Cytokine 72 (2015) 109–112

Contents lists available at ScienceDirect

Cytokine

journal homepage: www.journals.elsevier.com/cytokine

Short Communication

Serum IL-9, IL-17, and TGF-β levels in subjects with diabetic kidney disease (CURES-134)

Rathinam Vasanthakumar [a], Viswanathan Mohan [b], Gowrisankar Anand [c], Mohan Deepa [b], Subash Babu [d], Vivekanandhan Aravindhan [c,e,*]

[a] Department of Biotechnology, Prathyusha Institute of Technology and Management, Thiruvallur, Tamil Nadu, India
[b] Madras Diabetes Research Foundation & Dr. Mohan's Diabetes Specialties Centre, WHO Collaborating Centre for Non-Communicable Diseases Prevention and Control, International Diabetes Federation (IDF) Centre of Education, Chennai, Tamil Nadu, India
[c] AU-KBC Research Centre, MIT Campus of Anna University, Chennai, Tamil Nadu, India
[d] National Institutes of Health-International Center for Excellence in Research, National Institute for Research in Tuberculosis, Chennai, Tamil Nadu, India
[e] Department of Genetics, Dr. A.L.M. PG IBMS, University of Madras, Chennai, Tamil Nadu, India

ARTICLE INFO

Article history:
Received 1 August 2014
Received in revised form 4 September 2014
Accepted 27 October 2014
Available online 23 December 2014

Keywords:
Diabetic kidney disease
Nephropathy
IL-9
IL-17
TGF-β and insulin resistance

ABSTRACT

The role of inflammation in both diabetes and diabetic kidney disease (DKD) is becoming more widely accepted. However, the role of recently characterized T cell cytokines interleukin (IL)-9 and IL-17 in diabetes and especially DKD is less well studied. Transforming growth factor beta (TGF-β) controls the secretion of both of these cytokines. In this study, we estimated the levels of IL-9, IL-17, and TGF-β in the serum of subjects with normal glucose tolerance (NGT = 88) and subjects with type 2 diabetes without (diabetes mellitus (DM) = 65) and with DKD (DKD = 97) using enzyme-linked immunosorbent assay (ELISA), and we correlated these levels with the clinical risk factors of diabetes and DKD. IL-17 levels showed a serial decline and TGF-β levels showed a serial increase from NGT to DM to DKD ($p < 0.001$). However, the IL-9 levels were significantly reduced in the DM group compared to the NGT and DKD group ($p < 0.001$). While TGF-β and IL-17 showed a positive and negative correlation, respectively, with fasting and postprandial glucose levels and glycated hemoglobin (HbA1c), IL-9 showed positive correlation with urea and microalbuminuria. Apart from pro-inflammatory cytokines, T helper (Th) cytokines might play an important role in insulin resistance and DKD.

1. Introduction

Diabetic kidney disease (DKD) occurs in about 35–40% of patients with both type 1 and type 2 diabetes [1]. The classic view of metabolic and hemodynamic alterations as the main causes of renal injury in DKD has been transformed significantly [2]. One of the most important recent advances in our understanding of DKD is the participation of inflammatory processes in the pathogenesis of the disease [2]. Increased infiltration of activated T cells and aberrant expression of T cell cytokines have been reported in DKD [3]. However, no data are currently available on the recently characterized T cell-derived cytokine interleukin (IL)-9 in DKD. IL-9, initially recognized as a type 2 T helper (Th2) cytokine, was recently attributed to a novel CD4 T cell subset termed Th9 [4]. However, IL-9 can also be secreted by Th17 cells, and it may mediate aspects of the pro-inflammatory activities of Th17 cells [4]. The production of both IL-9 and IL-17 by T cells is under the control of TGF-β [4]. A resurgence of interest in IL-9 and IL-17 has been spurred by recent work demonstrating a role for IL-9 in obesity [5,6], insulin resistance [6], and kidney disease in general [7,8]. In the present study, our objective was to estimate the levels of IL-9 along with IL-17 and transforming growth factor beta (TGF-β) in subjects with type 2 diabetes with DKD and to correlate these levels with the clinical risk factors associated with diabetes mellitus (DM) (IR and glycated hemoglobin (HbA1c)) and with DKD (blood urea, serum creatinine, estimated glomerular filtration rate (eGFR), and microalbuminuria).

Abbreviations: NGT, normal glucose tolerance; DM, diabetes mellitus (type 2); IL-9, interleukin 9; IL-17, interleukin 17; TGF-β, transforming growth factor beta; HbA1c, glycated hemoglobin.

* Corresponding author at: Department of Genetics, Dr. A.L.M. PG IBMS, University of Madras, Taramani, Chennai - 600 113, Tamil Nadu, India. Tel.: +91 44 24547061; fax: +91 44 24547067.

E-mail addresses: cvaravindhan@gmail.com, cvaravindhan@mail.unom.in (V. Aravindhan).

http://dx.doi.org/10.1016/j.cyto.2014.10.009
1043-4666/

2. Methods

2.1. Study population

Study participants were recruited from the Chennai Urban Rural Epidemiology Study (CURES), a large epidemiological study conducted on a representative population of Chennai (formerly Madras City in southern India). The exclusion criteria were patients with type 1 diabetes and those previously diagnosed with urolithiasis, recent or current viral hepatitis or cirrhosis of liver, congestive heart failure, chronic lung disease, or acute or chronic infections. Institutional ethical committee approval was obtained from the Madras Diabetes Research Foundation Ethics Committee (Ref No-MDRF-EC/SOC/2009//05), and written informed consent was obtained from all the study participants. The study was conducted as per the Declaration of Helsinki.

2.2. Anthropometric and biochemical parameters

Anthropometric measurements including height, weight, and waist circumference were obtained using standardized techniques. The body mass index (BMI) was calculated as the weight in kilograms divided by the square of height in meters. Fasting plasma glucose (FPG) (the glucose oxidase–peroxidase method), serum cholesterol (the cholesterol oxidase–peroxidase–amidopyrine method), serum triglycerides (the glycerol phosphate oxidase–peroxidase–amidopyrine method), high-density lipoprotein cholesterol (HDL-C) (a direct method using polyethylene glycol-pretreated enzymes), urea, and creatinine (Jaffe's method) were measured using a Hitachi-912 AutoAnalyzer (Hitachi, Mannheim, Germany). Glycated hemoglobin (HbA1c) was estimated by high-pressure liquid chromatography using a variant machine (Bio-Rad, Hercules, CA, USA). The intra- and inter-assay coefficient of variation for the biochemical assays ranged between 3.1% and 5.6%.

2.3. Diagnosis of DKD

Urinary albumin concentration was measured in a fasting sample using an immunoturbidimetric assay (Hitachi 902 AutoAnalyzer; Roche Diagnostics). Subjects were classified based on albumin excretion per milligram of creatinine as normoalbuminuric ($\leqslant$29 μg), microalbuminuric (30–229 μg), or albuminuric/overt nephropathy ($\geqslant$300 μg). Data on serum creatinine, age, and sex were used to calculate the eGFR using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [9].

2.4. Estimation of serum cytokine levels

The levels of cytokines IL-9 (ebiosciences, USA), IL-17 (R&D System, USA), and TGF-β (R&D System, USA) were estimated by enzyme-linked immunosorbent assay (ELISA) as per the instructions of the kit. In brief, cytokine-specific monoclonal antibody was coated onto microplates followed by the addition of standards and samples. After washing and blocking, enzyme-linked monoclonal antibodies were added. After washing, the substrate solution was added and the absorbance was read at 450 nm. The intra- and inter-assay coefficients of variation for multiplex assay were <5% as determined in our laboratory. The lowest detection limit for IL-9, IL-17, and TGF-β were 1, 2, and 4 pg/ml.

2.5. Sample size calculation

Initially, 20 NGT, 20 DM, and 20 diabetic nephropathy (DN) subjects were screened for the serum cytokines studied. On the basis of the preliminary results, with a confidence interval of 95%, an estimated p-value <0.05, and a power of 80%, a sample size of 60 per group was calculated. However, we increased the numbers in each group to account for the wide variation generally seen among serum biomarkers.

2.6. Statistical analyses

Student's t-test was used to compare groups for continuous variables that followed normal distribution, whereas the χ^2 test or Fisher's exact test (as appropriate) was used to compare proportions. The Kruskal–Wallis test was used for multiple parameters that did not show normal distribution. Spearman's correlation analysis and multivariate logistic regression analysis were used to determine the association of cytokines with clinical parameters and study groups, respectively. Multiple comparisons were corrected using Holm's correction for each set of analysis. All the analyses were conducted using SPSS statistical package (Version 20.0; SPSS, Chicago, IL, USA) and a p-value <0.05 was considered significant.

3. Results and discussion

Table 1 presents the clinical and biochemical characteristics of the study subjects. The age was significantly higher in the DKD group compared to the NGT and DM groups ($p < 0.0001$). BMI showed a linear increase from NGT to DM to DKD ($p = 0.003$). Subjects with DKD had higher blood pressure (BP) (both systolic

Table 1
Clinical and biochemical characteristics of the study subjects.

Clinical parameters	NGT* ($n = 88$)	DM* ($n = 65$)	DKD* ($n = 97$)	p-Value
Age (years)	37.9 ± 12.4	51.3 ± 12.2	59.3 ± 12.1	<0.0001
Gender (M/F)	35/53	38/27	57/40	NS
BMI* (kg/m^2)	24.2 ± 4.9	26.3 ± 4.5	26.9 ± 5.5	0.0029
Systolic BP* (mm Hg)	116 ± 19	129 ± 17	138 ± 15	<0.001
Diastolic BP* (mm Hg)	74 ± 12	77 ± 8	83 ± 9	<0.001
FPG* (mg/dl)	82 ± 7	180 ± 35	166 ± 57	<0.001
PPBS* (mg/dl)	102 ± 22	199 ± 76	243 ± 81	<0.0001
HbA1c levels* (%)	5.5 ± 0.5	7.6 ± 1.7	9.5 ± 7.0	<0.001
Cholesterol (mg/dl)	175 ± 38	164 ± 41	158 ± 39	0.0114
Serum triglycerides (mg/dl)	114 ± 54	136 ± 55	160.7 ± 79	<0.001
HDL* cholesterol (mg/dl)	42.6 ± 10.2	40.7 ± 8.6	41.2 ± 10.5	0.5297
LDL* cholesterol (mg/dl)	109.3 ± 33.4	90.0 ± 26.3	84.1 ± 31.1	<0.0001
Serum creatinine (mg/dl)	0.8 ± 0.1	0.8 ± 0.1	1.0 ± 0.4	0.0031
Microalbuminuria (mg/dl)	15.3 ± 36.8	6.8 ± 5.7	116.1 ± 81.4	<0.001
eGFR*	96.0 ± 20.8	101.2 ± 20.4	86.9 ± 32.2	0.0031
Retinopathy	Nil	Nil	54.6%	NA

* NGT – normal glucose tolerance, DM – type 2 diabetes, DKD – diabetes kidney disease, BMI – body mass index, BP – blood pressure, FPG – fasting plasma glucose, Hb1Ac – glycated hemoglobin, HDL – high-density lipoprotein, LDL low-density lipoprotein, VLDL – very low-density lipoprotein, Malb – microalbuminuria, PPBS – postprandial blood sugar, GFR – glomerular filtration rate.