Peripheral Total and Differential Leukocyte Count in Diabetic Nephropathy

The relationship of plasma leptin to leukocytosis

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OBJECTIVE — Because of increasing evidence that white blood cells (WBCs) play a role in the development and progression of diabetes complications, this study aimed to investigate the relation of circulating total and differential leukocyte counts to nephropathy in patients with type 2 diabetes. Plasma leptin levels were also measured to investigate their role in peripheral leukocytosis.

RESEARCH DESIGN AND METHODS — For this study, 1,480 subjects with type 2 diabetes who were enrolled in a disease management program were stratified according to urinary microalbumin and serum creatinine measurements. The total and differential leukocyte profiles of peripheral blood were measured and plasma leptin was examined by enzyme-linked immunosorbent assay. Demographic and potential metabolic confounding factors were analyzed with linear and logistic regression to calculate the effects of leukocyte count on diabetic nephropathy.

RESULTS — The peripheral total WBC, monocyte, and neutrophil counts increased in parallel with the advancement of diabetic nephropathy. In contrast, the lymphocyte count decreased. When WBC counts were analyzed per quartile and as continuous variables after adjusting for age, sex, and other known risk factors with multiple regression analysis, peripheral total WBC, monocyte, neutrophil, and lymphocyte counts were independently and significantly associated with diabetic nephropathy. Plasma leptin levels increased in patients with nephropathy and correlated significantly with total WBC count ($r = 0.194$, $P = 0.014$).

CONCLUSIONS — Because leukocytes are activated and secrete cytokines in the diabetic state and leptin stimulates leukocyte proliferation and differentiation, our results suggest that circulating leukocytes contribute to the development and progression of nephropathy, partially through the effects of leptin, in patients with type 2 diabetes.

Diabetes Care 28:1710–1717, 2005

Peripheral white blood cell (WBC) count has been shown to be associated with insulin resistance, type 2 diabetes (1–4), coronary artery disease (CAD) (5–8), stroke (5,8), and diabetes micro- and macrovascular complications (9,10). An association between leukocytes counts and CAD has been observed in prospective and retrospective cohort studies as well as in case-control studies; this association persists after adjusting for multiple coronary heart disease (CHD) risk factors, including smoking (11).

Peripheral blood leukocytes are composed of polymorphonuclear cells, including monocytes as well as lymphocytes. Polymorpho- and mononuclear leukocytes can be activated by advanced glycation end products (12), oxidative stress (13,14), angiotensin II (15), and cytokines (16) in a state of hyperglycemia. Leukocytes may be activated through the release of cytokines, such as tumor necrosis factor-α (TNF-α) (17,18), transforming growth factor β1 (19), superoxide (20), nuclear factor κB (NF-κB) (21), monocyte chemoattractant protein 1, interleukin-1β, and others (17) to participate in the pathogenesis of diabetic micro- and macrovascular complications. Elevated differential cell counts, including counts of eosinophils, neutrophils, and monocytes, also predict the future incidence of CAD (11,22,23). However, there is no research concerning the differential leukocyte count in relation to diabetic nephropathy.

The mechanism responsible for leukocytosis in obesity, diabetes, or atherosclerosis is largely unknown. It has been determined that leptin, an adipocytokine, serves as a satiety factor and plays an important role in regulating body weight, homeostasis, and energy balance (24). Recent evidence suggests that leptin and the leptin receptor are parts of a pathway that stimulates hemopoiesis (25,26).

The purpose of the present study was to investigate the amounts of peripheral

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Received for publication 24 December 2004 and accepted in revised form 6 April 2005.

Abbreviations: CAD, coronary artery disease; CHD, coronary heart disease; DBP, diastolic blood pressure; NK-κB, nuclear factor κB; SBP, systolic blood pressure; TNF-α, tumor necrosis factor-α; UACR, urinary albumin-to-creatinine ratio; WBC, white blood cell; WHR, waist-to-hip ratio.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Table 1—*Clinical characteristics of study subjects*

	Normoalbuminuria	Microalbuminuria	Overt nephropathy	*P*
n	888	326	266	—
Sex (% female)	56.7	57.9	52.4	0.367
Age (years)	58.8 ± 11.6	62.0 ± 11.8	63.6 ± 11.2	<0.0001
Duration of diabetes (years)	7.7 ± 5.8	10.0 ± 6.9	14.4 ± 8.5	<0.0001
BMI (kg/m^2)	25.5 ± 3.6	25.8 ± 4.0	24.8 ± 3.9	0.004
WHR	0.94 ± 0.08	0.95 ± 0.07	0.95 ± 0.08	0.102
SBP (mmHg)	138 ± 21	150 ± 20	156 ± 20	<0.0001
DBP (mmHg)	83 ± 12	88 ± 13	88 ± 13	<0.0001
Fasting glucose (mmol/l)	9.0 ± 3.1	9.9 ± 3.6	10.5 ± 4.0	<0.0001
A1C (%)	8.2 ± 2.1	8.8 ± 2.1	9.0 ± 2.3	<0.0001
Total cholesterol (mmol/l)	5.06 ± 1.09	5.26 ± 1.15	5.46 ± 1.39	<0.001
Triglycerides (mmol/l)	1.72 ± 1.88	2.02 ± 1.61	2.50 ± 5.70	0.001
HDL cholesterol (mmol/l)	1.20 ± 0.34	1.16 ± 0.30	1.12 ± 0.32	0.002
LDL cholesterol (mmol/l)	3.07 ± 0.88	3.21 ± 1.00	3.31 ± 0.98	0.001
Uric acid (μmol/l)	333 ± 89	357 ± 101	410 ± 113	<0.001
Creatinine (μmol/l)	80 ± 27	80 ± 18	150 ± 115	<0.0001
Smokers (%)	20.0	21.7	21.4	0.780
Diabetes medication (%)				<0.0001
Oral hypoglycemic agent	93.6	86.5	67.2	
Insulin	1.4	5.3	12.0	
Oral hypoglycemic agent + insulin	5.0	8.2	20.8	

Data are means ± SD. See definition of normo- and microalbuminuria and overt nephropathy in RESEARCH DESIGN AND METHODS.

total and differential WBCs as they are altered and regulated by leptin in patients with diabetic nephropathy. We analyzed the peripheral total as well as differential WBC counts in type 2 diabetic patients in various stages of nephropathy to clarify the role of peripheral leukocytes in the pathogenesis of diabetic nephropathy and the role of plasma leptin in leukocytosis.

RESEARCH DESIGN AND METHODS

— Patients with type 2 diabetes who entered a disease management program at the Diabetic Clinic of the Pingtung Christian Hospital under the guidance of the National Health Bureau of Taiwan from January 2002 to June 2004 were studied (27,28). The diagnosis of type 2 diabetes was based on World Health Organization criteria (29). Patients presenting with symptoms suggestive of type 1 diabetes, defined as diabetic ketoacidosis, acute presentation with heavy ketonuria (3+), or continuous requirement of insulin within 1 year of diagnosis, were excluded (30). Patients with a previous diagnosis of urinary tract infection, urolithiasis, liver cirrhosis, congestive heart failure, chronic lung diseases, chronic otitis media, sinusitis, periodontitis (or undergoing dental treatment), chronic viral hepatitis, pelvic infection, or other known renal diseases were also excluded on the basis of interview, physical examination, and urinalysis.

Each patient participated in a detailed interview of his or her personal disease and smoking history. Information on smoking habits was assessed by a standardized questionnaire. Patients' smoking status was classified as never having smoked, former smoker (ceased smoking for at least 1 year), or current smoker. In this study, former and current smokers were analyzed as a group and compared with those who had never smoked. All of the study subjects were of Han Chinese origin, without any known ancestry of other ethnic origin, and lived in the same region at the time of the study. The Human Research Ethics Committee of our hospital approved this study and informed consent was obtained from each patient.

All of the patients underwent complete physical examination and routine biochemical analyses for blood and urine and were assessed for the presence and extent of macro- or microvascular diabetes complications. Anthropometric parameters were used to calculate BMI and the waist-to-hip ratio (WHR). A trained nurse measured blood pressure with a digital automatic blood pressure monitor (model HEM-907; Omron, Tokyo, Japan) after subjects had rested for 5 min. Fasting overnight blood and urine samples were collected for plasma biochemical parameters and urinary microalbumin analysis. Plasma biochemical parameters and urinary albumin concentrations were measured, as previously described (27,28).

Peripheral leukocyte analyses included total leukocyte counts and differential percentages of neutrophils, monocytes, lymphocytes, eosinophils, and basophils using an automated cell counter (XE-2100 Hematology Alpha Transportation System; Sysmex, Kobe, Japan). The absolute count of a leukocyte subtype was calculated as the product of its respective differential percentage and total leukocyte count. To minimize the confounding effect of infection, subjects with a WBC count $<4.0 \times 10^9$/l or $>10.0 \times 10^9$/l were rechecked for the analysis and examined extensively for possible occult chronic infections. Any specimen with abnormal or atypical leukocytes was reanalyzed and excluded. The laboratory analyses were under internal and external quality control at the laboratory of the College of American Pathologists surveys.

Table 2—*Total and differential leukocyte counts of study subjects*

	Normoalbuminuria	Microalbuminuria	Overt nephropathy	*P*
n	888	326	266	—
WBC count (10^9/l)				
Means ± SD	6,572 ± 1,647	6,984 ± 1,662	7,440 ± 1,769	<0.0001
Adjusted mean	6,555	6,962	7,407	<0.0001
Neutrophil count (10^9/l)				
Means ± SD	3,730 ± 1,283	4,101 ± 1,432	4,742 ± 1,590	<0.0001
Adjusted mean	3,728	4,074	4,660	<0.0001
Monocyte count (10^9/l)				
Means ± SD	440 ± 158	482 ± 173	553 ± 237	<0.0001
Adjusted mean	445	483	544	<0.0001
Lymphocyte count (10^9/l)				
Means ± SD	2,222 ± 736	2,206 ± 701	1,937 ± 769	<0.001
Adjusted mean	2,197	2,206	1,997	0.0003
Eosinophil count (10^9/l)				
Means ± SD	152 ± 139	171 ± 245	194 ± 146	0.002
Adjusted mean	157	175	191	0.017
Basophil count (10^9/l)				
Means ± SD	32 ± 19	35 ± 21	36 ± 25	0.040
Adjusted mean	32	35	36	0.031
Plasma leptin (ng/ml)*	7.8 ± 6.8	10.6 ± 9.1	15.8 ± 17.9	0.014

See definition of normo- and microalbuminuria and overt nephropathy in RESEARCH DESIGN AND METHODS. Adjusted means were adjusted by sex, age, BMI, WHR, and smoking status. *Plasma leptin levels were measured in 50 normoalbuminuric, 50 microalbuminuric, and 63 overt nephropathic subjects.

Diabetic patients were screened based on the results of the urinary analysis and urinary microalbumin and serum creatinine measurements. They were classified as having normal albuminuria (urinary albumin-to-creatinine ratio [UACR] <30 mg/g), microalbuminuria (UACR 30–300 mg/g, with at least two or more tests showing significant results), or overt nephropathy (UACR >300 mg/g and/or serum creatinine >1.5 mg/dl).

Table 3—*Univariate and multivariate analysis of risk factors associated with diabetic nephropathy*

	Simple		Multiple		
	Estimate	*P*	Estimate	*P*	95% CI
Age	0.015 ± 0.002	<0.0001	—	NS	—
Sex	−0.082 ± 0.050	0.104	—	NS	—
Duration of diabetes	0.048 ± 0.003	<0.0001	0.034 ± 0.003	<0.0001	0.027–0.040
BMI	−0.012 ± 0.007	0.079	—	NS	—
WHR	0.718 ± 0.318	0.024	—	NS	—
Smoking	0.039 ± 0.062	0.525	—	NS	—
SBP	0.014 ± 0.001	<0.0001	0.010 ± 0.001	<0.0001	0.008–0.012
DBP	0.012 ± 0.002	<0.0001	—	NS	—
Fasting glucose	0.003 ± 0.000	<0.0001	0.001 ± 0.000	0.001	0.001–0.002
A1C	0.064 ± 0.011	<0.0001	—	NS	—
Total cholesterol	0.002 ± 0.001	<0.0001	0.002 ± 0.001	<0.0001	0.001–0.003
Triglycerides	0.292 ± 0.041	<0.0001	—	NS	—
HDL cholesterol	−0.008 ± 0.002	<0.0001	−0.008 ± 0.002	<0.0001	−0.012 to −0.005
LDL cholesterol	0.002 ± 0.001	0.001	—	NS	—
WBC count	0.111 ± 0.014	<0.0001	0.063 ± 0.017	0.0002	0.029–0.097
Neutrophil count	0.000 ± 0.000	<0.0001	0.000 ± 0.000	<0.001	0.000–0.000
Monocyte count	0.001 ± 0.000	<0.0001	0.001 ± 0.000	<0.0001	0.000–0.001
Lymphocyte count	−0.000 ± 0.000	<0.0001	−0.000 ± 0.000	<0.0001	0.000–0.000
Eosinophil count	0.001 ± 0.000	0.0002	—	NS	—
Basophil count	0.003 ± 0.001	0.039	—	NS	—

Data are means ± SE. In multiple linear stepwise regression analysis, all covariates were used for analysis (r^2 = 0.280, n = 1,480). NS, variable not accepted as significant for stepwise analysis.

Table 4—*Prevalence of diabetic nephropathy categorized according to total and differential white cell count quartiles and multivariate analysis of the impact of leukocyte count on diabetic nephropathy*

	1st quartile	2nd quartile	3rd quartile	4th quartile	*P* for trend
Total WBC count	2.55–5.59	5.60–6.66	6.67–7.83	7.84–11.94	<0.001
Normoalbuminuria (%)	72.3	60.7	58.2	49.5	<0.0001
Microalbuminuria (%)	17.7	22.8	23.2	23.7	<0.0001
Overt nephropathy (%)	10.1	16.5	18.6	26.9	0.001
Multivariate	1.0	2.0 (1.33–3.10)	2.4 (1.59–3.70)	4.2 (2.77–6.40)	—
Neutrophil count	1,005–2,979	2,980–3,741	3,742–4,772	4,773–10,341	<0.001
Normoalbuminuria (%)	72.7	64.9	59.2	43.8	<0.0001
Microalbuminuria (%)	18.7	22.4	22.4	23.8	<0.0001
Overt nephropathy (%)	8.7	12.7	18.4	32.4	0.019
Multivariate	1.0	1.6 (1.00–2.41)	2.5 (1.65–3.87)	5.5 (3.64–8.40)	—
Monocyte count	47.9–341	342–433	434–559	560–1685	<0.001
Normoalbuminuria (%)	67.8	68.2	60.3	44.3	<0.0001
Microalbuminuria (%)	20.1	19.1	22.2	26.0	0.019
Overt nephropathy (%)	12.2	12.7	17.6	29.7	0.995
Multivariate	1.0	1.1 (0.69–1.62)	1.7 (1.13–2.56)	3.5 (2.36–5.30)	—
Lymphocyte count	221–1,622	1,623–2,067	2,068–2,580	2,581–5,958	<0.001
Normoalbuminuria (%)	54.3	60.0	63.8	62.4	0.0001
Microalbuminuria (%)	17.8	23.0	22.2	24.3	<0.0001
Overt nephropathy (%)	27.8	17.0	14.1	13.2	0.006
Multivariate	1.0	0.7 (0.46–0.99)	0.6 (0.38–0.83)	0.6 (0.43–0.95)	—

Data are odds ratios (95% CI) and are adjusted for age, sex, BMI, WHR, and smoking status. See definition and criteria of diabetic nephropathy in RESEARCH DESIGN AND METHODS. Patients with normoalbuminuria were used as a reference, and the sum of patients with microalbuminuria and overt nephropathy were used as cases.

Plasma leptin measurement

To investigate the mechanism responsible for leukocytosis associated with diabetic nephropathy, plasma leptin was measured from 163 randomly selected diabetic subjects who had fasted overnight. Samples were kept at −80°C and were diluted 100-fold before the assay. The concentration of plasma leptin was determined by a commercial, solid-phase enzyme-linked immunosorbent assay kit (Quantikine Human Leptin Immunoassay; R&D Systems, Minneapolis, MN). The dilution curve was parallel to the standard curve. The intra-assay coefficients of variation were 3.2–6.9% for values of 15.6–283.3 pg/ml. The leptin measurement was done in a single experiment.

Statistical analysis

Data are shown as means ± SD, unless otherwise noted. All statistical analyses were performed using the Statistical Package for Social Science program (SPSS for Windows, version 10.1; SPSS, Chicago, IL). ANOVA was used for between-group comparisons for continuous variables and the χ^2 test was used for categorical variables. Because the distribution of serum triglycerides and plasma leptin levels was skewed, logarithmically transformed values were used for statistical analysis. Mean total and differential WBC counts were calculated across the categorized diabetic nephropathy.

The general linear modeling function analysis was used to control for potential confounders other than age (e.g., sex, BMI, WHR, smoking status). The association between diabetic nephropathy and all other parameters was first analyzed by univariate analysis and then by multivariate linear stepwise regression analysis. In the regression analysis, known conventional risk factors for cardiovascular diseases were included as covariates in the final model. WBC counts were grouped into quartiles and the multivariate-adjusted odds ratios (ORs) were presented with 95% CI. These quartiles were computed with the lowest category as the reference group.

The relation between total and differential leukocytes counts and anthropometric and biochemical parameters, including plasma leptin concentrations, was examined using Pearson's correlation coefficients with two-tailed tests of significance. $P < 0.05$ was considered significant.

RESULTS

— A total of 1,480 type 2 diabetic patients were included in this cross-sectional study. The clinical characteristics of the patients stratified by nephropathy status are given in Table 1. The prevalence of normoalbuminuria, microalbuminuria, and overt nephropathy in the present study was 60.0, 22.0, and 18.0%, respectively. Patients with nephropathy were older and had a longer duration of diabetes; higher systolic blood pressure (SBP) and diastolic blood pressure (DBP); higher fasting glucose, HbA_{1c} (A1C), total and LDL cholesterol, triglycerides, uric acid, and creatinine levels; and lower HDL cholesterol concentrations and BMI values than those without albuminuria. Smoking profiles were not different among the study groups. Patients with overt nephropathy had a higher prevalence of insulin treatment.

There was a significant trend in total and differential leukocyte counts ($P < 0.05$ by ANOVA) across the three study groups, with the main difference being detected between overt nephropathic and normoalbuminuric subjects. The mean peripheral total WBC, neutrophil, monocyte, eosinophil, and basophil counts increased, whereas, in contrast, the lymphocyte count decreased parallel to the severity of nephropathy (Table 2). Adjust-

Table 5—*Pearson's correlation analysis of peripheral total and differential leukocyte counts with metabolic parameters*

	Total WBC count		Monocyte count		Neutrophil count		Lymphocyte count	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*
Age	−0.016	0.527	0.091	<0.001	0.082	0.002	−0.214	<0.001
Sex	−0.032	0.226	−0.169	<0.001	−0.028	0.284	0.055	0.035
BMI	0.119	<0.001	0.038	0.141	0.047	0.069	0.164	<0.001
WHR	0.069	0.009	0.056	0.032	0.099	<0.001	−0.045	0.091
Fasting glucose	0.056	0.032	0.032	0.221	0.088	0.001	−0.045	0.088
A1C	0.060	0.022	0.081	0.002	0.071	0.007	−0.013	0.622
SBP	0.091	<0.001	0.073	0.005	0.125	<0.001	−0.054	0.039
DBP	0.079	0.002	0.040	0.131	0.066	0.012	0.038	0.145
Total cholesterol	0.067	0.010	0.026	0.323	0.064	0.014	0.029	0.262
Triglycerides	0.095	<0.001	0.016	0.545	0.070	0.008	0.080	0.002
HDL cholesterol	−0.134	<0.001	−0.144	<0.001	−0.107	<0.001	−0.042	0.110
LDL cholesterol	0.057	0.031	0.036	0.166	0.075	0.004	−0.015	0.556
Uric acid	0.071	0.022	0.113	<0.001	0.080	0.010	−0.049	0.115
Creatinine	0.098	<0.001	0.174	<0.001	0.151	<0.001	−0.128	<0.001
UACR	0.078	0.007	0.071	0.013	0.137	<0.001	−0.103	<0.001
Smoking status	0.108	<0.001	0.154	<0.001	0.069	0.008	0.052	0.045

ments for age, sex, BMI, WHR, and smoking status did not affect the significance of these differences.

Univariate analyses revealed that age; duration of diabetes; WHR; systolic and DBP; fasting glucose; A1C; triglycerides; total, HDL, and LDL cholesterol; and total and differential leukocytes counts were all associated with diabetic nephropathy (Table 3). A multiple linear stepwise regression analysis was performed with the stages of diabetic nephropathy as the dependent variables; the nephropathy status was predictive of the following variables: age; sex; known duration of diabetes; smoking status; BMI; WHR; systolic and DBP; fasting plasma glucose; A1C; triglycerides; total, HDL, and LDL cholesterol; and total and differential leukocytes counts. Our analysis showed that the duration of diabetes, SBP, fasting plasma glucose, total and HDL cholesterol, and total leukocyte, neutrophil, monocyte, and lymphocyte counts were independent risk factors for diabetic nephropathy ($r^2 = 0.280$, $P < 0.001$) (Table 3).

When subjects were divided into quartiles according to total WBC, neutrophil, monocyte, and lymphocyte counts, the prevalence of diabetic nephropathy was significantly ($P < 0.001$) associated with the leukocyte count (Table 4). Multiple logistic regression analysis ORs for the risk of the presence/absence of nephropathy in the 1st, 2nd, and 3rd quartiles, adjusted for age, sex, BMI, WHR, and smoking status, were significantly and positively associated with total WBC, neutrophil, and monocyte counts and negatively associated with lymphocyte count. The total WBC count showed a significant correlation with all parameters of the metabolic syndrome, such as BMI; WHR; fasting plasma glucose; A1C; systolic and DBP; triglycerides; total, HDL, and LDL cholesterol; uric acid; creatinine; UACR; and smoking status (Table 5). The monocyte counts were correlated with age, sex, WHR, A1C, SBP, HDL cholesterol, uric acid, creatinine, UACR, and smoking status. In one interesting finding, the peripheral neutrophil count correlated well with all parameters included in this study except for sex and BMI. The lymphocyte count correlated negatively with age, SBP, serum creatinine, and the UACR, and positively with sex, BMI, triglyceride levels, and smoking status.

The mean plasma leptin concentrations in 50 patients with normoalbuminuria, 50 patients with microalbuminuria, and 63 patients with overt nephropathy were 7.8 ± 6.8, 10.6 ± 9.1, and 15.8 ± 17.9 ng/ml, respectively. There was a significant trend in serum leptin concentrations ($P = 0.014$ by ANOVA) (Table 2) across the three groups, with the main difference being detected between overt nephropathic and normoalbuminuric subjects. Using Pearson's correlation analysis, plasma leptin levels were significantly associated with total WBC counts ($r = 0.194$, $P = 0.014$), whereas plasma leptin levels were not correlated with individual differential leukocyte counts. The association still existed after adjusting for age, sex, WHR, and smoking status by multiple linear regression analysis (estimate = 0.028, $P = 0.0082$).

CONCLUSIONS — Our study showed that peripheral total leukocyte, neutrophil, monocyte, and lymphocyte counts, even within the normal range, are independently associated with nephropathy in subjects with type 2 diabetes in a dosage-related manner. The association between leukocytes and diabetic nephropathy persists even after controlling for conventional risk factors, including age, sex, smoking status, blood pressure, lipid profile, and glucose control, as well as obesity. Furthermore, the plasma leptin concentration was increased in patients with nephropathy and correlated well with the peripheral total leukocyte counts. These findings are consistent with current evidence regarding the association of inflammatory markers, including WBC count, with the development of diabetic microvascular and macrovascular complications (9,10).

Numerous epidemiological and clinical studies have shown leukocytosis to be an independent predictor of insulin resistance, type 2 diabetes, micro- and macrovascular complication of diabetes, and future cardiovascular events in patients with stable angina, unstable angina, or a history of myocardial infarction (1–8). The differential cell counts, including

counts of eosinophils, neutrophils, and monocytes, also predict the future incidence of CHD (11,22,23). Although there is no research reporting the differential leukocyte count in relation to diabetic nephropathy, our study clarifies that the composite members of WBC, especially the neutrophils and mononuclear cells (monocytes and lymphocytes), are associated with nephropathy in type 2 diabetic patients. To the best of our knowledge, ours is the first report to demonstrate that peripheral neutrophil, monocyte, and lymphocyte counts are associated with diabetic nephropathy.

The mechanism responsible for the increased total and differential leukocytes in diabetic patients with nephropathy is a matter of speculation. A plausible hypothesis is that leptin might be involved in increased leukocyte counts (25,26). Leptin has been reported to stimulate myeloid differentiation from human bone marrow CD34+ progenitors (25) and can induce proliferation, differentiation, and functional activation of hemopoietic cells (26). We measured plasma leptin concentrations and confirmed previous reports that plasma leptin levels are increased in patients with diabetic nephropathy (31). Furthermore, our results showed that plasma leptin levels correlate well with total leukocyte count. We propose that leptin may enhance the release and activation of leukocyte from bone marrow and contribute to the development and progression of diabetic nephropathy.

Other mechanisms contributing to leukocytosis in patients with nephropathy may be related to plasma cortisol and the changing insulin levels in renal disease. Both factors are known to increase WBC counts by increasing neutrophil influx from marrow storage and decreasing efflux from the blood stream (32,33). In addition, cortisol and insulin may increase the WBC count by stimulating leptin secretion from adipocytes (34).

The biological mechanisms by which leukocytes might influence the development and progression of diabetic nephropathy are multiple and protean. Data from research show that leukocytes play an important role in the initiation and progression of renal disease, including inflammatory mechanisms independent of infection, causing proteolytic and oxidative damage to the mesangial cells (17). Macrophages and lymphocytes are prominent in the human glomeruli of diabetic nephropathy, even in the earliest stages of the disease process (35). WBCs in diabetic patients may be activated by advanced glycation end products or reactive oxygen species (12–14) and cytokines (16). Activated leukocytes secrete many kinds of cytokines and transcription factors that have a crucial role in inflammation, including TNF-α, NF-κB, interleukin-1β, and transforming growth factor β, thereby contributing to glomerulosclerosis (17–21).

In addition, activated leukocytes can release superoxide radicals and proteases, all of which promote oxidative stress (20). The latter can then activate the transcription of NF-κB in peripheral mononuclear blood cells (14,21). All of these pathways can lead to diabetic nephropathy. Taken together, it is plausible that low-grade chronic inflammatory responses can interact with other risk factors, leading to widespread vascular damage, endothelial dysfunction, increased oxidative stress, and increased production of growth factors and cytokines, thereby causing renal damage.

The peripheral lymphocyte count was inversely related to the severity of diabetic nephropathy in the present study. In a retrospective study of CAD patients, the 5-year survival rate was significantly better for patients who had a normal lymphocyte count as compared with those with a relatively low lymphocyte count (36). Studies of patients with congestive heart failure have also identified lymphocyte counts or a low percentage of lymphocytes as an adverse prognostic factor (37). The mechanism for the decrease in lymphocyte counts in patients with diabetic nephropathy or cardiovascular disease is still largely unknown. Our study revealed that peripheral lymphocyte counts are negatively associated with SBP; serum uric acid levels may provide some clues into the role of peripheral lymphocytes in protection from diabetic nephropathy.

One limitation of our study was its cross-sectional design, which limited our ability to infer a causal relation between total and differential WBCs and diabetic nephropathy. Our analyses were based on a single measurement of WBC counts that may not reflect the relation over time. It would be interesting to measure the serial changes of WBC counts to further clarify the role of WBCs in the development of diabetic nephropathy. It would also be useful to know if the WBC measurements are stable over time. The bias effect of such measurement should be attenuated by a larger number of patients enrolled.

In addition, although we controlled for major risk factors, including potential confounders such as hypertension, dyslipidemia, BMI, WHR, and smoking, the existence of unrecognized confounding variables is always possible. Smoking status did not appear to have an additive effect on the prevalence of microvascular complications in our study, unlike the case with macrovascular complications. Although we extensively surveyed for the presence of a chronic infection state that could affect the WBC count, some chronic infections still could not be controlled for in the present study and might have been potential confounding factors. These include chronic infections with *Chlamydia* pneumonia, *Helicobacter pylori*, cytomegalovirus, and other organisms that have been postulated as potential risk factors for atherosclerosis (38).

Finally, this study did not provide serological data on infection or other markers of inflammation, such as C-reactive protein, TNF-α, or leukocyte adhesion molecules. Future studies should focus on whether differential WBCs are activated to secrete specific cytokine markers involved in the pathogenesis of diabetic nephropathy.

In conclusion, our study showed that the peripheral total WBC, neutrophil, monocyte, and lymphocyte counts are associated with diabetic nephropathy. Plasma leptin concentrations are increased in patients with nephropathy and associated with leukocytosis in patients with type 2 diabetes.

Acknowledgments— This work was supported by a grant from the National Science Council of Taiwan (93-2314-B-475-002).

We are grateful to the staff of the diabetes care team for their assistance in the various measurements and other organizational aspects of this study.

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