BANTAO Journal 2019; 17(2): 86-90

BJ
BANTAO Journal

Original article

Interleukin-19 in Diabetic Nephropathy

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Abstract

Introduction. Proinflammatory cytokines play an important role in the establishment of arteriolosclerosis and kidney injury. Inflammatory cytokines are involved in the development of microvascular diabetic complications, including diabetic nephropathy**.** IL-19 has vital functions in many inflammatory processes and also can induce the angiogenesis of endothelial cells. The aim of our study was to investigate the role of interleukin-19 in development of diabetic nephropathy.
Methods. A total number of 112 persons were included and classified into 4 main groups: group I: The control group; 28 age and sex matched subjects, group II: 28 patients with type 2 diabetes without nephropathy (normoalbuminuria), group III; 28 patients with type 2 diabetes with nephropathy (microalbuminuria) and group IV; 28 patient with type 2 diabetes with nephropathy (macroalbuminuria). All subjects were submitted to: complete blood count, complete urine analysis, fasting and random blood glucose, glycosylated haemoglobin (HbA1c), serum creatinine and urea, urinary albumin excretion rate (UAE), albumin creatinine ratio (ACR), lipid profile and serum Interleukin-19 levels assays.
Results. CRP and serum IL-19 levels were significantly higher in diabetic patients compared to controls. IL-19 levels were significantly positively correlated with serum creatinine, ACR, UAE, HbA1c and CRP. Multivariable logistic regression analysis showed that IL-19 levels were independently associated with patients with DN.
Conclusion. IL-19 levels were elevated in patients with diabetic nephropathy and were positively correlated with ACR, UAE, HbA1c and CRP. IL-19 may play an important role that contributes to the progression of diabetic nephropathy.

Keywords: interleukin-19, diabetic nephropathy

Introduction

Type 2 diabetes mellitus (T2DM) is a metabolic disease and characterized by hyperglycemia which is due to the deficiency in peripheral insulin effects (insulin resistance). Macrovascular and microvascular complications are the primary causes of morbidity and mortality in diabetes. It is important to understand the risk factors in order to prevent the development and progression of such complications [1]. Diabetic nephropathy (DN) or diabetic kidney disease is a syndrome characterized by the presence of pathological quantities of urine albumin excretion, diabetic glomerular lesions, loss of glomerular filtration rate (GFR), and arterial hypertension in diabetics [2].
The pathophysiology of diabetic nephropathy is caused by both metabolic alterations (hyperglycemia and possibly hyperlipidemia) and hemodynamic alterations (systemic and glomerular hypertension). Other factors, such as inflammation, endothelial dysfunction and oxidative stress, are also involved [3].
Inflammation plays some important roles in the pathogenesis of DN. Leukocytes, macrophages and monocytes all involve in the process of DN, and proinflammatory cytokines and inflammatory markers are strongly associated with the development of DN [4].
Interleukin (IL)-19 is a member of the IL-10 family of cytokines. Secreted IL-19 is composed of 159 amino acids that form α-helical structure. IL-19 is produced by activated monocytes, and to a lesser extent, by B cells [5]. It has been reported that IL-19 can promote the T-helper2 (Th2) response, which is associated with a wide variety of allergic conditions (i.e., asthma and atopic dermatitis), type 1 diabetes, and cardiovascular disease. IL-19 have indispensable functions in many inflammatory processes and also can induce the angiogenic potential of endothelial cells [6]. A previous study also reported that IL-19 is closely related to T2DM with vascular complications. However, whether there are some association between IL-19 concentration and DN have not been revealed clearly yet [7].

Material and methods

This is a case-control study that included 112 subjects after their written and informed consent. The study was cleared by the institutional ethics committee on human research and has been conducted in the departments of Internal Medicine and Clinical Pathology, Faculty of

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Participants and groups

A total number of 112 persons were included and classified into 4 main groups: group I: The control group; age and sex matched subjects included 16 males (57.1%) and 12 females (42.9%), group II: patients with type 2 diabetes without nephropathy (normo-albuminuria). It included 16 males (57.1%) and 12 females (42.9%), group III: patients with type 2 diabetes with nephropathy (microalbuminuria); It included 12 males (42.9%) and 16 females (57.1%), and group IV: patient with type 2 diabetes with nephropathy (macroalbuminuria); It included 12 males (42.9%) and 16 females (57.1%). **Inclusion criteria:** Co-operative patients and both sexes with type 2 diabetes mellitus (T2DM) were eligible. **Exclusion Criteria:** patients with type 1 diabetes, patients with confounding factors for proteinuria and those previously diagnosed with urolithiasis, recent or current viral hepatitis or cirrhosis of liver, medical history of clinical cardiovascular disease, chronic lung disease, acute or chronic infections, with autoimmune disorders or with malignancy and pregnant or lactating females.

Physical examination and measurements:

All subjects of the study were submitted to: 1-Full history talking and thorough physical examination. Fundus examination was performed to confirm diabetic retinopathy in participants with albuminuria to confirm the diagnosis of DN.
2-Investigations (to verify the inclusion and exclusion criteria of studied subjects) including: A) Routine investigations: complete blood count ((by Sysmex KX21N), Fasting and random blood glucose, glycosylated haemoglobin (HbA1c), serum creatinine and urea, liver function tests, lipid profile and C-reactive protein (CRP). Complete urine analysis by uriscane analyzer, determination of urinary albumin excretion (UAE) and creatinine then calculation of albumin creatinine ratio (ACR), C-reactive protein (CRP) and urinary albumin were determined by Immunoturbidimetric assay, these parameters were measured by Cobas 8000 (Roche diagnostics). B) Special investigation including: Serum Interleukin-19 levels determined by double antibody sandwich enzyme linked immunosorbent assay (ELISA), Kit provided by Glory Science Co., Ltd. (2400Veterans Blvd. Suite16-101, Del Rio, Tx 78840, USA).

Statistical Analysis

All data were collected, tabulated and statistically analyzed using SPSS 24.0 for windows (SPSS Inc., Chicago, IL, USA). Data were tested for normal distribution using the Shapiro Walk test. Qualitative data were represented as frequencies and relative percentages. Chi square test ($\chi 2$) and Fisher exact was used to calculate difference between qualitative variables as indicated. Quantitative data were expressed as mean ± SD (Standard deviation). One-way ANOVA test was used to compare between more than two dependent groups of normally distributed variables while Friedman's test ranks test was used for non-normally distributed variables. Pearson's and Spearman's correlation coefficient were used for correlating normal and non-parametric variables respectively. We considered values near to 1 as strong correlation & values near 0 as weak correlation. Regression analysis using the stepwise method was used to determine the association between IL-19 and diabetic nephropathy. All statistical comparisons were two tailed with significance level of P-value ≤ 0.05 indicating significant, $p<0.001$ indicating highly significant difference while, $P>0.05$ indicating Non-significant difference.

Results

There is a high statistically significant difference among the four studied groups regarding body mass index (BMI) and systolic blood pressure (SBP) (Table 1).
A high statistically significant difference is found among the four studied groups regarding fasting blood gluco-

Table 1. Demographic data of the four studied groups

Groups / Variables	Group I (N=28)	Group II (N=28)	Group III (N=28)	Group IV (N=28)	F / χ^2	P
Age(years) Mean ± SD	53.43±5.12	60.04±13.83	58.07±13.83	57.71±12.07	1.564	0.202
Sex Male: N(%)	16 (57.1%)	16 (57.1%)	12 (42.9%)	12 (42.9%)	2.294	0.514
Female:N(%)	12 (42.9%)	12 (42.9%)	16 (57.1%)	16 (57.1%)		
BMI (kg/m^2) Mean ± SD	24.36±1.32	27.88±1.43	26.82±1.69	29.12±1.46	**25.698**	**<0.001**
SBP Mean ± SD (mm Hg)	113.57±6.22	119.64±13.39	123.75±14.95	118.75±11.59	**3.402**	**0.020**
DBP Mean ± SD (mm Hg)	74.64±5.59	76.07±6.85	76.77±8.19	75.36±11.29	0.349	0.790

BMI: Body mass index, SBP: Systolic blood pressure, DBP: Diastolic blood pressure. p-value<0.05 is significant

Table 2. Comparison of different variables among the four studied groups

Groups Variables	Group I (N=28)	Group II (N=28)	Group III (N=28)	Group IV (N=28)	F	P
FBG(mg/dL) Mean ± SD	79.89±13.51	131.36±56.65	141.82±73.37	150.89±94.83	**6.354**	**0.001**
RBG(mg/dL) Mean ± SD	92.07±8.23	160.93±93.86	194.64±98.85	180.14±66.26	**10.020**	**<0.001**
HbA1c (%) Mean ± SD	5.19±0.335	7.77±1.74	8.64±1.49	8.49±1.18	**18.428**	**<0.001**
Duration of DM Mean ± SD (years)	-----	8.73±4.73	15.26±4.01	19.21±4.86	**16.525**	**<0.001**
S. Cr(mg/dL) Mean ± SD	0.882±0.136	1.13±0.533	3.48±1.16	5.45±2.91	**45.749**	**<0.001**
ACR(mg/g) Mean ± SD	18.54±4.39	25.82±4.26	39.79±4.4	64.46±2.52	**76.012**	**<0.001**
UAE (mg/24h) Mean ± SD	9.62±2.15	22.18±1.62	280.18±93.16	532.83±180.34	**38.629**	**<0.001**
TC (mg/dL) Mean ± SD	163.32±34.91	186.71±25.48	201.12±67.85	218.50±54.17	**6.490**	**<0.001**
TG (mg/dL) Mean ± SD	162.11±46.39	176.29±23.88	210.71±61.29	211.39±56.33	**7.167**	**<0.001**

FBG: Fasting blood glucose RBG: Random blood glucose HbA1c: HemoglobinA1C, DM: diabetes mellitus S.Cr: Serum Creatinine, ACR: Albumin/creatinine ratio, UAE: urinary albumin excretion,, TC: Total cholesterol, TG: Total triglycerides, p-value<0.05 is significant

Table 3. Comparison of different Inflammatory markers of the studied groups

Groups Variables	Group I (N=28)	Group II (N=28)	Group III (N=28)	Group IV (N=28)	F	P
CRP (mg/L) Mean ± SD	0.761±0.179	8.2±4.42	19.64±6.71	23.25±9.97	**73.318**	**<0.001**
IL-19 (pg/ml) Mean ± SD	224.36±139.61	240.32±178.43	1013.57±1202.08	1457.64±1387.71	**12.058**	**<0.001**

CRP: C-reactive protein, IL-19: Interleukin-19, p-value<0.05 is significant

se (FBG), random blood glucose (RBG), HbA1c, DM duration, serum creatinine, albumin creatinine ratio (ACR) and urinary albumin excretion (UAE), total cholesterol (TC) and triglycerides (TG) (Table 2). There is a high statistically significant difference among the four studied groups regarding CRP and serum IL-19 where they increased in diabetic patients (groups II, III, and IV) compared to controls (group I) (Table 3). A significant positive correlation is found between IL-19 and BMI, DM duration, serum creatinine, ACR, UAE, FBG, HbA1c, CRP, TC and TG (Table 4). Multivariable logistic regression analysis shows that IL-19 levels are independently associated with patients with DN (Table 5).

Table 4. Correlation between IL-19 and other variables in all patients' groups

IL-19 Variables	***r***	***p***
Age	0.040	0.672
Body mass index	0.213	0.024
DM duration	0.447	<0.001
Serum Creatinine	0.414	<0.001
Albumin/creatinine ratio	0.505	<0.001
Urinary albumin excretion	0.498	<0.001
Fasting blood glucose	0.323	0.001
HbA1c	0.375	0.001
C-reactive protein	0.467	<0.001
Total cholesterol	0.291	0.002
Total triglycerides	0.334	<0.001

P-value<0.05 is significant

Table 5. Multivariable logistic regression analysis, to detect the association between IL-19 levels and diabetic nephropathy

	β	S.E.	Wald	P-value	B	95% C.I. for B	
						Lower	*Upper*
IL-19	0.005	0.001	21.267	<0.001	1.005	1.003	1.007
Constant	-2.123						

P-value<0.05 is significant

Discussion

Diabetic nephropathy is a major microvascular complication of diabetes mellitus (DM), it is the leading cause of end-stage renal disease. In type 1 diabetes, it develop in about 20%-30% of patients, whereas it occurs in about 10%-20% of those with type 2 diabetes [8]. Pathogenesis of DN is multifactorial element, including genetic and environmental factors, which trigger more complex pathological processes [9]. Intensive research

on molecular and cellular aspects demonstrated that immunological and inflammatory factors play essential roles in DN and its progression [10].

Inflammatory cytokines are involved in the development of microvascular diabetic complications, including diabetic nephropathy [11]. However, the role of inflammatory cytokines in development and progression of DN is still lacking. Extending the knowledge regarding the role of inflammation in the development and progression of DN is useful to find novel therapeutic strategies. In line with this, our study aimed to investigate the role of interleukin-19 in diabetic nephropathy and its association with DN.

Our findings showed that there was a highly significant difference among the studied groups regarding BMI, where was higher in albuminuria patients than normoalbuminuric patients. Another study found that obesity-associated glomerular hyperfiltration, renal vasodilation, increases in the glomerular filtration rate and intraglomerular capillary pressure, and increased blood pressure also are characteristics of diabetic nephropathy [12].

Also, there was a highly significant difference among the studied groups regarding SBP, it was higher in macroalbuminuric and microalbuminuric patients than normoalbuminuric patients. In agreement with another study that suggested that microalbuminuria precedes hypertension more commonly in DM1 than DM2 [13].

A highly significant difference was found among the studied groups regarding DM duration which was higher in macroalbuminuric and microalbuminuric patients than normoalbuminuric patients. Also, a previous study reported that a long duration of diabetes and poor glycemic control is associated with increased production of glycosylation end products, metabolic derangements, endothelial injury, and oxidative products [14].

Regarding serum creatinine there was a highly significant difference among the four studied groups which was higher in macroalbuminuric and microalbuminuric patients than normoalbuminuric patients. In agreement with another study which reviewed aspects of the association of diabetes with renal disease, emphasizing that CKD and albuminuria are associated with increased rates of cardiovascular disease (CVD) and mortality [15].

Among the four studied groups regarding serum triglycerides (TG) and total cholesterol (TC), there were again highly significant differences, which were higher in macroalbuminuric and microalbuminuric patients than in normoalbuminuric patients. Also, a previuos study stated that DN is associated with an altered lipid profile characterized by elevated triglyceride rich lipoproteins even in the early stages of the renal disease [16].

In the current study, fasting blood glucose (FBG), random blood glucose (RBG), HbA1c, albumin creatinine ratio (ACR) and urinary albumin excretion (UAE) in the microalbuminuric and macroalbuminuric diabetic group were significantly increased compared to normoalbuminuric and control groups. This study is in agreement with a previous study, which has suggested that hyperglycemia is the driving force for the development of DN [17].

Positive correlation was seen between IL-19 and BMI, DM duration, serum creatinine, ACR, UAE, FBG, HbA1c, CRP, TC and TG. These results may suggest that long term hyperglycemia could increase the expression of IL-19 via stimulating endothelial cells, which results in local inflammation and accelerate endothelial damage and atherosclerosis. On the same hand, another study demonstrated a positive correlation between IL-19 and HbA1c and UAE [7].

Findings from the study also showed highly significant difference regarding C- reactive protein among studied groups, which were higher in macroalbuminuric and microalbuminuric patients than in normoalbuminuric patients. C-reactive protein, a marker of inflammation, has been reported to be associated with the risk of DM complications [17]. This result is consistent with a previous study that showed that CRP may deteriorate the inflammatory cascade in tissue injury in addition to initiating endothelial damage and atherosclerosis [18].

IL-19 concentrations were significantly higher in macroalbuminuric and microalbuminuric patients than normoalbuminuric patients. Similarly, a previous study [23] reported that inflammatory cytokines and inflammatory stimuli can prompted IL-19 to express, the expression of IL-19 is ascribed in injured and stimulated vascular smooth muscle cells [19]. Also, another study has revealed that the roles of IL-19 in development of vascular inflammatory diseases such as atherosclerosis, restenosis, and coronary artery transplant vasculopathy [8]. Similar observation was reported by another study which documented that chronic inflammation, characterized by elevated circulating levels of inflammatory markers, appears to play a critical role in the pathogenesis of T2DM and its associated complications [20].

On the same hand, a previous study reported that proinflammatory cytokines play an important role in the establishment of arteriolosclerosis and kidney injury and inflammatory cytokines are involved in the development of microvascular diabetic complications, including diabetic nephropathy [11].

Multivariable logistic regression analysis showed IL-19 levels were independently associated with DN which is similar to another study which reported the same results [7]. These results suggest that IL-19 involved in the inflammatory reaction and plays a significant role in the progression of DN.

Conclusion

The previous findings of this study showed that IL-19 levels were significantly high in patients with diabetic nephropathy and were associated CRP, ACR, UAE and HbA1c. The results suggest that IL-19 has a possible

role in the pathophysiology and progression of DN, providing further concepts as a therapeutic target for prevention or delaying progression of DN.

Conflict of interest statement: None declared.

Acknowledgments. The authors acknowledge all participants of the study, their colleagues and staffs.

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