The role of Serum Interleukine-6 level in type 2 diabetic nephropathy

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Abstract
Albuminuria is considered the gold standard for the detection of diabetic nephropathy (DN). Nevertheless, there are still discordances of albuminuria and progression of kidney function attenuation, raising the question of finding alternative indicators. This study aimed to evaluate the correlation of serum IL-6 levels with diabetes-related variables in type 2 diabetic (DM2) patients and the role of serum-IL-6 in the detection of DN in DM2. The cross-sectional study was done on 59 DM2 patients including a DN group (n = 30) and a non DN group (n=29). Serum IL-6 level was measured by electrochemiluminescent immunoassay. There was a significant and positive correlation between serum IL-6 and BMI, TC, LDL and ACR in DM2. A ROC-curve analysis showed an AUC_{IL-6} of 0.69 and an optimal cutoff value of 10.5 pg/mL was set with sensitivity of 79.3% and specificity of 56.7%.

In conclusions, serum IL6 levels significantly correlated with a number of diabetes-related variables in DM2 and can be used to differentiate type 2 diabetes with and without DN at an optimal cutoff value of 10.5 pg/mL with sensitivity of 79.3% and specificity of 56.7%.

Keywords: Albuminuria, diabetic nephropathy, type 2 diabetes, serum IL-6.

Introduction
Diabetic nephropathy (DN) which is characterized by progressive albuminuria and a decline in glomerular filtration rate is one of the major complications of diabetes. Nearly 40% of diabetic patients develop DN leading to chronic kidney disease (CKD).[7] Early diagnosis and interventions are vital in prevention and treatment of DN. So far, albuminuria or urinary albumin-to-creatinine ratio (ACR) is often the first indicator for early DN detection. However, a number of studies have revealed limitations of albuminuria such as the discordance of the ACR with CKD stages in some patients.[6]

Therefore, it is necessary to find alternative biomarkers to effectively detect DN at the onset for better prevention of CKD progression. A variety of markers including tubular damage indicators and inflammatory markers have been studied.[1]

Inflammation has been long known to play an important role in the development and the progression of type 2 diabetes and its complications. Interleukine-6 (IL-6), an inflammatory cytokine which participates in the onset and acceleration of the chronic inflammation process might contribute to the development of micro- and macro-vascular complications in diabetic patients.[10] Elevated IL-6 concentration was found associated with diabetes-related variables which could accelerate progression of microvascular complications in type 1 diabetic patients.[9]

The aims of this cross-sectional descriptive study are to evaluate the correlation of serum IL-6 levels with diabetes-related variables in type 2 diabetic patients and the role of serum-IL-6 in the detection of DN in type 2 diabetic patients.

Material and Methods
Patients: Type 2 diabetic patients of 20 - 80 years old admitted into the 108 Military Hospital (Hanoi, Vietnam) from July 2016 to December 2016 were enrolled in the study. The diabetes diagnosis was based on the American Diabetes Association criteria. Diabetic kidney disease was detected using urinary albumin-to-creatinine ratio (ACR). The exclusion criteria included severe and acute manifestations such as coma, ketoacidosis, uncontrolled hypertension, severe infections in the last 3 months, current acute infections, pregnant women, patients with liver diseases, patients with end-stage renal disease, unwillingness to participate in the study.

59 type 2 diabetic patients were enrolled in the study. All of them provided their written consent. The study complied to the Declaration of Helsinki and was approved by the Ethics Committee of the 108 Hospital with approval number 108-2016-017.

The type 2 diabetic patients were divided into two subgroups based on their renal status. A group of DM2 without DN (n=30) included the normoalbuminuria subjects with ACR of < 30 mg/g creatinine, a group of DM2 with DN (n=29) included those patients with ACR $\geq$ 30 mg/g.

Data collection: The serum levels of creatinine, triglycerides (TG), total cholesterol (TC), HDL cholesterol (HDL) and fasting plasma glucose (FPG) were determined using the commercially and fasting plasma glucose (FPG) using the commercially available assay kits (Beckman Coulter). The serum IL-6 level was measured by electrochemiluminescent immunoassay using the COBAS E601 system and Elecsys IL-6 assay kit (Roche). The

glomerular filtration rate (GFR) was estimated using the CKD EPI equations. An early morning urine sample of each subject was used for ACR measurement.

Statistical analysis: Characteristics of patients and clinical variables were summarized using descriptive statistics. The Kruskal-Wallis analysis was performed to compare continuous variables and the Chi-square test for categorical variables. Correlations between IL-6 and several diabetes-relating clinical and biochemical parameters were evaluated by Spearman's analysis. The clinical benefits of using IL-6 were assessed with receiver operating characteristic (ROC) curve analyses. A two-tail p value of less than 0.05 was considered significant. All data analysis was conducted using R software version 3.5.1.

Results and Discussion

The general characteristics and laboratory data of the study subjects are demonstrated in table 1. There was no difference in gender and age among the subjects. There was a significant increase of BMI, HbA1c, TG, ACR and IL6 from the non DN group to the DN group. Meanwhile, the eGFR decreased in the DM2 patients with DN (p=0.045).

The activation of inflammation pathways is very important to the progression of type 2 diabetes as well as its complications.[4] A number of acute-phase proteins and pro-inflammatory cytokines have been implicated in the development of diabetic kidney disease.[10] In a study by Wegner et al on type 1 diabetic patients, serum IL-6 level was positively correlated with FPG, LDL, TC levels and BMI. These correlations were stronger in the subgroup of patients with microvascular complications[9]. In order to reveal the role of IL-6 in the type 2 diabetes, a correlation analysis was done showing strong and positive correlations between the serum IL-6 level and BMI (r = 0.455, p<0.001), TC (r = 0.306, p=0.018), LDL (p = 0.308, p = 0.018) and ACR (r = 0.273, p = 0.037) (Table 2).

The positive correlation of IL-6 with BMI was consistent to the lipogenic upregulation effect of IL-6 in mice as reported by Vida et al[8]. Critical metabolic changes such as dyslipidemia, hyperaminoacidemia and hyperglycemia alter kidney hemodynamics, fibrosis and promote inflammation (reflected by IL-6 increase) in early diabetes.[2] The glomerular basement membrane (GBM) width which strongly correlated with IL-6 level also increased from normoalbuminuric to proteinuric patients.[3] Meanwhile, as reviewed by MacIsaac et al[5], diabetic patients with persistent microalbuminuria still progress to CKD of stages 3–5 and an inappropriate emphasis may be placed on the presence or absence of albuminuria or changes in albuminuria within the microalbuminuric range.

The correlation of IL-6 and ACR suggested the ability of using it for detection of DN in type 2 diabetic patients. Therefore, a ROC analysis was done among the DM2 patients with or without DN showing the ROC-AUC of 0.69 and an optimal cutoff value of 10.5 pg/mL IL-6 was set with sensitivity of 79.3% and specificity of 56.7% (Figure 1).

Table 1
Characteristics of study subjects

Variables	Type 2 diabetic patient groups		*P*
	Without DN (n=30)	**With DN (n=29)**	
Gender			0.678
Males	16	18	
Females	14	11	
Age (years)	65.00 (11.5)	64.00 (15)	0.493
BMI (kg/m^2)	22.67 (1.24)	23.51 (2.28)	**0.017**
HbA1c (%)	6.75 (1.40)	7.20 (1.80)	**0.024**
FPG (mmol/L)	6.90 (1.25)	7.10 (4.5)	0.108
TC (mmol/L)	5.27 (1.54)	4.97 (2.09)	0.649
TG (mmol/L)	1.69 (1.07)	2.68 (1.66)	**0.026**
HDL (mmol/L)	1.13 (0.29)	1.09 (0.60)	0.367
LDL (mmol/L)	3.10 (1.15)	2.60 (1.70)	0.249
IL-6 (pg/mL)	10.03 (10.44)	18.80 (22.96)	**0.013**
eGFR (ml/min/1,73m^2)	88.50 (13.75)	78.00 (35.00)	**0.045**
ACR (mg/g)	10.01 (50.00)	80.09 (11.01)	**0.000**

BMI: Body Mass Index; FPG: Fasting plasma glucose; TC: Total cholesterol; TG, Triglycerides; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; IL-6: Interleukine 6; eGFR: estimated Glomerular Filtration Rate; ACR: albumin-to-creatinine ratio; DN: diabetic nephropathy.

Categorical data are presented as numbers and continuous data are presented as median (interquartile range)

Table 2
The correlations between serum IL-6 level and several diabetes-relating clinical and biochemical parameters in type 2 diabetic patients

Variables	r	*P*
Age (years)	0.252	0.054
BMI (kg/m^2)	0.455	**0.000**
HbA1c (%)	0.044	0.739
FPG (mmol/L)	0.047	0.721
TC (mmol/L)	0.306	**0.018**
TG (mmol/L)	0.032	0.812
HDL (mmol/L)	0.076	0.568
LDL (mmol/L)	0.308	**0.018**
ACR (mg/g)	0.273	**0.037**
eGFR (ml/min/1,73m^2)	-0.209	0.114

BMI: Body Mass Index; FPG: Fasting plasma glucose; TC: Total cholesterol; TG, Triglycerides; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; IL-6: Interleukine 6; eGFR: estimated Glomerular Filtration Rate; ACR: albumin-to-creatinine ratio; r: Spearman correlation coeficient
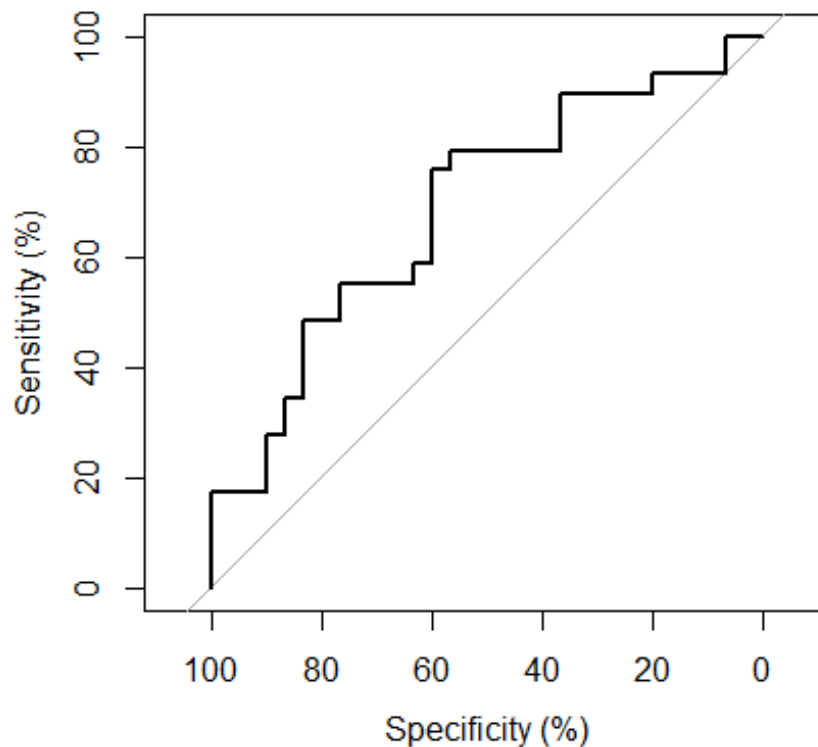

Figure 1: ROC curve analysis for IL-6 in the DM2 with DN vs DM2 without DN

The ROC-AUC of IL6 in the current study was comparable to that reported by Al-Rubeaan et al[1] in a cross-sectional study on Saudi DM2 patients. An IL6 optimal cutoff value was set. This value might be used for screening and detecting DN in DM2 patients. The involvement of IL-6 in DM2 opens a perspective in DN diagnosis and treatment. In fact, db/db mice were treated with tocilizumab, an IL-6 receptor antibody exhibited reduced proteinuria and glomerular mesangial matrix accumulation.[12]

A limitation of this study is the cross-sectional design which only provides the basis for associations and not causality.

Another limitation is the small size of samples. It is worth doing a comprehensive prospective cohort at large scales to reveal the diagnosis value of IL-6 in type 2 diabetic nephropathy.

Conclusion
Serum IL6 levels significantly correlated with BMI, TC, LDL levels and ACR in DM2. IL6 level can be used to differentiate type 2 diabetes with and without DN at an optimal cutoff value of 10.5 pg/mL with sensitivity of 79.3% and specificity of 56.7%.

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