Original Article

Inflammatory markers mediated diabetic nephropathy in patients with type 1 and type 2 diabetes mellitus

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Abstract:

Background: Diabetic nephropathy (DN) represents the most common cause of end stage renal disease (ESRD) worldwide. Diabetic nephropathy occurs as a result of an interaction between hemodynamic and metabolic factors, however recent evidence shows an increase growing support for the notion that inflammation plays a key role in the pathogenesis of diabetic nephropathy.

Objectives: To speculate the role of IL-18 and TNF-α proinflammatory cytokines in the initiation and development of diabetic nephropathy in T1DM and T2DM.

Materials and methods: Eighty seven T1DM and T2DM patients with or without DN were enrolled. IL-18 and TNF-α cytokines were measured by solid phase immunosorbent assays.

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Results: IL-18 had increased significantly in patients with DN compared to those without DN in T1DM and T2DM patients whereas TNF-α had exhibited a significant elevation among patients with DN compared to those without DN in T2DM but not T1DM.

Conclusion: IL-18 is suggested to play a crucial role in the initiation, development, and progression of DN in T1DM and T2DM patients whereas TNF-α is playing a similar role but only in T1DM patients with DN.

Key words: Diabetic nephropathy, interleukin-18, tumor necrotic factor-alpha.

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Introduction:

Diabetic nephropathy (DN) represents the most common cause of end stage renal disease (ESRD) worldwide, and patients with DN are at a higher risk of mortality, mostly from cardiovascular complications, than other patients with diabetes [1]. Around 50% of individuals with type 1 diabetes will develop nephropathy within 10 years of having the disease and around 20% of those with type 2 diabetes will develop it within 20 years [2]. DN causes pathologic abnormalities in all of the major structural compartment of the kidney including the glomeruli, extra-glomerular vessels, interstitium, and tubules [3]. The pathologic abnormalities are noted in patient with long standing DM [4]. Diabetic nephropathy occurs as a result of an interaction between hemodynamic and metabolic factors [5]. Hemodynamic factors that contribute to the development of DN include increased systemic and intra-glomerular pressure, as well as activation of vasoactive hormone pathways including the renin angiotensin system and endothelin [6]. These hemodynamic pathways activate intracellular second messengers such as protein kinase C (PKC), Mitogen-activated protein (MAP kinase) [7], NF-kB and various growth factors such as TGF-β, and vascular endothelial growth factor (VEGF). Glucose dependent pathways are also activated within the diabetic kidney and result in enhanced oxidative stress, renal polyol formation [8] and the accumulation of advanced glycation end products (AGEs). In combination, these pathways ultimately lead to increased renal albumin permeability and extracellular matrix accumulation, resulting in increasing proteinuria, glomerulosclerosis and ultimately tubulointerstitial fibrosis. Diabetic nephropathy has traditionally been considered a non-immune disease; however, recent evidence shows an increase growing support for the notion that inflammation plays a key role in the pathogenesis of diabetic nephropathy [9] and circulating inflammatory markers and proinflammatory cytokines are strongly associated with the risk of developing of diabetic complications [10].

IL-18 as a member of the IL-1 family is a potent inflammatory cytokine that induces IFN-γ [11], production TNF-α, upregulation of ICAM-1, as well as apoptosis of endothelial cells [10]. IL-18 is constitutively expressed in renal tubular epithelia and recent studies demonstrate that infiltrating monocytes, macrophages, and T cells, along with proximal tubular cells, are potential sources of this cytokine [12]. The hypothesis that the serum level of IL-18 is a common predictor of nephropathy and atherosclerosis in patients with type 2 diabetes was investigated in one study [13]. In another study, it had been hypothesized that IL-18 specifically contributes to the progression of nephropathy by directly affecting kidney function in addition to its proinflammatory effect [14].

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Tumor necrosis factor-alpha (TNF-α), may play a critical role in the development of microvascular diabetic complications, including nephropathy [15]. This cytokine is cytotoxic to glomerular, mesangial and epithelial cells, and may induce significant renal damage [16]. In addition, other relevant TNF-α effects have been reported, such as induction of apoptosis and necrotic cell death [12]. The aim of this study was to investigate and compare the serum level of TNF-α and IL-18 among Type 1 and Type 2 diabetes mellitus patients with or without diabetic nephropathy.

Patients Material and Method:

Patients: Eighty-seven Type1 and Type2 diabetic Iraqis patients (45 female and 42 male), with (57 patients) and without (30 patients) diabetic nephropathy as it was indicated by positive microalbuminuria (30-300 mg/L), were enrolled in this study. The age range of all patients were from 14 year to 55 year. All enrolled patients were attending the Endocrinology and Diabetic Center/ AL-Kindy teaching hospital/ Baghdad during the period from November 2013 to march 2014.

Materials and methods: Urine samples were used for the evaluation of microalbuminuria as a diagnostic and discriminative test between patients with or without diabetic nephropathy for both T1DM and T2DM. Blood samples were taken from all patients and the sera were separated for the estimation of interleukin-18 (IL-18) and tumor necrotic factor-alpha (TNF-α) serum levels for all diabetic patients whether they were with or without nephropathy. The estimation methods had used ELISA kits from MBL (medical & biological laboratories CO, China) and R & D system, USA & Canada for IL-18 and TNF-α respectively. The principle of the IL-18 kit depends on using two monoclonal antibody against two different epitopes of human IL-18 by sandwich ELISA whereas the principle of the TNF-α employs the quantitative sandwich enzyme immunoassay technique in which a monoclonal antibody specific for TNF-α has been pre-coated onto a microplate for the detection of serum TNF-α.

Results:

In this study, the alterations in the levels of two cytokines (IL-18 and TNF-α) in groups of patients representing kidney vascular complications (diabetic nephropathy) in type 1 and 2 diabetes mellitus were measured.

In (Table 1), the serum IL-18 level exhibited a significant elevation among patients with diabetic nephropathy compared to patients without diabetic nephropathy for both T1DM (99.8 X 72.4 pg/ml) and T2DM (125.3 X 73.6 pg/ml). The same table illustrates a significant elevation in the level of IL-18 among T2DM patients with DN (125.3 pg/ml) in comparison with T1DM patients with DN (99.8 pg/ml).

Table 1: Serum IL-18 level in all diabetic patients with or without diabetic nephropathy.

Test	Studied groups		№	Mean pg/ml	SD	SE	Range		(P-value)
							Min	Max	
IL-18	T1DM	WO	15	72.4	20.6	5.7	47.6	106.8	0.044 S
		W	27	99.8	32.2	6.7	63.0	188.2	
	T2DM	WO	15	73.6	21.0	5.8	37.2	104.6	0.00 HS
		W	30	125.3	54.9	11.2	74.2	280.1	
	Total		87	T1DM with DN VS T2DM with DN					0.026 S

S= Sig.,HS= highly Sig., T1DM= Type 1 diabetes mellitus, T2DM= T2 diabetes mellitus, W= with nephropathy, WO= without nephropathy, SD= Standard deviation, SE= Standard error, DN= diabetic nephropathy.In Table 2, the serum TNF-α expressed a significant increased level among patients with diabetic nephropathy compared to patients without diabetic nephropathy for T2DM (51.3 X 15.2 pg/ml) whereas there were no difference in the level of TNF-α between patients with or without diabetic nephropathy for T1DM. However the difference in the TNF-α serum level between T1DM patients with diabetic nephropathy and T2DM patients with diabetic nephropathy was highly significant (p= 0.00).

Table 2: Serum TNF-α level in all diabetic patients with or without diabetic nephropathy.

Test	Studied groups		№	Mean pg/ml	SD	SE	Range		(P-value)
							Min	Max	
TNF- α	T1DM	WO	15	22.1	5.3	1.5	12.6	30.4	0.816 NS
		W	27	22.7	5.1	1.0	11.8	30.4	
	T2DM	WO	15	15.2	3.6	1.0	11.8	24.4	0.00 HS
		W	30	51.3	12.6	2.8	37.6	84.2	
	Total		87	T1DM with DN VS T2DM with DN					0.00 HS

S= Sig., HS= highly Sig., NS= not significant, T1DM= Type 1 diabetes mellitus, T2DM= T2 diabetes mellitus, W= with nephropathy, WO= without nephropathy, SD= Standard deviation, SE= Standard error, DN= diabetic nephropathy.

Discussion:

In the current study, there was a significant elevation in the serum IL-18 among diabetic patients (T1 and T2 DM) with nephropathy complication compared to those without nephropathy complication. Similar results were documented in a Japanese study in which serum and urinary IL-18 levels were significantly elevated in patients with type 2 diabetes as compared with control subjects (serum IL-18 179 ± 62 vs. 121 ± 55 pg/ml, $P < 0.001$; urinary IL-18 97 ± 159 vs. 47 ± 54 pg/ml, $P = 0.035$) [13]. In the same study, significant positive correlations were found between serum IL-18 and urinary albumin excretion rate ($P < 0.001$), HbA_{1c} ($P = 0.029$), high-sensitivity C-reactive protein ($P = 0.031$), and urinary β-2 micro globulin ($P = 0.036$). They concluded that serum levels of IL-18 might be a predictor of progression of diabetic nephropathy as well as cardiovascular diseases. A similar result was also observed in one another study in which the IL-18, TNF-α and IL-6 were compared between Type 2 diabetes mellitus patients with variable degrees of nephropathy and controls of normal healthy individuals [17]. Indeed, IL-18 is also known to have a proinflammatory effect in systemic lupuserythematosus [18]. Moreover, IL-18 has been reported to play a role in the formation of inflammation and endothelial dysfunction [19]. Tucci et al. [20] reported that IL-18 induced the accumulation of dendritic cells in the glomerulus, resulting in kidney injury, whereas Araki et al. [21] reported predictive impact of IL-18 on diabetic renal dysfunction in a follow-up study. Conclusively, several lines of evidence therefore implicate IL-18 in the direct induction of renalinjury in diabetic nephropathy, in addition to its inflammatoryeffect and role in oxidative stress. Concerning TNF-α, this type of cytokines is mainly produced by monocytes, macrophages, and T cells. However, resident renal cells are also able to produce TNF-α, including mesangial, glomerular, endothelial, dendritic, and renal tubular cells [22]. The effects of TNF-α include promotion of local reactive oxygen species (ROS) generation [23], increasing albumin permeability, and the induction of cytotoxicity, apoptosis, and necrosis [24]. TNF-α is implicated in the recruitment of monocyte-macrophages, reducing glomerular filtration rate (GFR) by hemodynamic changes, as well as altering endothelial permeability [25]. In line with experimental data, patients with type 2 diabetes have 3-4 times greater serum levels of TNF-α compared to non-diabetic patients, and these levels are higher in diabetic patients with microalbuminuria compared with those that have normoalbuminuria [26]. Similarly, urinary TNF-α excretion correlates well with the clinical markers of DN and progression of disease [27]. It is believed that TNF-α inhibits insulin transduction, and has an effect on glucose metabolism. Disturbances in the TNF-α metabolism have been implicated in metabolic disorders, such as obesity and insulin resistance, indicating that perturbations of TNF-α metabolism may affect the onset of type 2 diabetes mellitus and the progression of the disease [28]. It had been suggested that TNF-α has stimulatory effects on sodium uptake by proximal tubule cells, contributing to sodium retention and renal hypertrophy, typical alterations that occur during the early stage of DN [26]. The results of the current study would come in consistency with other study which stated that the activity of the TNF-alpha system is increased in subjects with type 1 diabetes mellitus and diabetic neuropathy, regardless of their glycemic control and cardiovascular risk factors associated with insulin resistance. These results suggest that TNF-alpha may play a pathogenic role in the development of diabetic neuropathy [29]. In conclusion, IL-18 is an inflammatory cytokines that increased significantly in patients with DN compared to those without DN in T1DM and T2DM patients suggesting a crucial role of this cytokine in the initiation, development, and progression of nephropathy in diabetic patients. On the other hand, TNF-α is another inflammatory cytokine which exhibited a significant elevation among patients with DN compared to those without DN in T2DM but not T1DM. The elevation for both cytokines was greater significantly for patients with DN in T2DM than those in T1DM. Recommendation: it is highly recommended to survey more serum factors as an early predictor markers for the possible occurrence of DN in T1DM and T2DM.

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Authors' contribution:

Madha Mohammed Sheet Saleh: Critical revision, acquisition of data analysis and interpretation of data, Ghuroob Dalil Dhamad: Study design, Study conception, Laith Abul-Ellah Kamel: Drafting of manuscript.

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