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Smoking cessation predicts amelioration of microalbuminuria in newly diagnosed type 2 diabetes mellitus: a 1-year prospective study

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ABSTRACT

The objective of the study was to assess the effect of smoking cessation on microalbuminuria in subjects with newly diagnosed type 2 diabetes mellitus (DM). From 500 smokers newly diagnosed with type 2 DM and microalbuminuria, only 193 (96 men/97 women; age, 56.4 ± 7.8 years) agreed to participate and were educated on smoking cessation, diet, and exercise. Pharmacological interventions were not different among the studied groups. All subjects were contacted by phone monthly with emphasis on smoking cessation. Anthropometric, biochemical parameters and urine specimens were obtained at baseline and at 12-month follow-up. *Microalbuminuria* was defined as an albumin to creatinine ratio of 30 to 299.9 μg/mg creatinine. Ankle brachial pressure index was determined by ultrasound. A total of 120 (62.2%) subjects quit smoking. Prevalence of microalbuminuria was reduced at 1 year to 72.6% in the subjects who quit smoking and to 22.5% in those who continued smoking ($P = .015$). Multivariate logistic regression analysis demonstrated that independently associated with the reduction in albumin to creatinine ratio (84.8 vs 28.7 μg/mg creatinine) were amelioration of glycemic control ($P < .001$), blood pressure ($P = .02$), dyslipidemia ($P = .02$), and insulin resistance ($P = .05$). Smoking cessation also reduced the prevalence of peripheral vascular disease ($P = .03$) and neuropathy ($P = .04$). From the pharmacological and lifestyle interventions, smoking cessation had the highest and an independent contribution to the reduction of microalbuminuria ($P < .001$). Smoking cessation in newly diagnosed type 2 DM patients is associated with amelioration of metabolic parameters, blood pressure, and the reduction of microalbuminuria. Stricter counseling about the importance of quitting smoking upon type 2 DM diagnosis is necessary to protect against the development of diabetic nephropathy and vascular complications.

1. Introduction

Diabetic nephropathy is the primary cause of chronic kidney disease and is associated with increased cardiovascular mortality [1]. Previous studies revealed that microalbuminuria is predictive of the later development of proteinuria in type 2 diabetes mellitus (DM) [2]. In the United Kingdom Prospective Diabetes Study, the cumulative incidence of microalbuminuria in type 2 DM was 2% per year; and the prevalence rate 10 years after diagnosis was 25% [3]. Proteinuria occurred in 5% to 20% of the patients [3].

In the last decade, implementation in clinical practice of several measures that contribute to the early diagnosis and prevention of diabetic nephropathy has slowed down the rate of microalbuminuria and its progression to established renal

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disease. However, the adoption of these measures is far less than the desirable goals [4].

Screening for diabetic nephropathy must be initiated at the time of diagnosis in type 2 DM because approximately 7% of them already have microalbuminuria at that time [5]. Genetic susceptibility contributes to the development of diabetic nephropathy in type 2 DM, whereas the main potentially modifiable factors to its progression are sustained hyperglycemia, hypertension, dyslipidemia, and smoking [6].

Diabetes is considered among the target groups for smoking cessation treatment because of the increased health risks associated with smoking [7]. Smoking cessation is also recommended as an effective mean of preventing diabetes in the general population [7]. However, epidemiological data have demonstrated that 27% of patients with type 2 DM are daily cigarette smokers and show a lack of awareness of the microvascular and macrovascular complications associated with smoking [8]. In contrast, they are most concerned about the impact on weight and/or diet and diabetes management upon cessation [8].

Microalbuminuria frequently coexists with other cardiovascular risk factors, such as hypertension and dyslipidemia. In the Steno-2 study, in 160 patients with type 2 DM and microalbuminuria, multifactorial intervention, which consisted of lifestyle changes including smoking cessation and pharmacological therapy, was able to reduce by 61% the risk of developing macroalbuminuria compared with the conventional drug therapy [9]. Although several diabetic complications may be exacerbated by smoking, it has not yet been established that this is due to a direct impact of smoking or primarily due to the detrimental effects of smoking on diabetes-related metabolic factors, such as hyperglycemia and insulin resistance.

This study was designed to evaluate the effect of smoking cessation treatment in a cohort of newly diagnosed patients with type 2 DM and microalbuminuria on the progression of microalbuminuria as well as other metabolic and vascular parameters.

2. Methods

2.1. *Study participants and design*

From December 2008 to September 2009, all smokers newly diagnosed with type 2 DM and microalbuminuria attending the outpatients' clinics of our hospital were considered as eligible and were invited to take part in a 1-year prospective study assessing the effect of cigarette smoking on renal function. Diagnosis of type 2 DM was based on the American Diabetes Association criteria [10]. To ensure that the subjects were newly diagnosed (incident) with type 2 DM, a patient had to have normal glucose testing results (fasting plasma glucose, glycated hemoglobin [HbA_{1c}], or 2-hour 75-g oral glucose tolerance test) for a maximum of 1 year before the date of diagnosis.

The study was contacted according to the ethical guidelines of the 2008 revised Declaration of Helsinki. Written informed consent was obtained from all willing participants, and the final protocol was approved by the Ethics Committee of our Hospital.

Inclusion criteria were age of at least 18 years, smoking at least 10 cigarettes a day for at least 1 year, presence of microalbuminuria measured on at least 2 of 3 urine specimens examined for urinary albumin to creatinine ratio (ACR) over a 3-month period prior or after diabetes diagnosis, and estimated glomerular filtration rate (eGFR) greater than 90 mL/(min 1.73 m^2). Smokers consuming less than 10 cigarettes a day and nonsmokers were excluded from the study. During screening and at the follow-up examination, none of the participants included experienced acute febrile illness, urinary tract infection (excluded by microscopic examination and culture), hematuria, short-term serious hyperglycemia (plasma glucose values ≥250 mg/dL), and uncontrolled hypertension (sitting systolic blood pressure [BP] >160 mm Hg or sitting diastolic BP >100 mm Hg). All subjects had normal serum creatinine levels (<1.4 mg/dL for men and <1.2 mg/dL for women). Patients with acute or chronic heart failure, acute renal failure, chronic glomerulonephritis, polycystic kidney disease, use of steroids, or lithium were excluded. Secondary hypertension, pregnancy, breast-feeding, and malignancy were also among the exclusion criteria.

Out of 500 eligible patients, only 193 smokers (96 men/97 women; mean age, 56.4 ± 7.8 years) agreed to participate in the study. Subjects who agreed to participate in the study were evaluated by a smoking assessment questionnaire, which estimated their desire to smoke, their anticipation of a positive outcome and relief from nicotine withdrawal, and their intention to smoke using a scale of 1 to 10. Participants scored 10 if they were evaluated as absolutely positive and ready to stop smoking and 1 if they were not ready. Those who scored 5 or less were not included in the study.

Complete physical examination was performed early in the morning in all studied participants during screening and at the 12-month follow-up visit. Blood pressure was measured 3 consecutive times 5 minutes apart, in the sitting position, using an appropriate cuff size. The mean value of the last 2 measurements was used in the statistical analysis. Ankle brachial pressure index (ABI) was measured by a pulsed, continuous Doppler (Mini Doppler DSOO; Huntleigh Technology, Cardiff, United Kingdom) in the supine position. Peripheral vascular disease (PVD) was defined by an ABI less than 0.9. Direct fundoscopy was performed through dilated pupils by an experienced ophthalmologist; and the findings were classified as normal, background (including maculopathy), and proliferative retinopathy. Assessment of peripheral neuropathy (PN) was based on symptoms (neuropathy symptom score) and signs (neuropathy disability score), as described previously [11]. A 12-lead electrocardiogram (Cardio Control NV, Rijswijk, the Netherlands) was recorded in the supine resting position, and electrocardiographic and/or clinical findings of coronary artery disease were determined in all studied subjects.

At baseline, participants were asked to fill out a 30-minute survey questionnaire. Participants were given an opportunity to answer and/or clarify survey questions and adequate time to complete it. Demographic and social characteristics, years of smoking, and current smoking frequency were recorded. Cumulative smoking exposure was assessed by pack-years,

which were calculated by multiplying the number of packs smoked per day (1 pack = 20 cigarettes) by the number of years over which that amount was smoked. Three self-reported measures of environmental smoke exposure were evaluated at baseline: whether smoking was allowed inside the home (home smoking ban), whether the subject lives with a partner/spouse who smokes, and whether smoking was allowed in the workplace. A strong relationship exists between self-reports of home and work smoke exposure and cotinine levels [12]. Physical activity was assessed by the International Physical Activity Questionnaire [13].

At baseline and during the study, pharmacological interventions for hyperglycemia, dyslipidemia, and BP control did not differ in the group of patients who quit smoking compared with the group who continued smoking. Participants received counseling from a dietician on the usual dietary guidelines. Finally, participants were invited to take part in a face-to-face 45- to 60-minute motivational interviewing session and then were offered monthly telephone support and were asked to report their smoking status. At the end of the study, smoking status was assessed by self-reported 7-day point-prevalence abstinence and was confirmed by an expired carbon monoxide (CO) level of less than 10 ppm. Cotinine was not used to assess abstinence because, when used with self-report to indicate whether a person has smoked, CO and cotinine levels show high agreement [14].

2.2. Analytical methods

Blood was collected after a 12-hour overnight fast. Serum glucose, lipids (total cholesterol, high-density lipoprotein [HDL] cholesterol, triglycerides), and creatinine were measured on an automatic analyzer. Low-density lipoprotein (LDL) cholesterol was calculated. Estimated GFR was calculated using the Modification of Diet in Renal Disease study equation [15]. Glycated hemoglobin was measured with a latex immunoagglutination inhibition method (Bayer Healthcare, Elkhart, IN) with a nondiabetic range of 4.0% to 6.0%. Albumin to creatinine ratio was measured by radioimmunoassay (Pharmacia and Upjohn Diagnostics, Uppsala, Sweden) in 2 of 3 random urine specimens over a 3-month period. Before screening, subjects were advised to avoid vigorous exercise. An ACR of less than 30 μg/mg creatinine was considered as normoalbuminuria; 30 to 299.9 μg/mg creatinine, as microalbuminuria; and at least 300 μg/mg creatinine, as macroalbuminuria. High-sensitivity C-reactive protein (hsCRP) was determined using ADVIA 1650 (Bayer). Plasma insulin was measured with radioimmunoassay (Biosure, Brussels, Belgium; coefficient of variation = 3.3% ± 1.2%). Insulin resistance was estimated using the homeostasis model assessment equation for insulin resistance (HOMA-IR).

2.3. Statistical analysis

Statistical analyses were performed using the SPSS statistical package (Version 15.0; SPSS, Chicago, IL). Continuous variables are given as mean ± SD; categorical variables are presented as percentages. The Student *t* test was used to assess differences in continuous variables, whereas the χ^2 test was used for categorical variables; differences in nonparametric variables were compared using the Mann-Whitney test.

The time course of demographic, clinical, and laboratory data was investigated using a linear mixed model analysis. For each outcome variable, a model was constructed with time (baseline and 1 year) as the repeated measured factor. Smoking status was included in the model as a fixed effect. Age at baseline, sex, drug therapy, and all annually recorded data were included as covariates. The ID of the subjects was included as a random effect to account for the variability resulting from individual differences among subjects.

Post hoc differences were assessed using a Bonferroni correction to the overall α level of .05. Univariate logistic regression analyses were performed to examine for associations between absence of microalbuminuria and the studied variables. In addition, a total of 5 models of multivariate logistic regression analysis were created to look for independent associations between amelioration of microalbuminuria and the variables of interest. Model 1 included the variables that were found to be associated significantly ($P < .05$) or to be suggestive of an association ($P < .10$) with amelioration of microalbuminuria at study end in the univariate analysis and included age, sex, body mass index (BMI), waist, fasting glucose, HbA_{1c}, systolic BP, diastolic BP, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, HOMA-IR, hsCRP, eGFR, and physical activity, assessed by metabolic equivalent (MET)–hours per week. Models 2 to 5 included the variables of model 1 and use of angiotensin-converting enzyme (ACE) inhibitors (model 2); use of ACE inhibitors and metformin (model 3); use of ACE inhibitors, metformin, and statins (model 4); and use of ACE inhibitors, metformin, statins, and 1-year smoking abstinence (model 5). Separate models of multivariate linear regression analysis were performed to examine the effect of smoking cessation upon microalbuminuria as a continuous variable (ACR). To avoid multicollinearity, Pearson or Spearman correlations were performed to identify variables with a high correlation coefficient value (>0.80). Highly correlated variables were entered in the models of multivariate regression analyses sequentially. $P < .05$ (2-tailed significance) were considered statistically significant.

3. Results

3.1. Social-clinical data and smoking habits of the studied population

The majority of the participants who agreed to quit smoking had received a higher (college or university) education (81.8%, n = 158), whereas a minority had completed only elementary school (18.2%, n = 35). Marital status differed among the participants, with the majority of the smoking quitters being married (48.1%, n = 93), followed by single subjects (32.1% n = 62) and finally divorcees and widowers (19.8%, n = 38). Regarding baseline environmental smoke exposure, no significant differences existed between our studied arms: 19.2% (n = 37) lived with a spouse/partner who

smoked, 37.8% (n = 73) had a home smoking ban, and 43.0% (n = 83) had smoking bans at work.

Duration of smoking in the studied population was as follows: less than 36 years (29%, n = 56), 36 to 41 years (26%, n = 50), and at least 41 to 44 years (45%, n = 87). Furthermore, daily current smoking frequency and cumulative smoking exposure did not differ significantly between the participants, with 36.4% (n = 75) of the studied sample smoking at baseline 14 to 24 packs of cigarettes per month (9-16 cigarettes per day) and 38.8% (n = 70) smoking more than 45 packs of cigarettes per month (30 cigarettes per day), whereas 24.8% (n = 48) smoked at baseline less than 14 packs of cigarettes per month (<9 cigarettes per day).

None of the participants consumed more than 18 ± 4 g of ethanol per day. At baseline, prevalence of PN was 25.9% (n = 35), whereas background retinopathy was diagnosed in 23.3% (n = 45) and proliferative retinopathy in 2.1% (n = 4) of the subjects. Peripheral vascular disease was present in 17.7% (n = 24) of the participants. Although absent at baseline in both groups, at the end of the study, angina pectoris was present in 8.2% (n = 6) of the individuals who continued smoking.

Table 1 – Time course of demographic and clinical data in subjects who stopped smoking vs continuing smokers

Variables	Baseline data M (SE)	1-y change (adjusted M)[a]	Bonferroni test for 1-y change
BMI (kg/m^2)			
Stopped smoking	31.7 (5.6)	+0.8 (32.5)	
Continued smoking	31.5 (5.8)	−1.7 (29.8)	
Difference between groups[b]	+0.0 (P = 1.00)	−2.5 (P = .04)	P = .002
Waist circumference (cm)			
Stopped smoking	109.8 (12.9)	−7.4 (102.4)	
Continued smoking	109.7 (12.9)	−8.3 (101.4)	
Difference between groups[b]	+0.1 (P = .78)	−1.0 (P = .01)	P = .03
Systolic BP (mm Hg)			
Stopped smoking	143.5 (32.0)	−26.8 (116.7)	
Continued smoking	143.3 (34.0)	−13.6 (129.7)	
Difference between groups[b]	+0.2 (P = .56)	−13.2 (P = .03)	P = .001
Diastolic BP (mm Hg)			
Stopped smoking	77.4 (13.9)	−9.9 (67.5)	
Continued smoking	77.5 (14.0)	−6.2 (71.3)	
Difference between groups[b]	−0.1 (P = .78)	−3.7 (P = .02)	P = .01
ABI			
Stopped smoking	1.00 (0.10)	+0.1 (1.10)	
Continued smoking	0.99 (0.12)	+0.03 (1.02)	
Difference between groups[b]	+0.01 (P = 1.00)	+0.07 (P = .007)	P < .001
PVD (ABI <0.9) n (%)			
Stopped smoking	16 (13.3)	−5.8 (7.5)	
Continued smoking	8 (10.9)	−2.7 (8.2)	
Difference between groups[b]	+2.4 (P = .27)	−0.7 (P = .03)	P = .003
PN n (%)			
Stopped smoking	22 (18.3)	−7.4 (10.9)	
Continued smoking	13 (17.8)	−2.8 (15.0)	
Difference between groups[b]	+0.5 (P = .87)	−4.1 (P = .04)	P = .05
Retinopathy n (%)			
Stopped smoking	30 (25.0)	−3.4 (21.6)	
Continued smoking	19 (26.0)	−13.7 (12.3)	
Difference between groups[b]	−1.0 (P = .18)	−0.3 (P = .24)	P = .57
Physical activity (MET-h/wk)			
Stopped smoking	9.6 (1.6)	+0.4 (10.0)	
Continued smoking	9.6 (3.8)	+0.1 (9.7)	
Difference between groups[b]	+0.0 (P = .71)	+0.3 (P = .40)	P = .06

All data are adjusted for mean baseline age (56.4 years) and sex, daily ethanol consumption, regular diet, use of metformin, use of ACE inhibitors, and use of statins.

[a] Change from baseline data.

[b] For 1-year changes, these values represent the effect of smoking cessation on each variable compared with smoking continuance.

3.2. *Clinical differences between the participants who quit and those who did not quit smoking during the study*

At the end of the 12-month period, 62.2% (n = 120) of the initial population reported successful cessation. Smoking abstinence was accompanied by a small but significant increase in BMI. However, central obesity was significantly ameliorated in both treatment groups. Towards baseline, patients who ceased smoking increased their physical activity; achieved better glycemic, BP and lipids control; and improved their renal function compared with those who continued smoking (Table 1). Smoking cessation was associated with amelioration of insulin resistance parameters and reduction of PVD and of PN prevalence, but had no effect on retinopathy (Table 2).

3.3. *Contribution of different variables in the prevalence of microalbuminuria*

Multivariate logistic regression analysis, after controlling for the effect of age, sex, and all the variables significantly associated with amelioration of microalbuminuria in the univariate logistic regression analysis, verified that the reduction in systolic BP and the improvement in insulin resistance as well as glycemic and lipids profile were all independent predictors of the absence of microalbuminuria at study end. Among the pharmacological and lifestyle interventions, the highest contribution to the reduction of microalbuminuria was achieved by smoking cessation treatment. Moreover, multivariate logistic regression analysis further confirmed that smoking cessation affected microalbuminuria independently from the effect of antihypertensive, hypolipidemic, and diabetic treatment. No significant differences were observed when multivariate analysis was repeated with ACR as a continuous variable (Table 3).

4. Discussion

The main finding of the present study is that smoking cessation upon diagnosis of type 2 DM predicts the improvement of patient's glycemic and lipids profile and is associated with reduction in BP and the prevalence of microalbuminuria, independent of other pharmacological or lifestyle interventions.

Table 2 – Time course of laboratory data in subjects who stopped smoking vs continuing smokers

Variables	Baseline data M (SE)	1-y change (adjusted M) [a]	Bonferroni test for 1-y change
Fasting glucose (mg/dL)			
Stopped smoking	156.7 (45.6)	−36.1 (120.6)	
Continued smoking	156.7 (42.6)	−21.7 (135.0)	
Difference between groups [b]	+0.0 (P = 1.0)	−14.4 (P = .02)	P = .02
HbA_{1c} (%)			
Stopped smoking	7.7 (0.9)	−0.5 (7.2)	
Continued smoking	7.8 (0.8)	−0.3 (7.5)	
Difference between groups [b]	−0.1 (P = .87)	−0.2 (P = .04)	P = .004
HOMA-IR			
Stopped smoking	12.4 (3.1)	−4.2 (8.2)	
Continued smoking	12.5 (3.1)	−2.1 (10.4)	
Difference between groups [b]	−0.1 (P = .97)	−2.1 (P = .02)	P < .001
Total cholesterol (mg/dL)			
Stopped smoking	221.3 (29.5)	−39.6 (181.7)	
Continued smoking	223.2 (29.6)	−23.2 (200.0)	
Difference between groups [b]	−0.9 (P = .62)	−16.4 (P = .01)	P = .02
HDL cholesterol (mg/dL)			
Stopped smoking	37.4 (7.8)	+6.1 (43.5)	
Continued smoking	37.8 (6.3)	+3.0 (40.8)	
Difference between groups [b]	−0.4 (P = .51)	+2.7 (P = .05)	P = .004
LDL cholesterol (mg/dL)			
Stopped smoking	149.4 (22.4)	−31.6 (117.8)	
Continued smoking	150.8 (23.6)	−22.9 (127.9)	
Difference between groups [b]	−1.4 (P = .21)	−8.7 (P = .007)	P = .01
Triglycerides (mg/dL)			
Stopped smoking	151.4 (54.0)	−7.6 (143.8)	
Continued smoking	155.6 (53.3)	−7.6 (148.0)	
Difference between groups [b]	−0.7 (P = .34)	−0.0 (P = 1.0)	P = .08
hsCRP (mg/dL)			
Stopped smoking	1.72 (0.13)	−0.59 (1.13)	
Continued smoking	1.72 (0.15)	−0.59 (1.13)	
Difference between groups [b]	0.0 (P = 1.0)	−0.0 (P = 1.0)	P = 1.0
ACR (μg/mg creatinine) [c]			
Stopped smoking	84.8 (30-299.9)	−56.1 (28.7)	
Continued smoking	82.3 (30-299.9)	−10.2 (72.1)	
Difference between groups [b]	+1.2 (P = .44)	−45.9 (P < .001)	P = .006
Estimated GFR (mL/[min 1.73 m^2])			
Stopped smoking	101.0 (37.0)	+16.2 (117.2)	
Continued smoking	101.0 (34.5)	+13.9 (114.9)	
Difference between groups [b]	+0.0 (P = 1.0)	+2.3 (P = .06)	P = .08
Microalbuminuria n (%)			
Stopped smoking	120 (100.0)	−77.5 (22.5)	
Continued smoking	73 (100.0)	−27.4 (72.6)	
Difference between groups [b]	+0.0 (P = 1.0)	−50.1 (P < .001)	P < .001

All data are adjusted for mean baseline age (56.4 years) and sex, daily ethanol consumption, regular diet, use of metformin, use of ACE inhibitors, and use of statins.

[a] Change from baseline data.

[b] For 1-year changes, these values represent the effect of smoking cessation on each variable compared with smoking continuance.

[c] Median values (interquartile range).

Table 3 – Independent predictors of the absence of microalbuminuria 1 year after target smoking quit date by multivariate logistic regression analysis

Variable (mean difference over 1 y)	Odds ratio (95% CI)	P	Adjusted R^2
Model 1			
Age (increase by 1 y)	0.92 (0.84-1.09)	.07	
Sex (male vs female)	1.18 (0.78-1.22)	.08	
Systolic BP (decrease by 5 mm Hg)	1.25 (1.18-1.39)	.02	
HbA_{1c} (decrease by 1%)	1.32 (1.17-1.61)	<.001	
Fasting glucose (decrease by 10 mg/dL)	1.22 (1.10-1.63)	<.001	
HDL cholesterol (increase by 10 mg/dL)	1.22 (1.16-1.29)	<.001	
Triglycerides (decrease by 10 mg/dL)	1.04 (1.00-1.08)	.02	
HOMA-IR (decrease by 1 U)	1.18 (1.08-1.23)	.05	
eGFR (increase by 10 mL/[min 1.73 m^2])	1.04 (1.02-1.06)	.001	0.60
Model 2			
Model 1 + use of ACE inhibitors	1.25 (1.10-1.44)	.04	0.65
Model 3			
Model 1 + use of ACE inhibitors + use of Metformin	3.17 (0.98-4.43)	.08	0.65
Model 4			
Model 1 + use of ACE inhibitors + use of metformin + use of statins	1.20 (0.72-2.38)	.30	0.65
Model 5			
Model 1 + use of ACE inhibitors + use of metformin + use of statins + smoking (abstinence status after 1 y)	2.10 (1.24-3.25)	<.001	0.76

Additional variables adjusted in model 1 were as follows: waist (decrease by 1 cm), BMI (decrease by 1 kg/m^2), diastolic blood pressure (decrease by 5 mm Hg), total cholesterol (decrease by 10 mg/dL), LDL cholesterol (decrease by 10 mg/dL), hsCRP (decrease by 1 mg/dL), physical activity (increase by 10 MET-hours of moderate-vigorous activity per week). The same analysis was repeated with absence of microalbuminuria for the outcome of interest as a dichotomous variable (yes vs no) and as a continuous variable (ACR) with comparable results.

Although previous studies reached mixed results [16,17], evidence on the efficacy of counseling and motivational interviewing in helping patients with diabetes to quit smoking is provided. Furthermore, telephone support successfully increased the smoking cessation rate compared with that previously reported by the usual care in patients with diabetes [17]. Finally, our results further verify those of a recent study where American Diabetes Association recommendations were applied for the treatment of tobacco dependence in patients with diabetes, with the successful result of increased smoking cessation rates and continued abstinence maintenance [18].

In contrast with previous reports [19], the majority of our smokers were highly educated and single. This may be explained by the adoption of an unhealthy lifestyle, including smoking, physical inactivity, and obesity, finally leading to type 2 DM and by the increased psychosocial stress often

found in people with higher socioeconomic status and those living alone that may hamper smoking cessation. However, the higher quit rate achieved in this study may also be partially explained by the higher educational level and marital support of our studied participants [20].

In accordance with previous studies [21], we found smoking continuance to be associated with worse glycemic control compared with smoking abstinence. Although this may be partly explained by the impact of smoking on insulin resistance and of the metabolic syndrome in diabetes [22], the beneficial glycemic effect of smoking cessation was independent of the improvement in insulin resistance parameters in our studied population. Finally, in the present study, regardless of any weight gain, 1-year smoking cessation together with the appropriate drug therapy was sufficient to ameliorate hypertension and dyslipidemia and help quitters to achieve earlier their metabolic treatment goals compared with those who continued smoking.

Regarding the microvascular and macrovascular complications, the salutary effect of weight loss, physical activity, and 1-year on-target drug therapy did not outweigh the adverse effect of smoking. One recent study also demonstrated a beneficial effect of 1-year smoking abstinence on the endothelial function of ex-smokers; however, in accordance with the present study, no significant detrimental change upon the endothelium vasculature was observed with 1-year smoking continuance [23]. Moreover, although 1-year smoking continuance did not increase the prevalence of vascular disease in the present study, it however blunted the positive effect of the antihypertensive and diabetic treatment, as well as the salutary effect of increased physical activity, in the studied population. This is in accordance with previous studies which demonstrated that smoking blunts the beneficial effect of antihypertensive medication on the management of BP [24] and on amelioration of arterial stiffness [25].

Diabetic microalbuminuria reflects glomerular endothelial dysfunction, and previous studies showed that its presence in type 2 DM is associated with a 20% increased risk of all-cause death, stroke, and acute myocardial infarction and a 10% increased risk of leg amputation if an intensified and target-driven treatment is not initiated early [9]. Except for microalbuminuria, smoking cessation in our study successfully predicted amelioration of PVD and of PN prevalence, whereas 1 year of smoking continuance contributed to the development of angina pectoris in 8.2% of the studied participants.

The results of the present study are in accordance with those of a global cross-sectional study that recently evaluated patients with type 2 DM and without known albuminuria and added further information regarding the early stage of diabetic nephropathy [26]. Among several potentially modifiable risk factors such as glycemic and BP control, smoking was an independent risk factor for increased urine albumin excretion [26]. In addition, our study in a smaller, community-based population also revealed a significant association between microalbuminuria and several vascular risk factors, such as HbA_{1c}, systolic and diastolic BP, smoking, and eGFR.

Another recent study also addressed the effect of cigarette smoking on urinary albumin excretion in a susceptible subgroup of premenopausal women with type 2 DM and demonstrated an increased level of urinary albumin excretion associated with current smoking [27]. In accordance with our findings, smoking was independently associated with increased insulin resistance, higher serum triglycerides, and lower HDL cholesterol levels [27].

Although smoking has been shown to exacerbate macroalbuminuria in diabetes, whereas smoking cessation reduces its progressive renal damage [28], to our knowledge, this is the first study to investigate prospectively the effect of smoking cessation on microalbuminuria upon type 2 DM diagnosis. Moreover, the beneficial effect of smoking cessation from other confounding variables and lifestyle interventions besides drug therapy was isolated; and an independent relationship was established. Finally, this is the longest study to determine the detrimental effect of smoking continuation in patients with type 2 DM and microalbuminuria, regardless of a multifactorial management.

Estimated GFR has been expected to decrease when macroalbuminuria is established, but not before. In accordance with a recent follow-up study of patients with type 1 and type 2 DM with and without nephropathy [29], we found that smoking is independently associated with a decrease of eGFR, regardless of the concomitant presence of macroalbuminuria. Furthermore, in the present study, although eGFR was comparable between those who continued and those who quit smoking, the higher frequency of microalbuminuria in the patients with type 2 DM who continued smoking compared with those who quit also indicates potential kidney dysfunction. Thus, our results indicate that smoking cessation is effective and crucial at the indolent course before eGFR drastically declines and provides the opportunity for preventive therapy in patients with diabetes and increased renal failure risk.

Previous studies have verified the effect of smoking on the vasculature via several different interactive mechanisms [30-32]. Among the hemodynamic mechanisms previously demonstrated as potential mediators of smoking-induced renal damage, nicotine and CO levels produce tachycardia, hypertension, and vasoconstriction; and both damage the endothelium directly [33]. Cardiovascular autonomic dysfunction is also involved in endothelial dysfunction and the development of microalbuminuria [34].

Smoking also affects vaso-occlusive factors such as activation of growth factors [35], platelet aggregation, plasma viscosity, and fibrinogen levels [36]. Recently, in human mesangial cells, nicotine induced cell proliferation and doubled fibronectin production [37], both important players in the progression of renal damage. Impaired lipoprotein and glycosaminoglycan metabolism, modulation of immune mechanisms, vasopressin-mediated antidiuresis, and insulin resistance all are affected by smoking exposure and promote kidney dysfunction [38].

Smoking is also associated with systemic markers of inflammation such as CRP through several pathophysiological mechanisms [39]. Certain compounds of smoke, such as free radicals [40] and phenol-rich glycoproteins [38], directly promote mesangial cell proliferation and hypertrophy, which may trigger the production of inflammatory cytokines such as tumor necrosis factor α, interleukin 1, and interleukin 6 [41]. There may also be an indirect effect of nicotine-induced catecholamine release [42], which modulates both the

systemic and local cytokine balances [43] and a direct release of endothelial progenitor cells from the bone marrow [44]. Although significant knowledge of the detrimental effect of smoking on kidney function is provided, few studies have focused on the pathophysiological substrate of smoking-induced diabetic nephropathy [34,45,46]. Therefore, our results indicate that further research addressing smoking-induced diabetic nephropathy from a pathological point of view is needed.

Although methodological problems that hampered several previous studies addressing the effect of smoking on diabetic microalbuminuria [26] are carefully presently addressed, this study is not without limitations. Firstly, because microalbuminuria was only measured at baseline and after 1 year, we could not evaluate the time course of renal function improvement with quitting. Secondly, although the 77.5% absolute regression of microalbuminuria in newly diagnosed patients with type 2 DM is as dramatic as that reported in a 6-year prospective study of patients with type 1 DM and proteinuria [47], even so, our treatment effect may be slightly underestimated because smoking quitters may still be exposed to second-hand cigarette smoking, which was not evaluated in this study.

The risk of PVD is increased in patients with diabetes, occurs earlier, and is often more severe and diffuse [48]. Endothelial dysfunction, vascular smooth muscle cell dysfunction, inflammation, and hypercoagulability are the key factors in diabetic arteriopathy [49]. The presence of PVD is associated with increased risk of claudication, ischemic ulcers, gangrene, and possible amputation [50]. Moreover, it is a marker of atherosclerosis and a strong predictor of diabetic cardiomyopathy and ischemic heart disease [51]. Recently published trials pointed out that lowering glycemic targets to nearly normal glycemia does not further reduce cardiovascular events in individuals with type 2 DM [52]. The Steno-2 study demonstrated that significant reduction in PVD, cardiovascular disease, and mortality in type 2 DM is manifested through intensified multifactorial treatment of all modifiable risk factors [53].

In the present study, 1-year smoking cessation was associated with amelioration of insulin resistance parameters and with reduction of PVD and of PN prevalence. On the contrary, 1-year smoking continuance was associated with presence of angina pectoris in previously asymptomatic and free from macrovascular complications patients with type 2 DM. Recent studies demonstrated that, in patients with type 2 DM, presence of angina pectoris is an independent high-risk predictor of myocardial ischemia [54]. Finally, higher smoking rates were recently associated with higher rates of diabetic neuropathy among different ethnic groups [55]. Therefore, our results further demonstrate that to prevent macrovascular together with microvascular complications in patients with type 2 DM, smoking cessation should be initiated upon diabetes diagnosis.

In conclusion, diabetic nephropathy in type 2 DM is a leading cause of end-stage renal disease worldwide [1,5]. Its early clinical sign is microalbuminuria, an independent predictor for progression of nephropathy [56] as well as an independent risk factor for cardiovascular disease [57,58]. Although, initially, diabetic nephropathy was believed to be progressive and irreversible, recent studies provided growing evidence regarding its remission and/or regression [1,5]. In accordance with previous studies, we verified microalbuminuria as the earliest clinical manifestation of diabetic nephropathy. Furthermore, our results support that the early screening and diagnosis of microalbuminuria, together with on-target glycemic, lipids, and BP control, remain important in decreasing diabetic nephropathy and its progression. We also demonstrated that smoking cessation is among the main therapeutic objectives for type 2 DM because it not only is associated with the prevention of the progression of microalbuminuria to overt macroalbuminuria, but also has a beneficial effect on the diabetic microvascular and macrovascular damage. Moreover, together with ACE inhibitors, statins, and other targeted therapies of diabetic nephropathy, smoking cessation appears to be a crucial and optimal treatment for the amelioration of kidney exposure and of the other diabetic complications upon diagnosis of diabetes.

Smoking cessation is recently reported as among the highest very cost-effective interventions intended to prevent and/or control diabetes [59]. Although extended follow-up would be necessary to estimate the relationship between smoking cessation and amelioration of future microvascular as well as macrovascular disease events, our results underline the importance of an early smoking cessation education upon diagnosis of diabetes to possibly decrease the rate of the clinical and economical burden of diabetic complications. Health keepers should therefore consider giving this therapeutic intervention a higher priority. Finally, prospective and randomized intervention studies are needed to further provide sufficient evidence that smoking cessation slows the rate of the diabetic renal and vascular function decline.

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