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1: CXCL1/KC, and CXCl

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cholesterol, and glycosylated haemoglobin (HbAlc) were measured by using routine clinical laboratory procedures. Insulin was determined by radioimmunoassay Kit (DPC, Los Angeles, CA). e insulin sensitivity was determined by Homeostasis Model Assessment Model (HOMA) index with formula: HOMA-IR=fasting insulin (U/ml) × fasting glucose (mmol/l)/22.5 [13]. Low HOMA-IR values indicate high insulin sensitivity, whereas high HOMA values indicate low insulin sensitivity (insulin resistance).

PBMC Isolation from blood samples

Peripheral blood mononuclear cells (PBMC) were isolated from heparinized peripheral blood obtained from normal subjects and patients by density gradient centrifugation using Ficoll-Hypaque (density: 1.077; Pharmacia, Dübendorf, Switzerland). Mononuclear cells at the interface were carefully transferred into a Pasteur Pipette, and washed twice in PBS (137 mmol/L NaCl, 2.7 mmol/L KCL, 10 mmol/L Na_2HPO_4, 2 mmol/L KHPO$_4$). Cells were suspended at a density of 2×10^6 cells/ml and used for RNA isolation.

ELISA for serum IL-17

Serum IL-17 levels were assayed by ELISA Kit (Human IL-17 immunoassay, eBioscience, San Diego, USA) according to the manufacturer's instructions. e optical density was measured at 450 nm with an automatic ELISA reader. e minimum detection limit was 4 pg/ml for IL-17. To minimize the e ect of inter-assay variation, samples from diabetes patients and controls were equally represented on each ELISA plate. All samples were analyzed in duplicates, and the mean of the duplicates was used for the statistical analysis. e intra-assay and inter-assay coe cients of variation for IL-17 were 6.4 and 14.3%, respectively.

Quantitative real time PCR

Total RNA was isolated from cell pellets using RNeasy Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Genomic DNA was removed from total RNA using the RNase-free DNase set (Qiagen, Hilden, Germany). e rst strand cDNA was synthesized by using the cDNA synthesis kit (Promega, Madison, WI). All reactions were performed on ABI Prism 7500 Sequence Detection System (Applied Biosystems, Foster City, CA). e gene expression levels were analyzed by real time PCR using SYBR Green master mix (Applied Biosystems, Foster City, USA). e PCR conditions comprised an initial holding at 50°C for 2 min, and 95°C for 10 min followed by a two-step PCR program consisting of 95°C for 15 s, and 60°C for 60 s for 40 cycles. For each sample, mRNA expression level was normalized to the level of GAPDH gene. e sequences of primers were showed in the Table 1.

Statistical analysis

Data were expressed as mean ± standard deviation (SD) or

creatinine (Cr), blood urea nitrogen (BUN), blood uric acid (UA), urinary microquantitative albumin (Ualb) and urinary albumin/ creatinine ratio (Ualb/Ucr). ese clinical parameters re ecting liver and renal function did not exceed the upper limits of normal range in patients with type 2 diabetes (data not shown).

In all of patients, glutamic acid decarboxylase antibody (GAD) and islet cell antibody (ICA) and insulin antibody (IAA) were negative. No patients have apparent diabetic complications including microvascular and macrovascular complications.

Serum levels of IL-17 in patients with type 2 diabetes

To investigate the role of IL-17 in type 2 diabetes, we examined serum concentrations of IL-17 in patients with newly diagnosed type 2 diabetes. As shown in Figure 1, serum IL-17 levels in patients with type 2 diabetes were signi cantly elevated compared to healthy subjects (10.44 ± 6.47 *vs* 2.99 ± 1.68 pg/ml, $P<0.01$).

mRNA expression of IL-17and ROR t in PBMC from diabetic patients

PBMC is a major source of IL-17. To test whether serum IL-17 is secreted by PBMC, we further investigated mRNA levels of IL-17 and its upstream regulator ROR t in PBMC from patients with type 2 diabetes. As shown in Figure 2A, mRNA levels of IL-17 were dramatically higher in diabetic patients than in control subjects ($P<0.001$); and the expression of ROR t gene in diabetic patients were also markedly increased compared with control subjects ($P<0.001$).

Relationship between IL-17 and IL-6 or TNF- or IL-1 mRNA expression levels in PBMC from diabetic patients

To clarify the relationship between IL-17 and other in ammation cytokines on mRNA levels, we rst examined mRNA expression of

A

B

Figure 2: *HQH H[SUHVVLRQ LQ SHULSKHUDO EORRG PRQRQXFOHDU FHOOV IURP SDWLHQWV ZLWK W\SH 'FRQWUROV DQG VXEMHFWHG WR UHDO WLPH 3&5 DQDO\VLV IRU P5,1$ H7[S)UHVVLRQ RI ,DWD DQG HLSVUHWHD H[SUHVVLRQ RI JHQHV LQ UHIHUHQFH WR *$3'+ '0 W\SH GLDEHWLF SDWLHQWV &RQWURO +HDOWK\ VWDWLVWLFDO GLIIHUHQFH EHWZHHQ JURXSV P<0.01

experiment, the average level of BMI in all patients is about 25.42 kg/m^2; and BMI levels were similar between diabetic patients and healthy subjects. As a consequence of narrow selection criteria, the subdivision of the patients' groups was hardly enough to make clear estimations. Further study should be performed in a large number of diabetic patients including diabetic patients with low-level BMI (BMI<25) and diabetic patients with high-level BMI (BMI 25)in order to investigate the relationship between IL-17 and clinical parameters. Moreover, further studies is required to investigate the circulating levels of IL-17 in groups with impaired glucose tolerance (IGT) and diabetic patients with complications. Serum IL-17 level might be useful to predict an early detection of risk for type 2 diabetes, as well as being potential marker of established diabetic complications. On the other hand, we need to further explore the detailed mechanism of IL-17 involved in the pathogenesis of type 2 diabetes.

In conclusion, we demonstrated that serum levels and mRNA levels of IL-17 are increased in patients with newly diagnosed type 2 DM. Our results suggested IL-17 might promote the in ammatory state of patients, and participate in the pathogenesis of type 2 DM. Further studies are necessary to clarify the crucial role of IL-17 in the pathogenesis of type 2 DM and whether IL-17 is a prognostic factor for the development of type 2 DM.

References

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