ± 0.19 [a,b**]	3.04 ± 0.33
Urea	7.13 ± 2.04	30.03 ± 11.74 [a,b**]	5.14 ± 0.44
Creatinine	65.94 ± 19.81	416.36 ± 101.26 [a,b**]	62.59 ± 9.86

The concentration of all parameters in mmol/l, except for IL-6 (ng/ml), neopterin (nmol/l), and creatinine (μ mol/l). *P* value derived from ANOVA test. Significant, *$P < 0.05$; Highly significant, **$P < 0.001$; No significant, $P > 0.05$; T2DM, type 2 diabetes mellitus; ESRD, end stage renal disease; IL-6, interleukin-6; SD, standard deviation; TG, triglycerides; TC, total cholesterol; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; [a]ANOVA test, diabetic vs diabetic with ESRD; [b]ANOVA test, diabetic or diabetic with ESRD vs control.

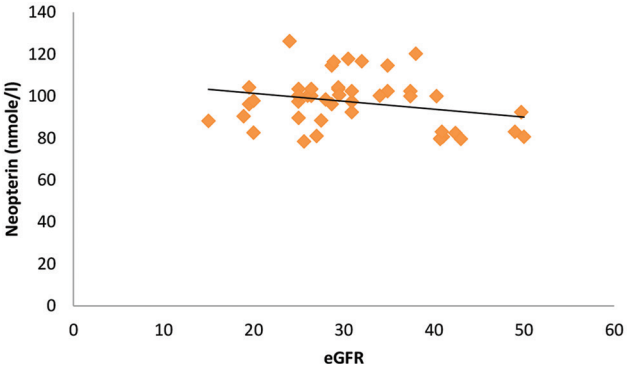

Fig. 3 **Correlation between eGFR and neopterin in type 2 diabetes mellitus with ESRD ($r = -0.314$, $P = 0.016$).**

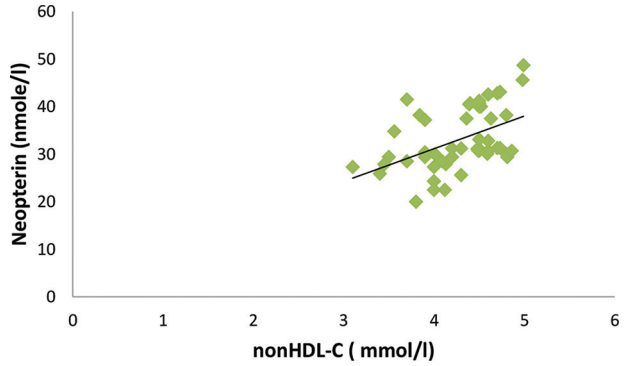

Fig. 4 **Correlation between serum non-HDL-C and neopterin in type 2 diabetes mellitus with ESRD ($r = 0.275$, $P = 0.037$).**

Discussion

This study presented that women with T2DM display an increase in serum neopterin concentration when compared with healthy women, and this significant elevation is correlated with the rising circulating levels of inflammation marker IL-6. Neopterin concentration shows continuous elevation with lowering eGFR in diabetic women with ESRD. Previous reports were consistent with the findings of inverse association of renal function with serum neopterin and a stepwise increase in serum neopterin with increasing stages of chronic kidney disease.[14,15] As well as the present results showed an increase in neopterin levels in T2DM with ESRD two fold as compared to T2DM without renal complication.

The cardiovascular morbidity and mortality has been related to the activation of inflammatory in renal disease.[14,16,17] Activated T cell release γ-interferon that causes mononuclear macrophage excitation and neopterin synthases. Hence, neopterin concentration reflects directly the activation of macrophages, cellular immunity and T lympho cells.[18] Further to the link between neopterin and immunity, the cell-mediated immunity marker 'neopterin' can easily enter to the blood stream and its level can be determined in peripheral blood because of its low molecular weight and chemical stability. So it can be used as a reliable and high-sensitive marker to evaluate the degree of lesions in coronary artery disease in published literature.[19] The positive correlation between IL-6 and serum neopterin in T2DM propose a relation with the total inflammatory events. This study also demonstrated a twofold increase in neopterin levels in T2DM with ESRD as compared to those with T2DM without renal complication, so that it was used as a diagnostic marker in T2DM with ESRD and as predictor for cardiac disease.

Studies had shown that the association between chronic inflammation and ESRD, which proceed to cardiovascular disease, and atherosclerotic disease had a magnificent advantage in comprehension with the mechanism of this association.[15,20] The reasonable explanation of such relationship is that the cause of inflammation activated T cells formation, and this cell led to elevated plasma neopterin that played a beneficial role in CVD mediation.[20] In conclusion, the recent study shows that the higher increase in plasma neopterin levels was related to more impaired in renal function.[21]

The presence of a significant positive correlation among IL-6, neopterin and non-HDL-C in T2DM women with ESRD group is the interest points of this study (Figs. 2 and 4).

Liu et al. match the diagnostic role of non-HDL-C as a prediction marker for coronary disease 'myocardial infarction and acute coronary events' among healthy subjects and diabetics. It founded that the increasing 1 mg/dl in the non-HDL-C level led to increase the cardiovascular mortality by 5% and looks to be a good predictive indicator than other lipid risk factors. Significantly higher concentrations of non-HDL-C and higher relative risk of coronary events among patients with diabetes were observed, and the risk in particular grade levels of non-HDL-C was more than one half to two half times higher in diabetes mellitus patients than in healthy control subjects.[22]

This study concluded that marked difference in the neopterin and IL-6 levels between T2DM without renal complications and T2DM with renal complications. This may be due to the abnormality in clearance pathway of both neopterin and IL-6 or due to the differences in the stage of the inflammations between these two types of patient groups the inflammation increase with increasing the severity of renal disease, or related to or mixing of both and study the correlation of these marker with non-HDL-C as predictors for CVD.

Conflict of Interest

None.

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