Risk Factors for Development of Incipient and Overt Diabetic Nephropathy in Type 1 Diabetic Patients

A 10-year prospective observational study

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OBJECTIVE — To evaluate prospectively putative risk factors for development of microalbuminuria and macroalbuminuria in type 1 diabetes.

RESEARCH DESIGN AND METHODS — Prospective observational study of a cohort of type 1 diabetic patients followed in the outpatient clinic at Steno Diabetes Center for ≤10 years (median 9 years). We followed 537 patients aged ≥18 years with type 1 diabetes, with duration of diabetes ≥5 years, with normoalbuminuria (urinary albumin excretion rate ≤30 mg/24 h), and who were not taking antihypertensive medication. Risk factors for development of microalbuminuria and macroalbuminuria were evaluated.

RESULTS — The mean progression of urinary albumin excretion rate was 7.6% (SE 0.8) per year. During follow-up, 134 patients (25%) progressed to persistent microalbuminuria or macroalbuminuria (>30 mg/24 h in two of three consecutive urine samples). Cox multiple regression analysis using baseline values of putative predictors of progression showed the following significant predictors of progression from normoalbuminuria to microalbuminuria or macroalbuminuria: baseline log urinary albumin excretion rate 2.63 (relative risk; 95% CI 1.65–4.19), HbA_{1c} 1.13% (1.04–1.23), presence of any retinopathy 1.90 (1.26–2.88), and smoking 1.61 (1.11–2.33). Sex, duration of diabetes, arterial blood pressure, serum creatinine, height, and social class were not risk factors.

CONCLUSIONS — Our study suggests that several potentially modifiable risk factors predict the development of microalbuminuria and macroalbuminuria in type 1 diabetic patients.

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In patients with diabetes, the development of persistent microalbuminuria (>30 mg/24 h in two of three consecutive urine samples) is an established predictor of development of overt diabetic nephropathy in both type 1 and type 2 diabetic patients (1–4). A total of ~30% of type 1 diabetic patients may develop diabetic nephropathy within 25 years of diabetes duration (5), although recent data suggest a declining incidence (6). Development of microalbuminuria is also a risk marker for increased morbidity and premature death from cardiovascular diseases (7), and measurement of urinary albumin excretion has become an integral part of routine diabetes care. Primary prevention of incipient nephropathy is only feasible if modifiable risk factors are known. Unfortunately, only limited information is available from large longitudinal studies of the initiation and progression of incipient nephropathy (8–11). Furthermore, the individual risk has been difficult to estimate based on the available studies.

We conducted a 10-year observational follow-up study of a large cohort of normoalbuminuric adult type 1 diabetic patients to elucidate putative risk markers for the development of incipient and overt diabetic nephropathy. The baseline and 10-year mortality data have been reported (7,12).

RESEARCH DESIGN AND METHODS

Patients

All 1,024 patients with type 1 diabetes who satisfied certain criteria and were attending the outpatient clinic at Hvidöre Hospital (now Steno Diabetes Center) in 1984 were asked to participate in the study (12). The criteria were age ≥18 years, duration of diabetes ≥5 years, and age ≤40 years at onset of diabetes. A total of 42 patients were excluded because they had been referred by one of us (H.H.P.). Among the remaining 982 patients, we obtained one or more 24-h urine collections from 957 (97%). A total of 593 patients had normoalbuminuria at baseline (≤30 mg/24 h in 83% of the patients based on a single sample). During the observation period, 12 patients were lost to follow-up. Patients who were taking antihypertensive agents at baseline (n = 44) were excluded from analysis to avoid the influence of antihypertensive medication on urinary albumin excretion rate; a post-hoc analysis showed that 15 (34%) of the 44 excluded patients progressed in albumin excretion during follow-up. Therefore, 537 patients were followed until 1 January 1995, until death (77 patients), or until emigration (20 patients). Clinical data at baseline are presented in Table 1. All the patients were white. Patients gave

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

Table 1—*Baseline variables and risk factors in 537 type 1 diabetic patients according to development of incipient/overt diabetic nephropathy*

	Normoalbuminuria	Incipient/overt diabetic nephropathy
n	403	134
Sex (% male)	52	51
Age (years)	40 ± 12	41 ± 13
Duration of diabetes (years)*	20 (5–60)	20 (5–55)
HbA_{1c} (%)	8.6 ± 1.7	9.2 ± 1.8†
Retinopathy (%)	63 (58–68)	78 (71–85)†
Systolic BP (mmHg)	133 ± 18	133 ± 17
Diastolic BP (mmHg)	80 ± 9	80 ± 9
Albuminuria (mg/24 h)‡	9 (9–10)	13 (12–15)†
Serum creatinine (μmol/l)	71 ± 13	68 ± 68†
Smokers (%)	57	70†

Data are means ± SD, unless otherwise indicated. *Median (range); †$P < 0.05$; ‡geometric mean (95% CI). BP, blood pressure.

informed consent, and the local ethics committee approved the study.

Procedures and methods in the baseline study were as described previously (12). Radioimmunoassay was used to measure 24-h urinary albumin excretion in sterile urine samples as they were obtained; the same method was used throughout the study, with a sensitivity of 0.5 mg/l and an interassay coefficient of variation of 9%. Measurements were performed at baseline and during regular follow-up in our outpatient clinic. Microalbuminuria and macroalbuminuria were defined as urinary albumin excretion 31–299 mg and ≥300 mg, respectively, per 24 h in at least two of three consecutive samples (13). Urine samples were collected routinely at least once per year. When values were abnormal, samples were requested at subsequent visits. Time of onset of microalbuminuria and overt nephropathy during follow-up was defined as the first recorded positive urine sample in a series of three fulfilling the above criteria.

Arterial blood pressure was measured once on the right arm with a standard clinical sphygmomanometer (cuff size 25 × 12) after the patient had been sitting for 10 min. During follow-up, blood pressure was measured at least yearly; hypertension was defined and treated according to World Health Organization criteria (≥160/95 mmHg). HbA_{1c} concentration was measured at least yearly with an isoelectric focusing method (normal range 4.1–6.1%, data comparable to Diabetes Control and Complications Trial values) (14). Owing to technical errors, HbA_{1c} values in 1984 were obtained for only 353 of the 537 patients; for the remaining patients, values from early 1985 were used. The same observer performed ophthalmoscopy through dilated pupils. Patients were categorized as having "any" retinopathy in the presence of background or proliferative retinopathy. Patients were classified as smokers if they smoked one or more cigarettes daily in 1984, based on case records. Socioeconomic classes I (i.e., university degree) through V (unskilled) were determined from length of education and occupation, based on case record information (15).

Statistical methods

The unpaired Student's *t* test was used to compare continuous variables, and χ^2 test was used to evaluate proportions. The urinary albumin excretion rate was logarithmically transformed before analysis, due to its skewed distribution, and is presented as the geometric mean and 95% CI. *P* values <0.05 (two-sided) were considered significant.

Cox's proportional hazards multiple regression analyses were used to examine the baseline variables predictive of progression to microalbuminuria or macroalbuminuria. Results are described as relative risk (hazard ratio). The models included those baseline variables that were a priori considered to be potentially important predictors of progressors, or that were found to be significantly different at baseline when we compared the two groups. These variables included sex, age, known duration of diabetes, presence of retinopathy, arterial blood pressure, log urinary albumin excretion rate, HbA_{1c}, height, social class, serum creatinine, and smoking. Stepwise backward selection was used. The time-scale was time since 1 January 1985, but the analysis was also performed for time since diagnosis of diabetes (implying that times were truncated). The hazards were smoothed using kernel smoothing with a bandwidth of 10 years.

The annual progression rate for urinary albumin excretion rate was evaluated by a mixed model. The patient levels reflect past experiences and were treated as separate parameters. The slope of albumin excretion rate on time was modeled as a function of the explanatory factors and random variation. The measurements were also subject to measurement error (including short-term variation in excretion rate). To avoid the confounding effect of antihypertensive medication on the progression rate, only observations before initiation of any antihypertensive medication, including diuretics, was included.

The software used was SAS version 6.11, proc mixed (SAS Institute, Cary, NC). For each year of follow-up, the median value for all urine collections during that year was used.

RESULTS — We followed 537 normoalbuminuric type 1 diabetic patients for a median (range) of 9 years (0–10)

Table 2—*Risk factors for progression to incipient or overt diabetic nephropathy in 537 type 1 diabetic patients followed for 10 years, by means of Cox multiple regression analysis*

Variable	Relative risk	95% CI	*P*
Log_{10} urinary albumin excretion rate*	2.63	1.65–4.19	<0.0001
HbA_{1c} (%)	1.13	1.04–1.23	<0.01
Retinopathy (any)	1.90	1.26–2.88	<0.01
Smoking	1.61	1.11–2.33	<0.02

Sex, duration of diabetes, arterial blood pressure, serum creatinine, height, and social class were not selected in the final model. *Relative risk corresponds to 10-fold increase in variable.

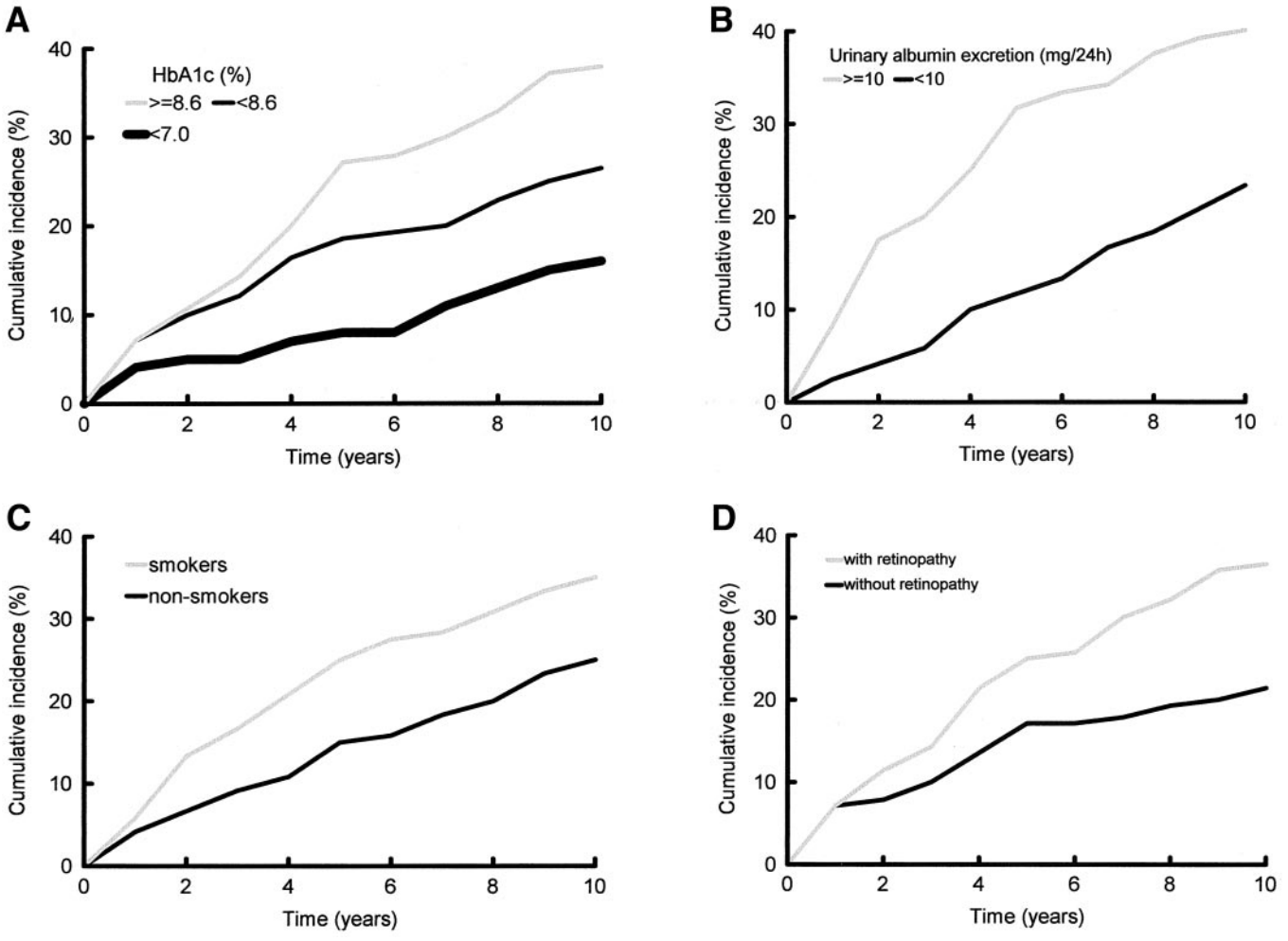

Figure 1—*Cumulative incidence of incipient diabetic nephropathy with respect to median (8.6%) and American Diabetes Association goal (7.0%) of HbA_{1c} concentration* (A)*, median urinary albumin excretion rate* (B)*, smoking* (C)*, and retinopathy* (D) *according to duration of follow-up in 537 type 1 diabetic patients with normoalbuminuria at baseline* ($P < 0.05$).

with a total of 4,475 patient-years of follow-up. During follow-up, 134 patients (25%; 95% CI 21–29) progressed to persistent microalbuminuria or macroalbuminuria (>30 mg/24 h in two of three consecutive samples). Macroalbuminuria developed in 34 patients, who tended to have higher HbA_{1c}: 9.7 (2.0) vs. 9.0 (1.7), $P = 0.051$. Otherwise, these patients were comparable to the patients in whom microalbuminuria developed.

A total of 13 (9.7%) patients in whom persistent microalbuminuria developed later regressed to normoalbuminuria.

Table 1 shows the clinical details at entry for the patients. Smokers had lower social class but no differences in sex or other risk markers compared with nonsmokers.

The putative risk factors for development of abnormally increased urinary albumin excretion rate were examined in backward stepwise Cox regression analysis (Table 2). No additional risk factors were included in the analysis if baseline albuminuria was excluded.

A similar analysis was performed by neglecting information for patients after initiation of antihypertensive medication (the observation period was reduced for 64 patients), which revealed the same risk markers.

To illustrate the impact of the risk markers and their combination, we arbitrarily chose baseline values above the median for HbA_{1c} (8.6%) and urinary albumin excretion rate (10 mg/24 h) as risk markers for progression to microalbuminuria, in addition to the presence/absence of smoking and diabetic retinopathy (Fig. 1). Based on a Cox regression model using these four binary risk markers, the individual risk for development of microalbuminuria during 10 years of follow-up was estimated (Fig. 2).

The results were similar when using the time-scale of duration of diabetes. The hazard of developing microalbuminuria according to duration of diabetes is shown in Fig. 3. A peak is seen at diabetes duration of 10–15 years. Dividing patients according to a duration of diabetes above or below the mean of 20 years showed no difference in prevalence of progressors (25 vs. 29%, $P = 0.30$).

Albumin progression rate

The mixed model revealed a mean progression in urinary albumin excretion rate of 7.6% (5.7–9.2) per year. Using the above-mentioned putative risk factors for the rate of progression (percent per year) in a regression model with stepwise ex-

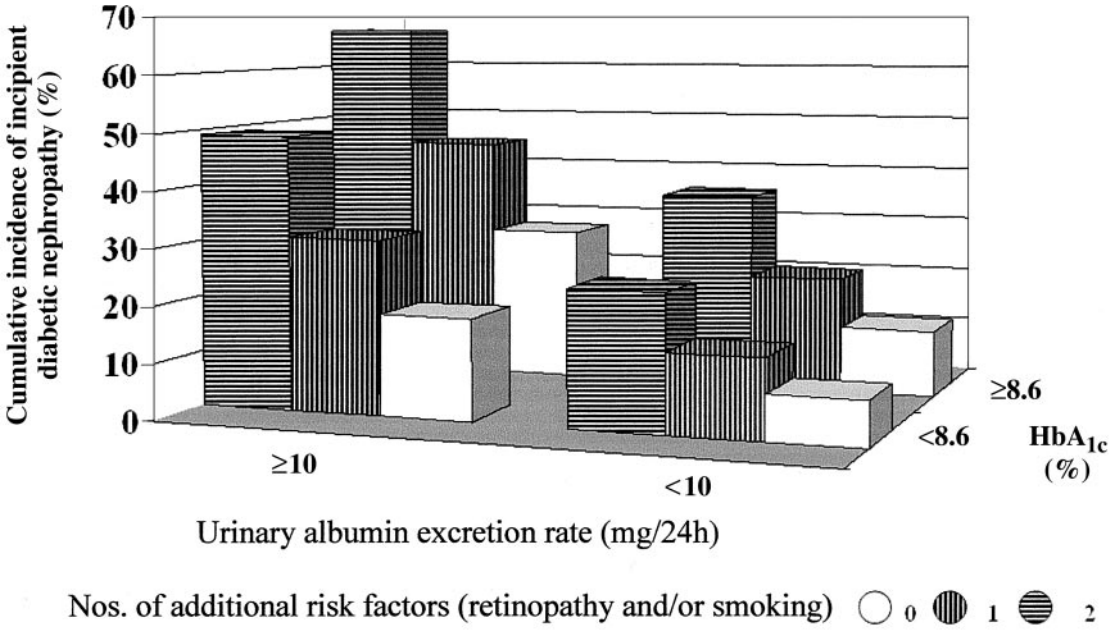

Figure 2—*Individual risks of progression to microalbuminuria during 10-year follow-up in type 1 diabetic patients with normoalbuminuria. Based on the presence or absence of HbA_{1c} >8.6%, urinary albumin excretion rate >10 mg/24 h, and number of additional risk markers: smoking and/or retinopathy.*

clusion demonstrated that only age (0.135 ± 0.066 [coefficient ± SE], $P <$ 0.05) and HbA_{1c} (1.81 ± 0.47, $P <$ 0.001) were significantly associated with the progression rate of albumin excretion. Data included 514 patients with 3,855 measurements, with variation between patient slopes (SD) of 14.3% per year and measurement error of 70.0% (including short-term variation in excretion rate).

CONCLUSIONS — During follow-up, persistent microalbuminuria developed in 134 patients (25%); macroalbuminuria developed in 34 of these patients (6% of all patients). This is higher than the 11% incidence of microalbuminuria during 7 years in the U.K. Microalbuminuria Collaborative Study (9) but similar to the results of 24% in 9 years in the conventionally treated group in the Diabetes Control and Complications Trial (16). In our study, the duration of diabetes at baseline was 20 (5–60) years, and patients taking antihypertensive medication at baseline were excluded. The incidence of microalbuminuria (peaking with 10–15 years' duration) would most likely be higher in a cohort of newly diagnosed patients compared with our results.

Elevated baseline urinary albumin excretion rate, within the normoalbuminuric range, has a clear effect, in accordance with previous examinations (8–11). The effect of this variable seems to reflect a higher level of urinary albumin excretion at baseline, implying that a small increase will bring the patient above the threshold for microalbuminuria. This fits with the fact that albumin level does not influence the slope-model. In a recent follow-up of an incident cohort followed from onset of type 1 diabetes, a higher level of albumin/creatinine ratio was demonstrated already within the first 2 years after diagnosis in

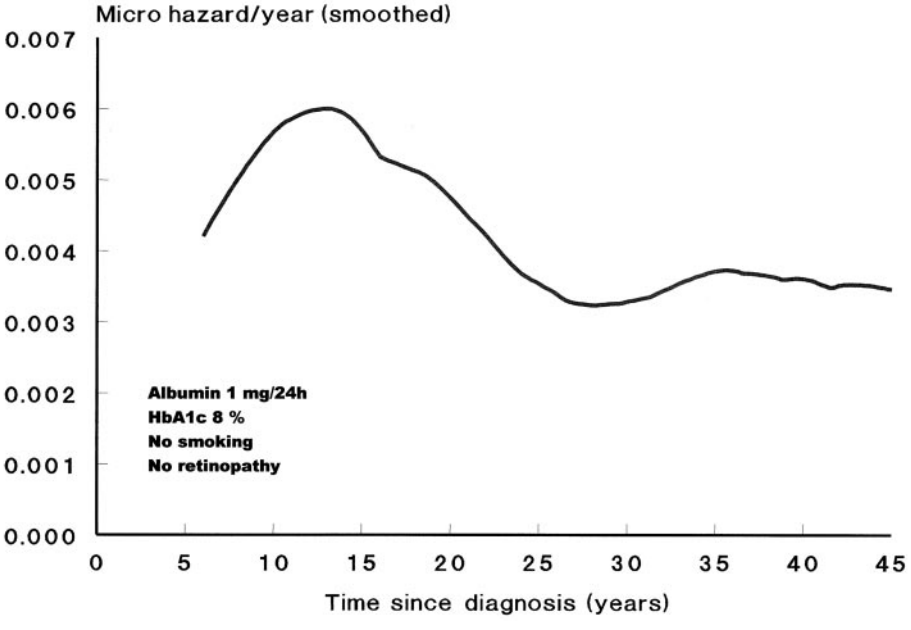

Figure 3—*The hazard (per year) of developing persistent microalbuminuria according to duration of diabetes modeled for a fictive patient with arbitrarily selected values for risk markers (urinary albumin excretion rate of 1 mg/24 h, HbA_{1c} 8%, no smoking or retinopathy), based on 537 type 1 diabetic patients with normoalbuminuria at baseline during 10-year follow-up.*

subjects in whom microalbuminuria subsequently developed (17). The cutoff value between normoalbuminuria and microalbuminuria has been discussed in several articles; we have chosen the values according to a consensus article (13).

The impact of glycemic control is also in accordance with other observational studies in type 1 diabetic patients (8,9,18) as well as intervention studies (16,19). We found the presence of retinopathy to be predictive of onset of microalbuminuria (with no difference between background or proliferative retinopathy, data not shown); this may reflect another sign of previous exposure to poor glycemic control or susceptibility to the development of diabetic late complications. In a cross-sectional study from the EURODIAB study, it was suggested that patients with retinopathy had increased urinary albumin excretion with increasing blood pressure, whereas in those without retinopathy, an increase in blood pressure was not associated with increasing albuminuria (20). Alternatively, because the variable does not enter in the slope analysis, it may be an artifact due to the time analysis measuring the time to diagnosis of microalbuminuria; that is, the patients with retinopathy are examined more frequently, reducing the delay from onset of microalbuminuria to diagnosis.

We were not able to demonstrate any impact of baseline blood pressure, systolic or diastolic, regardless of retinopathy status. It is possible that blood pressure only increases in relation to onset of microalbuminuria, either shortly before or after, and thus only is a short-term risk factor. In previous observational studies, some studies found blood pressure to be a risk marker for progression to nephropathy (9), whereas others, including the recent EURODIAB study including 1,134 patients (21), did not (18,22). Another possibility is that the changes in blood pressure initially are so small that they are not detectable with a baseline office measurement and that 24-h ambulatory blood pressure measurements are necessary to demonstrate their presence (23).

In the present study, the prevalence of smokers was high, and smoking was associated with progression in albuminuria. This was also found in some previous cross-sectional or prospective studies (24,25), but not in all studies (9,18). It has been suggested that smoking induces acute increases in blood pressure (26,27) and increases vascular permeability in nondiabetic subjects, but this was not confirmed in other studies (27).

The main analyses of this paper use the time since baseline as the basic time-scale, but additional analyses use the time since diagnosis of diabetes. An advantage of the slope analysis is that it is not sensitive toward the choice of time-scale. The analysis of Table 2 is sensitive, but we have found similar results.

Based on the presence or absence of only four dichotomized risk markers, it was possible to assess the individual risk of progression to microalbuminuria ranging from ~10% (no risk markers) to 70% (all four risk markers present) during the 10-year follow-up period. Such a risk estimate can be used in the individual patient to decide on intervention strategy, as known from similar models used in the prevention of ischemic heart disease. Second, they can be used when primary prevention trials are designed to identify high-risk patients, who are most likely to benefit from intervention, thereby reducing the necessary sample sizes.

We previously found stature to be a risk marker for nephropathy, which was supported by the U.K. study (9) but not in the present study. Lipid data were not available but has recently been suggested to be important for progression (21).

In conclusion, our study suggests that several potentially modifiable risk factors, such as smoking, poor glycemic control, and urinary albumin excretion rate, predict the development of incipient and overt diabetic nephropathy in normoalbuminuric type 1 diabetic patients.

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