

**Risk imparted by various parameters of smoking in Japanese men with type 2 diabetes on their development of microalbuminuria: Analysis from the Tsukuba Kawai Diabetes Registry**

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**Short Running Title:** smoking and microalbuminuria

**Abbreviations:** CS, current smokers; XS, ex-smokers; NS, never smokers; ACR, urinary albumin-creatinine ratio

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## INTRODUCTION

Whether smoking is an independent risk factor for the development of microalbuminuria has yet to be established. Inconsistencies in previous studies (1-12) might have been due to inadequacies in assessing smoking status of patients (i.e. current, ex- or never) (13). Moreover, although detailed quantitative assessment is critical for evaluating smoking risks (14), the dose effect of smoking on the development of nephropathy is not known (13). Therefore, we examined a wide variety of smoking parameters to clarify their relationship to microalbuminuria in a cohort of Japanese patients with type 2 diabetes.

## RESEARCH DESIGN AND METHODS

Data were derived from the ongoing Tsukuba Kawai Diabetes Registry database of the Kawai Clinic, which began collecting patient information in 1995. The Kawai Clinic is a typical diabetes clinic located in a suburb of Tokyo. All type 2 diabetic patients were consecutively registered for this study at their first visit. Study protocol was consistent with the Japanese Government's "Ethical Guidelines Regarding Epidemiological Studies" in accordance with the Declaration of Helsinki. Data from 357 normoalbuminuric male patients with type 2 diabetes (diagnosed according to the World Health Organization criteria (14)) who had been followed for at

least three years were analyzed. No data from female patients were included because their smoking frequency was quite low (9.4%). Patients were said to be normoalbuminuric if their first and second sample urinary albumin-creatinine ratios (ACR) were  $<30$  mg/g and were considered microalbuminuric if the ACR was  $\geq 30$  mg/g in at least two of three consecutive urine samples. The observation period was from the patients' first clinic visit to the date that they developed microalbuminuria or to their last ACR measurement. Patients with a history of cancer, high serum creatinine ( $>130$   $\mu\text{mol/l}$ ), hyperpotassemia, continuous microscopic hematuria and/or pyuria were excluded.

The mean age of our 357 study subjects was  $53.7 \pm 9.7$  years. At study entry, 285 patients used antidiabetic agents (268, oral hypoglycemic agents; 17, insulin), and 43 patients used antihypertensive agents. Mean values of baseline HbA<sub>1c</sub>, blood pressure, total cholesterol, plasma creatinine and calculated creatinine clearance (based on Cockcroft-Gault formula (15) ) were  $8.6 \pm 1.9\%$ ,  $126 \pm 15/72 \pm 10$  mmHg,  $5.1 \pm 0.9$  mmol/l,  $78.1 \pm 12.7$  mmol/l, and  $92.7 \pm 26.8$  ml/min, respectively. During the follow-up period, urinary albumin excretion was examined every 6 months using the turbidimetric immunoassay (Microalbumin-HA Test, Wako Pure Chemicals, LTD., Osaka, Japan). Patient

information vis-à-vis smoking habits was collected through interviews with registered nurses. Smoking status was classified into one of three categories: current smokers (CS), ex-smokers (XS), and never smokers (NS).

Data are expressed as means  $\pm$  SD. A one-way ANOVA followed by a Tukey's HSD test was used to compare the means of the three groups. Survival curves divided by baseline smoking status were constructed using Kaplan-Meier estimates. Cox proportional hazards modelling was used to determine independent predictors of microalbuminuria. *P* values  $<$  0.05 were considered to be significant. All statistical analyses were performed using SPSS version 14.0 for Windows (SPSS, Chicago, IL).

## RESULTS

At the time of study entry, 179 of our 357 patients (50.1%) were classified as CS and 74 (20.7%) as XS. CS smoked for a significantly longer time than XS ( $31.7 \pm 9.6$  vs.  $22.7 \pm 11.2$  years), though pack-years did not differ statistically ( $43.3 \pm 25.9$  vs.  $37.4 \pm 30.8$  pack-years). During the mean follow up period of  $5.7 \pm 2.1$  years, 106 patients (NS/XS/CS: 23/23/60) developed microalbuminuria, suggesting a crude incidence of 52.5/1000 patient-years. Final mean values of either serum creatinine (mean  $69.9 \pm 13.2$  mmol/l) or creatinine clearance (mean  $97.3 \pm 30.7$  ml/min) did not

differ significantly between those who did or did not develop microalbuminuria. Only one death occurred during the observation period, that due to a neoplasm.

Kaplan-Meier analysis revealed a difference in the incidence of microalbuminuria among NS, XS and CS, with that between CS and NS being statistically significant by log-rank testing (Figure). Even after adjustment for known predictors of nephropathy (i.e., age, diabetes duration, ACR, glycemic and blood pressure control), total and HDL cholesterol levels and alcohol consumption, differences between XS and NS or between CS and NS were still statistically significant (Figure). Furthermore, of all quantitative parameters determined, the number of cigarettes smoked per day (1.02 (1.01–1.03) cigarettes/day), duration of smoking (1.02 (1.01–1.03) per year) and pack-years smoked (1.01 (1.01–1.02) per pack-year) were also significant.

## CONCLUSIONS

Previous studies (4-8) that grouped XS and NS together as “non-smokers” or grouped CS and XS together as “ever smokers” (9-12) probably misestimated the risk of smoking because the effects of past exposure were eliminated. In fact, Kaplan-Meier curves of CS and XS were very close to each other supported by Cox regression results that pack-years smoked, which is an independent and strong

risk factor, did not differ between CS and XS.

As far as we know, only two prospective studies (16, 17) have investigated the relationship between the progression of diabetic nephropathy and lifetime smoking dose (pack-years of smoking); in these studies, only *progression* and not *development* of renal disease was quantified. Both studies found that the number of pack-years was a dose-dependent risk factor for the *progression* of diabetic nephropathy. These, combined with our results regarding *development*, suggest that smoking is a dose-dependent risk factor for

both the development and progression of diabetic nephropathy.

In conclusion, our study clarified that smoking, both past and current, is a dose-dependent risk factor for the development of microalbuminuria in type 2 diabetic patients. Detailed smoking history including dose-related parameters should be ascertained when evaluated.

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Figure legend

Survival probability curves showing the proportion of patients with normoalbuminuria and hazard ratios adjusted by known predictors in never, ex- and current smokers. Data were analyzed using Kaplan-Meier analysis followed by log-rank testing and Cox proportional hazards modeling.

