

Eosinophil Count Is Positively Correlated with Albumin Excretion Rate in Men with Type 2 Diabetes

Michiaki Fukui, Muhei Tanaka, Masahide Hamaguchi, Takafumi Senmaru, Kazumi Sakabe, Emi Shiraishi, Ichiko Harusato, Masahiro Yamazaki, Goji Hasegawa, and Naoto Nakamura

Department of Endocrinology and Metabolism, Kyoto Prefectural University of Medicine, Graduate School of Medical Science, Kyoto, Japan

Background and objectives: Patients with allergic disorders such as allergic rhinitis or asthma have been reported to be at increased risk for atherosclerosis. In this study, we evaluated the relationships between peripheral eosinophil count and degree of albumin excretion rate, which is a useful marker of cardiovascular mortality as well as diabetic nephropathy in patients with type 2 diabetes.

Design, setting, participants, & measurements: We evaluated relationships of peripheral eosinophil count to degree of albumin excretion rate as well as to major cardiovascular risk factors, including age, BP, serum lipid concentration, and glycemic control (glycosylated hemoglobin); body mass index; current treatment for diabetes; smoking status; and presence of cardiovascular disease in 783 patients (416 men and 367 women) with type 2 diabetes.

Results: Log(eosinophil count) was positively associated with systolic BP ($r = 0.124$, $P = 0.0108$), serum triglyceride concentration ($r = 0.108$, $P = 0.0284$), and log(albumin excretion rate) ($r = 0.301$, $P < 0.0001$) in men; however, no association was found between log(eosinophil count) and log(albumin excretion rate) ($r = 0.085$, $P = 0.1050$) in women. Multivariate linear regression analysis demonstrated that log(eosinophil count) ($\beta = 0.260$, $P < 0.0001$), duration of diabetes ($\beta = 0.203$, $P = 0.0003$), glycosylated hemoglobin ($\beta = 0.117$, $P = 0.0238$), systolic BP ($\beta = 0.205$, $P = 0.0001$), and serum triglyceride concentration ($\beta = 0.162$, $P = 0.0038$) were independent determinants of log(albumin excretion rate) in men.

Conclusions: Allergic disorders may be associated with microalbuminuria in men with type 2 diabetes.

Clin J Am Soc Nephrol ●●: ●●●-●●●, 2009. doi: 10.2215/CJN.03330509

Cardiovascular disease (CVD) is the leading cause of mortality and morbidity in patients with type 2 diabetes (1,2). Evidence has accumulated that inflammation such as chronic infection or collagen disease is crucially involved in atherogenesis (3). In addition, patients with allergic disorders such as allergic rhinitis or asthma have also been reported to be at increased risk for atherosclerosis (4). Indirect support for this clinically relevant concept is derived from previous studies that showed disorders such as eosinophilia, elevated serum IgE level, positive skin-prick test, and self-reported asthma are associated with increased risk for CVD (5). Male gender is an independent risk factor for CVD (6). Moreover, elevated albumin excretion rate, which is a useful marker of diabetic nephropathy, has been reported to be associated with increased risk for cardiovascular mortality (7). No study has investigated the association between eosinophil count in peripheral blood and diabetic nephropathy. In this study, we evaluated the relationships between peripheral total leukocyte

count or eosinophil count and degree of albumin excretion rate in patients with type 2 diabetes.

Materials and Methods

Patients

Peripheral total leukocyte count and eosinophil count were measured in 783 consecutive patients (416 men and 367 women) who had type 2 diabetes and were recruited from the outpatient clinic at the Kyoto Prefectural University of Medicine. Blood samples were obtained during a period of high-allergen exposure in Japan (from March to May). Type 2 diabetes was diagnosed according to the Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus (8). Retinopathy was assessed by chart review and was graded as follows: No diabetic retinopathy, simple diabetic retinopathy, and proliferative diabetic retinopathy. Nephropathy was graded as follows: Normoalbuminuria, urinary albumin excretion <30 mg/g creatinine (Cr); microalbuminuria, 30 to 300 mg/g Cr; or macroalbuminuria, >300 mg/g Cr. Sitting BP was measured after a 5-min rest. CVD was defined as a previous myocardial or cerebral infarction on the basis of the clinical history or physical examination. Patients were classified as nonsmokers, past smokers, or current smokers according to a self-administered questionnaire. Patients with malignant disease, liver cirrhosis, hematologic disease, or advanced renal dysfunction (serum Cr >2.0 mg/dl) were excluded from this study. Patients were also excluded when they were taking any medications that are known to affect allergic reaction (e.g., corticosteroid, antiallergy agents).

Received May 16, 2009. Accepted August 17, 2009.

Published online ahead of print. Publication date available at www.cjasn.org.

Correspondence: Dr. Michiaki Fukui, Department of Endocrinology and Metabolism, Kyoto Prefectural University of Medicine, Graduate School of Medical Science, 465 Kajii-cho, Kawaramachi-Hirokoji, Kamigyo-ku, Kyoto 602-8566, Japan. Phone: +81-75-251-5505; Fax: +81-75-252-3721; E-mail: sayarinapm@hotmail.com

Table 1. Clinical characteristics of patients with diabetes

Characteristic	Men	Women
<i>n</i>	416	367
Age (yr; mean ± SD)	63.6 ± 11.1 ^a	65.2 ± 12.0
Age at onset (yr; mean ± SD)	49.7 ± 12.3 ^a	52.0 ± 13.6
Duration of diabetes (yr; mean ± SD)	13.9 ± 11.5	13.2 ± 9.8
BMI (kg/m ² ; mean ± SD)	23.3 ± 3.5	23.1 ± 3.8
HbA _{1c} (%; mean ± SD)	7.2 ± 1.2 ^b	7.4 ± 1.3
SBP (mmHg; mean ± SD)	135 ± 16 ^b	131 ± 18
DBP (mmHg; mean ± SD)	77 ± 10 ^c	71 ± 11
Total cholesterol (mmol/L; mean ± SD)	4.97 ± 0.83	5.28 ± 0.86
Triglyceride (mmol/L; mean ± SD)	1.53 ± 1.01	1.46 ± 1.03
HDL cholesterol (mmol/L; mean ± SD)	1.32 ± 0.36 ^c	1.55 ± 0.41
Smoking (none/past/current)	89/192/135 ^c	276/41/50
Retinopathy (NDR/SDR/PDR)	286/59/71	239/59/69
Nephropathy (normo-/micro-/macroalbuminuria)	230/130/56	207/123/37
Cardiovascular disease (-/+)	339/77 ^a	322/45
Current treatment (diet/OHA/insulin)	52/268/96	31/226/110
Total leukocyte count (10 ⁶ /L; mean ± SD)	6730 ± 1830	6260 ± 1740
Eosinophil count (10 ⁶ /L; mean ± SD)	210 ± 150 ^b	180 ± 140

BMI, body mass index; DBP, diastolic BP; NDR, no diabetic retinopathy; OHA, oral hypoglycemic agents; PDR, proliferative diabetic retinopathy; SDR, simple diabetic retinopathy.

^a*P* < 0.05, ^b*P* < 0.01, ^c*P* < 0.0001 versus women.

Experimental Design

We evaluated relationships of peripheral eosinophil count to degree of albumin excretion rate as well as to major cardiovascular risk factors, including age, BP, serum lipid concentration, and glycemic control (glycosylated hemoglobin [HbA_{1c}]); body mass index; current treatment for diabetes; smoking status; and presence of CVD. This study was approved by the local research ethics committee and was conducted in accordance with the Declaration of Helsinki, and informed consent was obtained from all participants.

Biochemical Analysis

Peripheral leukocyte analyses included total leukocyte counts and percentages of eosinophils using an automated cell counter (XE-2100

Hematology Alpha Transportation System; Sysmex, Kobe, Japan). The absolute count of eosinophil was calculated as the product of its percentage and total leukocyte count. Serum total cholesterol, HDL cholesterol, and triglyceride concentrations were assessed using standard enzymatic methods. HbA_{1c} was assayed using HPLC. Urinary albumin and Cr concentration were determined in an early morning spot urine sample. Albumin excretion rate was measured with an immunoturbidimetric assay. A mean value for urinary albumin excretion was determined from three urine collections.

Statistical Analysis

StatView 5.0 (SAS Institute, Cary, NC) and R 2.9.0 were used for statistical analyses, and *P* < 0.05 was considered statistically significant.

Table 2. Correlation between log(eosinophil count) and other variables in both genders

Variable	Men		Women	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
Age	0.062	0.2024	-0.054	0.3020
Age at onset	0.099	0.0523	0.043	0.4365
Duration of diabetes	-0.033	0.5238	-0.082	0.1362
BMI	0.057	0.2487	0.099	0.0724
HbA _{1c}	0.044	0.3600	0.077	0.1409
SBP	0.124	0.0108	-0.017	0.7528
DBP	0.050	0.3104	-0.003	0.9607
Total cholesterol	0.077	0.1127	0.016	0.7573
Triglyceride	0.108	0.0284	0.153	0.0037
HDL cholesterol	-0.039	0.4157	-0.085	0.1080
Log(albumin excretion rate)	0.301	<0.0001	0.085	0.1050

Means, medians, or frequencies of potential confounding variables were calculated. Skewed variable such as eosinophil count is presented as median (interquartile range), and continuous variables are presented as the mean ± SD. Unpaired *t* tests, Mann-Whitney *U* tests, χ^2 tests, or Kruskal-Wallis tests were conducted as appropriate to assess statistical significance of differences between groups. Because both total leukocyte count or eosinophil count and albumin excretion rate showed skewed distributions, logarithmic transformation was carried out before performance of correlation and regression analysis. The relationships between log(eosinophil count) and log(albumin excretion rate), age, glycemic control, or other variables were examined by Pearson correlation analyses. For examination of the effects of various factors on log(albumin excretion rate), the following factors were considered simultaneously as independent variables for multivariate linear regression analysis in both genders: Log(total leukocyte count), log(eosinophil count), age, duration of diabetes, body mass index, HbA_{1c}, systolic BP (SBP), serum total cholesterol concentration, and smoking status.

Results

Characteristics of the 783 patients (416 men and 367 women) who had type 2 diabetes and were enrolled in this study are shown in Table 1. Relationships between log(eosinophil count) and other variables for both genders are shown in Table 2. SBP, serum triglyceride concentration, and log(albumin excretion rate) were positively associated with log(eosinophil count) in men. Serum triglyceride concentration was positively associated with log(eosinophil count), but no association was found between log(albumin excretion rate) and log(eosinophil count) in women. Log(eosinophil count) differs significantly according to diabetic retinopathy and nephropathy in men but not in women (Table 3). In addition, log(eosinophil count) did not differ between patients with or without CVD or between patients who were treated with and without insulin for both genders. At first, we investigated the association of log(albumin) excretion with gender, log(eosinophil count), and the interaction term gender * log(eosinophil count) in linear regression analyses. The interaction term was significant (*P* < 0.001), and then the multivariate analyses were stratified by gender. Multivariate linear regression analysis demonstrated that log(eosinophil count), duration of diabetes, HbA_{1c}, SBP, and serum triglyceride concentration were independent determinants of log(albumin excretion rate) in men (Table 4).

Discussion

Log(eosinophil count) correlated positively with degree of urinary albumin excretion in men with type 2 diabetes, which suggests that eosinophil has a potential role for progression of diabetic nephropathy. It is interesting that log(eosinophil count) differs significantly according to diabetic retinopathy as well as diabetic nephropathy in men. Multiple regression analysis also identified log(eosinophil count) as an independent determinant of log(albumin excretion rate). Our data suggest a possible link between allergic disease, as manifested by elevated eosinophil count, and diabetic nephropathy in men with type 2 diabetes. To our knowledge, this is the first report of such a relationship and suggests new avenues for research into the pathogenesis of diabetic nephropathy.

For explaining plausible mechanisms of eosinophils in pro-

Table 3. Comparisons of eosinophil count in various groups

Group	Men	Women
Retinopathy (NDR/SDR/PDR)	170 (100 to 250)/190 (120 to 290)/190 (110 to 340) ^a	150 (90 to 220)/130 (80 to 200)/150 (90 to 250)
Nephropathy (normo-/micro-/macroalbuminuria)	140 (90 to 210)/190 (120 to 310)/250 (170 to 350) ^b	150 (90 to 210)/140 (90 to 220)/170 (90 to 320)
Smoking (none/past/current)	160 (110 to 270)/180 (110 to 290)/170 (100 to 250)	150 (90 to 220)/130 (60 to 220)/150 (110 to 290)
Cardiovascular disease (-/+)	170 (100 to 280)/190 (130 to 280)	150 (90 to 230)/140 (90 to 180)
Insulin treatment (-/+)	160 (100 to 270)/190 (110 to 280)	150 (90 to 210)/150 (90 to 240)

Data are median (interquartile range).

^a*P* < 0.05.

^b*P* < 0.0001.

Table 4. Multivariate linear regression analysis on log(albumin excretion rate) in both genders

Parameter	Men		Women	
	β	<i>P</i>	β	<i>P</i>
Age	0.100	0.0854	0.182	0.0091
Duration of diabetes	0.203	0.0003	0.073	0.2895
BMI	-0.028	0.6323	0.131	0.0546
HbA _{1c}	0.117	0.0238	0.138	0.0419
SBP	0.205	0.0001	0.158	0.0150
Total cholesterol	-0.066	0.2182	0.006	0.9199
Triglyceride	0.162	0.0038	0.314	<0.0001
Smoking status	0.068	0.1819	-0.046	0.4795
Log(total leukocyte count)	0.041	0.4334	-0.067	0.3353
Log(eosinophil count)	0.260	<0.0001	0.042	0.5216

moting arteriosclerosis, several mechanisms can be suggested. Marone *et al.* (9) proposed that eosinophils can secrete other eosinophil cationic protein and major basic protein, which can activate mast cells. These activated mast cells can then release histamine, which can result in arterial spasm. Hallgren *et al.* (10) suggested the active participation of eosinophils in the inflammatory process in patients with acute myocardial infarction, and Trillo *et al.* (11) found eosinophils in the aortic fatty streaks of African green monkeys. Moreover, the enzyme arachidonate 15-lipoxygenase is expressed in significant quantities in eosinophils (12). This enzyme may be involved in oxidative modification of LDL in the early phase of atherogenesis and may contribute to the development of arteriosclerotic lesions. Taken together, it seems plausible that eosinophils have a role in endothelial inflammation.

The nature of the association observed between allergy and degree of urinary albumin excretion remains speculative; however, allergic disorders may constitute a true risk factor. More than 30 yr ago, Wittig *et al.* (13) and Reeves *et al.* (14) reported seasonal nephritic syndrome. There is ample evidence that localized allergic disease elicits a systemic inflammation response that is mediated by the release of vasoactive peptides and cytokines into the circulation. Endothelial cells at locations distinct from the site of allergen exposure were found to enhance adhesion molecule expression, thereby facilitating leukocyte trafficking into the vessel wall and potentially promoting arteriosclerosis. In a subgroup of men with type 2 diabetes ($n = 206$), log(eosinophil count) tended to be positively associated with serum high-sensitivity C-reactive protein ($r = 0.120$, $P = 0.085$), although it did not reach statistical significance. Among men, allergic rhinitis, which was self-reported, was diagnosed in 25.1% of patients with diabetes in this study population. Log(eosinophil count) and log IgE in patients with allergic rhinitis were significantly higher than those in patients without allergic rhinitis (2.28 ± 0.28 versus 2.21 ± 0.30 [$P = 0.0449$] and 2.05 ± 0.76 versus 1.79 ± 0.75 [$P = 0.0197$], respectively). Moreover, log(eosinophil count) was positively associated with log(IgE) ($r = 0.239$, $P = 0.0003$). Because allergy is a common disease and increases in Western countries and in Japan, our findings may have important implications for diabetic ne-

phropathy. Surprising, the strength of log(eosinophil count) as an independent determinant of log(albumin excretion rate) was greater than those of known factors such as SBP, HbA_{1c}, and duration of diabetes in multivariate linear regression analysis.

Limitations of our study include a cross-sectional design, which does not permit the determination of causality. Allergic rhinitis is seasonal; however, it must be considered that blood samples were obtained during a period of high-allergen exposure. Log(albumin excretion rate) was not significantly associated with log(eosinophil count) in women with type 2 diabetes. We cannot explain this gender difference; however, Criqui *et al.* (15) also demonstrated that IgE may be an independent marker for CVD in men but not in women. Peripheral leukocyte count has been shown to be associated with insulin resistance, type 2 diabetes (16), CVD (17), and diabetic microangiopathy and macroangiopathy (18,19); however, the correlation coefficient between log(total leukocyte count) and log(eosinophil count) was 0.33, which means that 0.67 of the variation in eosinophils was not explained by its association with total leukocytes. Multivariate linear regression analysis also identified log(eosinophil count) as an independent determinant of log(albumin excretion rate) after adjustment for log(total leukocyte count). Moreover, log(eosinophil count) was as an independent determinant of log(albumin excretion rate) after adjustment for log(total leukocyte count) and other different leukocyte counts (data not shown). To our knowledge, this is the first study to investigate the association between log(eosinophil count) and degree of albumin excretion rate in patients with type 2 diabetes. Although we are unable to determine whether eosinophil has a causative effect, these findings suggest that eosinophilia combined with diabetes might be associated with an increased risk for the development and progression of diabetic nephropathy.

The clinical relevance relates to potential preventive and therapeutic approaches, whereas the diagnostic relevance concerns the diagnostic utility of eosinophil count, as a provisional new marker of diabetic nephropathy as well as arteriosclerosis, that can be measured easily in the clinical laboratory and applied in medical practice. The intriguing concept of a role for eosinophils in diabetic nephropathy holds great promise for the

development of new preventive measures involving antiallergy agents. Large prospective trials are needed to assess better the effects of allergic disorders on diabetic nephropathy in men with type 2 diabetes.

Acknowledgments

We thank Mr. Masao Shimotsuma for technical assistance.

Disclosures

None.

References

1. Isomaa B, Almgren P, Tuomi T, Forsen B, Lahti K, Nissen M, Taskinen MR, Groop L: Cardiovascular morbidity and mortality associated with the metabolic syndrome. *Diabetes Care* 24: 683–689, 2001
2. Multiple Risk Factor Intervention Trial Research Group: Multiple Risk Factor Intervention Trial: Risk factor changes in mortality results. *JAMA* 248: 1465–1470, 1982
3. Wick G, Knoflach M, Xu Q: Autoimmune and inflammatory mechanisms in atherosclerosis. *Annu Rev Immunol* 22: 362–403, 2004
4. Togias A: Systemic effects of local allergic disease. *J Allergy Clin Immunol* 113: S8–S14, 2004
5. Hespers JJ, Rijcken B, Schouten JP, Postma DS, Weiss ST: Eosinophilia and positive skin tests predict cardiovascular mortality in a general population sample followed for 30 years. *Am J Epidemiol* 150: 482–491, 1999
6. European Atherosclerosis Society Study Group: The recognition and management of hyperlipidaemia in adults: A policy statement of the European Atherosclerosis Society. *Eur Heart J* 9: 571–600, 1988
7. Dinneen SF, Gerstein HC: The association of microalbuminuria and mortality in non-insulin-dependent diabetes mellitus: A systematic overview of the literature. *Arch Intern Med* 157: 1413–1418, 1997
8. Expert Committee on the Diagnosis and Classification of Diabetes Mellitus: Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care* 25: S5–S20, 2002
9. Marone G: Asthma: Recent advances. *Trends Immunol Today* 19: 5–9, 1998
10. Hallgren R, Venge P, Cullhed I, Olsson I: Blood eosinophils and eosinophil cationic protein after acute myocardial infarction or corticosteroid administration. *Br J Haematol* 42: 147–154, 1979
11. Trillo AA: The cell population of aortic fatty streaks in African green monkeys with special reference to granulocytic cells: An ultrasound study. *Atherosclerosis* 43: 259–275, 1982
12. Sigal E, Sloane DL, Conrad DJ: Human 15-lipoxygenase: Induction by interleukin-4 and insights into positional specificity. *J Lipid Mediat* 6: 75–88, 1993
13. Wittig HJ, Goldman AS: Nephrotic syndrome associated with inhaled allergens. *Lancet* 1: 542–543, 1970
14. Reeves WG, Cameron JS, Johansson SG, Ogg CS, Peters DK, Weller RO: Seasonal nephritic syndrome. *Clin Allergy* 5: 121–137, 1975
15. Criqui MH, Lee ER, Hamburger RN, Klauber MR, Coughlin SS: IgE and cardiovascular disease. *Am J Med* 82: 964–968, 1987
16. Ohshita K, Yamane K, Hanafusa M, Mori H, Mito K, Okubo M, Hara H, Kohno N: Elevated white blood cell count in subjects with impaired glucose tolerance. *Diabetes Care* 27: 491–496, 2004
17. Kannel WB, Anderson K, Wilson PW: White blood cell count and cardiovascular disease: Insights from the Framingham Study. *JAMA* 267: 1253–1256, 1992
18. Cavalot F, Massucco P, Perna P, Traversa M, Anfossi G, Trovati M: White blood cell count is positively correlated with albumin excretion rate in subjects with type 2 diabetes [Letter]. *Diabetes Care* 25: 2354–2355, 2002
19. Tong PC, Lee KF, So WY, Ng MH, Chan WB, Lo MK, Chan NN, Chan JC: White blood cell count is associated with macro- and microvascular complications in Chinese patients with type 2 diabetes. *Diabetes Care* 27: 216–222, 2004

Access to UpToDate on-line is available for additional clinical information
at <http://www.cjasn.org/>