Proteinuria Is an Independent Risk Factor for Ischemic Stroke in Non–Insulin-Dependent Diabetes Mellitus

Fernando Guerrero-Romero, MD; Martha Rodríguez-Morán, MD

Background and Purpose—Proteinuria is an independent risk factor for cardiovascular disease in patients with NIDDM. The aim of this study was to assess the relationship between proteinuria and ischemic stroke in subjects with NIDDM, and to determine whether proteinuria is an independent risk factor for stroke.

Methods—We performed a case-control study of 59 diabetic patients with first-ever ischemic stroke due to thrombotic arterial occlusion, who were considered cases, and 180 diabetic patients without stroke, matched by gender, age, and diabetes duration, as a control group. WHO criteria for verified definite or possible stroke were used to ascertain the diagnosis of stroke. For the purpose of this study proteinuria was defined as a 24-hour urinary protein excretion rate of ≥20 and <200 mg/min. Risk factors included were smoking, blood pressure, body mass index, serum total cholesterol, hyperglycemia, and proteinuria.

Results—Subjects with stroke had higher proteinuria proportion and systolic and diastolic blood pressures. Both frequency of antihypertensive treatment and antihypertensive drugs used were similar among subjects with and without stroke. In multivariate logistic regression analysis, the ORs and 95% CIs for the variables identified as risk factors for stroke were as follows: systolic pressure (OR 3.10; 95% CI 3.01 to 4.21; P=0.03); diastolic pressure (OR 3.30; 95% CI 1.04 to 4.48; P<0.0001); fasting glucose ≥11.1 mmol (OR 1.82; 905% CI 1.4 to 3.8; P=0.04), HbA1c ≥9.5% (OR 1.7; 95% CI 1.3 to 5.1; P<0.01), and proteinuria (OR 3.23; 95% CI 1.06 to 4.36; P<0.0001).

Conclusions—Our case-control study gives evidence that proteinuria is an independent risk factor for ischemic stroke in patients with NIDDM. (Stroke. 1999;30:1787-1791.)

Key Words: diabetes mellitus ♦ proteinuria ♦ stroke, ischemic

The incidence of cardiovascular and atherosclerotic vascular diseases such as stroke is higher in patients with non–insulin-dependent diabetes mellitus (NIDDM) than in nondiabetic patients. The cardiovascular risk has been found to be associated with an increased urinary protein excretion rate. Clinical proteinuria is an ominous development in a subject with diabetes. It leads to a decline in the glomerular filtration and premature cardiovascular mortality.

Albuminuria is a strong predictor of renal disease progression, premature death of cardiovascular origin, and foot ulcers in patients with NIDDM. It may reflect a generalized vascular process that affects the glomeruli and intima of large vessels simultaneously. The relationship between microalbuminuria and cardiovascular disease mortality in patients with NIDDM is well established; however, less is known about the association between total proteinuria and vascular disease. In particular, few data are available regarding the relationship between proteinuria and stroke.

Cardiovascular and neurological diseases were the most significant related complications with the cause of death in a study that included 1320 diabetic subjects. Stroke has a great impact on public health; its annual incidence in the United States is at least one-half million, with 3 million stroke survivors living. Determination of risk factors for ischemic stroke is the basis for stroke prevention strategies. The aim of this study was to assess the relationship between proteinuria and ischemic stroke in patients with NIDDM and to determine whether proteinuria in the range ≥20 and <200 μg/min is an independent risk factor for stroke.

Subjects and Methods

Design and Setting

The study protocol was approved by the Mexican Social Security Institute (MSSI) Research Committee. After obtaining informed consent from the patients, a case-control study was performed. Cases were recruited from the General Hospital Urgencies Department and control subjects from the offices at First Medical Level Care of the MSSI in Durango, Mexico, from January 1993 to June 1998.

Patients

Consecutive NIDDM Hispanic-Mexican subjects with first-ever ischemic stroke due to thrombotic arterial occlusion were considered cases and compared with a control group of NIDDM subjects.
without stroke, who were matched by age, gender, and duration of diabetes. A control-case ratio of 3:1 was considered.

The study was planned to include 48 cases and 144 controls on the basis of the following criteria: \( \alpha = 0.05, \beta = 0.10, \) frequency of proteinuria in the cases (\( p' = 0.68, 13 \)) frequency of proteinuria in the controls (\( p'' = 0.41, 14 \)) and difference of the exposure between cases and controls (\( \delta = 0.27, \)) with a control/case ratio of 3:1 (\( r \)). Sample size was calculated applying the formula\(^15\) \( n = \frac{(\alpha z^2 + z \beta')^2}{\delta^2} (1 - r) + \frac{(1 + \beta') r}{1 - \delta} \), in which \( p'' = p' + p'' \delta (1 + r) \).

When 71 cases were obtained, we had identified 265 potential control subjects; 192 of them fulfilled the matching criteria, which was done by sex, age, and diabetes duration. At this step of the process, we lost 12 patients in the case group because of death. Finally, 59 cases and 180 controls were included. After we obtained informed consent from the participants, 5 mL of venous blood was drawn to measure plasma glucose and cholesterol levels and a 24-hour urine sample was collected to determine the urinary protein excretion. All the measurements were done within 10 days after inclusion at study.

The WHO criteria for verified definite or possible stroke were used in the ascertainment of the diagnosis of first-ever ischemic stroke, which was defined as a clinical syndrome consisting of neurological symptoms persisting >24 hours\(^14\) and confirmed by an area of low density on CT scan of the head.\(^11\) Subjects with a history of previous stroke were excluded. The emergency department neurologist performed the physical examination and made the clinical diagnosis.

Stroke due to intracranial or subarachnoid hemorrhage and cerebral infarction associated with a major cardiac source of emboli or with significant atherosclerotic disease in an appropriate extracranial artery were excluded. Furthermore, the following subjects were excluded: those with heart failure, renal disease, acute febrile illness, infection of the urinary tract, and overt proteinuria (\( \geq 200 \mu \text{g} / \mu \text{L} \)) and those receiving treatment with angiotensin-converting enzyme inhibitors or pentoxifylline.

Risk factors included in the current study were smoking, blood pressure, body mass index (BMI), serum total cholesterol, hyperglycemia, and proteinuria. These were assessed in each patient by a standardized questionnaire, physical examination, and laboratory evaluation.

**Measurements**

Plasma glucose and cholesterol levels were measured with an Express 550 clinical chemistry autoanalyzer (Ciba Corning Diagnostic Corp) in fasting conditions of 8 to 10 hours. Glycosylated hemoglobin A\(_1c\) (HbA\(_1c\)) percentage was measured by HPLC, and the normal range was 4.5% to 6.5%.

Proteinuria was measured, on a 24-hour urine collection, by turbidity with glycine precipitation with 3% sulfasalicylic acid and determination of protein excretion by measuring absorbance at a wavelength of 550 nm with a spectrophotometer. For the purpose of this study, proteinuria was defined as a 24-hour urinary protein excretion rate of \( \geq 200 \mu \text{g} / \mu \text{L} \) and those receiving treatment with angiotensin-converting enzyme inhibitors or pentoxifylline.

Risk factors included in the current study were smoking, blood pressure, body mass index (BMI), serum total cholesterol, hyperglycemia, and proteinuria. These were assessed in each patient by a standardized questionnaire, physical examination, and laboratory evaluation.

**Statistical Analysis**

Comparison between groups was made with the unpaired Student \( t \) (Mann-Whitney \( U \)) test for numeric variables and \( \chi^2 \) test for differences among proportions. By calculating the odds ratio, the relationship between proteinuria and stroke was estimated. Multivariate logistic regression analyses was performed to determine the independent effect of microalbuminuria on stroke.

Differences were considered statistically significant at \( P < 0.05 \). Data were analyzed by the Statistica package (StatSoft, Inc; 1992).

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### Table 1. Characteristics of NIDDM Patients

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>With (n=59)</th>
<th>Without (n=180)</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI, kg/m(^2)</td>
<td>28.3±4.9</td>
<td>28.5±4.0</td>
<td>NS*</td>
</tr>
<tr>
<td>Systolic pressure, mm Hg</td>
<td>131.9±17.0</td>
<td>125.3±19.4</td>
<td>0.02*</td>
</tr>
<tr>
<td>Diastolic pressure, mm Hg</td>
<td>92.2±14.4</td>
<td>81.5±14.2</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Fasting glucose, mmol/L</td>
<td>10.4±4.6</td>
<td>9.7±3.5</td>
<td>NS*</td>
</tr>
<tr>
<td>Glycosylated HbA(_1c), %</td>
<td>11.4±2.1</td>
<td>10.7±2.0</td>
<td>NS*</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>5.9±2.6</td>
<td>6.3±3.6</td>
<td>NS*</td>
</tr>
<tr>
<td>Proteinuria, ( \mu \text{g} / \text{min} )</td>
<td>88.3±69.9</td>
<td>29.2±53.7</td>
<td>0.005†</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SD.

*By unpaired Student \( t \) test and †Mann Whitney \( U \) test.

### Results

Two hundred thirty-nine patients, 59 cases (37 women and 22 men) and 180 controls (118 women and 62 men) were included. The average age was 59.1±8.3 years versus 59.3±10.4 years (\( P = NS \), unpaired Student \( t \) test) and the average duration of diabetes 8.6±6.9 years versus 8.6±7.6 years (\( P = NS \), Mann-Whitney \( U \) test), for the subjects with and without stroke, respectively.

Prevalence of hypertension was of 52.7%. Antihypertensive treatment was similar among subjects with and without stroke. Antihypertensive drugs used in subjects with and without stroke were \( \beta \)-blockers (58% and 53%), calcium channel blockers (37% and 42%), thiazides (4% and 3%), and others (1% and 2%), respectively.

Men and women were of similar age and had similar levels of serum cholesterol and blood pressure. The frequency of hypertension and antihypertensive treatment drug was similar among men and women. More men (42.4%) than women (5.2%) were smokers (\( P = 0.0001 \), \( \chi^2 \) test).

Proteinuria was identified in 70 subjects (29.3%), 45 (76.3%) with ischemic stroke and 25 (13.9%) of the control group (\( P < 0.0001 \), \( \chi^2 \) test). In both groups, all the subjects with proteinuria had a positive microalbumin test, which was negative in all the subjects without proteinuria.

The vast majority of the patients had others vascular risk factors. These included age \( \geq 60 \) years (in 52.7%), obesity (BMI \( \geq 30 \text{ kg/m}^2 \); 31.3%), diabetes duration \( \geq 10 \) years (36.8%), arterial hypertension (52.7%), smoking (18.0%), and hypercholesterolemia (58.1%).

The main characteristics of the target population are presented in Table 1. Subjects with and without stroke had similar BMI, smoking, and hypertension. The vast majority of the subjects with stroke had proteinuria and higher systolic/diastolic blood pressures. Both frequency of antihypertensive treatment and antihypertensive drugs used were similar among subjects with and without stroke.

Because the great majority of the target population had elevated fasting glucose, HbA\(_1c\), and total cholesterol serum levels, to determine the association of these variables with stroke a subanalysis of subjects on the basis of quartile distribution was performed. Subjects within the upper quartile of the distribution were considered at risk.
Bivariate analysis demonstrated that proteinuria, elevated blood pressure, fasting glucose $\geq 11.1$ mmol, and HbA1c $9.5\%$ were factors significantly associated with stroke ($P<0.05$, $\chi^2$ test), Table 2.

To determine which factors were independently associated with the risk of stroke, multivariate logistic regression analyses were performed. High systolic/diastolic pressures, fasting glucose $11.1$ mmol, gHbA1c $9.5\%$, and proteinuria remained as independent predictors for stroke. The ORs (with 95% CIs and $P$ values) for the factors were as follows: systolic pressure $3.10$ (1.01 to 4.21, $P=0.03$); diastolic pressure $3.30$ (1.04 to 4.48, $P=0.0001$); fasting glucose $11.1$ mmol/L 1.82 (1.4 to 3.8, $P=0.04$); gHbA1c $9.5\%$ 1.7 (1.3 to 5.1, $P<0.01$); and proteinuria 3.23 (1.06 to 4.36, $P<0.0001$).

**Discussion**

Stroke is a special problem that is particularly tragic because of the potential for a lifetime of disability and the high risk of death. The most important risk factors for ischemic stroke are aging, smoking, high systolic and diastolic blood pressures, diabetes, hyperglycemia, transient ischemic attacks, and previous history of stroke.

Our findings shown that proteinuria ($\geq 20$ and $<200$ $\mu$g/min) is an independent risk factor for stroke in patients with NIDDM. This support the hypothesis that proteinuria reflects a more generalized vascular process, in agreement with a previous report which, based on a 7-year follow-up, showed that proteinuria is a predictor for stroke in nondiabetic and diabetic subjects.

Proteinuria seems to be an underlying disorder itself, predicting all-cause and cardiovascular disease mortality. We determined the total urinary protein excretion and qualitatively assessed the albumin concentration. Thus, we can assume that the subjects we studied, who had proteinuria, excreted urinary albumin. If we had quantitatively measured the albumin excretion rate, our findings concerning its relationship with stroke might have been even stronger.

The mechanisms of the association between proteinuria and cardiovascular disease are poorly understood. It has been proposed that albuminuria is associated with the increase of both albumin and fibrinogen transcapillary escape rate, which reflects widespread vascular damage, or endothelial dysfunction. Furthermore, albuminuria has been shown to be related to increased extravascular coagulation, which leads to an increased release of von Willebrand factor, contributing to the formation of microthrombi and platelet plugs, followed by areas of non-perfusion. These support the hypotheses that increased urinary protein or albumin excretion is associated with increased mortality and widespread vascular injury, which contribute to the risk of stroke. Nevertheless, only a few studies regarding this association have previously been published.

High systolic and diastolic blood pressures are risk factors associated with stroke, a relationship that was demonstrated in our study. It is well known that diabetic patients have an increased risk of both hypertension and death from stroke. It has been previously reported that some of the effects of hypertension status as well as duration on the risk of stroke can be attributed to factors other than high blood pressure itself. In this matter, although several antihypertensive drugs have adverse effects on glucose control and lipid profile, the relationship between hypertension and stroke risk is under debate. On this respect, recent studies have...
demonstrated that there may be no possible advantage to a particular antihypertensive drug in terms of worsening glucose control in diabetic subjects. Because ACE inhibitors modify albuminuria, in this study the subjects who received these drugs were not included. Furthermore, we have not evaluated the effects of different treatment modalities for hypertension on the risk of stroke.

The relationship between hyperglycemia and stroke, which has been previously reported, was established in our study. However, because the vast majority of the target population had high serum glucose and gHbA1c levels, a subanalysis of subjects in the extreme values of the quartile distribution was necessary to demonstrate the relationship. The importance of hyperglycemia with respect to macrovascular disease is controversial. Hyperglycemia is related to atherogenic lipoprotein changes and is also a procoagulant state. Hyperglycemia can decrease prostacyclin synthesis, increase thrombosis formation, and cause glycosylation of proteins in the artery wall. These conditions, which are related to poor metabolic control, can cause atherosclerosis, which could contribute to increase the risk of stroke. Regarding this concern, further studies are needed to determine whether activation of the coagulation system is the cause or the result of vascular disease in poorly controlled diabetic subjects.

Because aging and duration of diabetes have previously been reported as strong predictors of both stroke and albuminuria, in this study we controlled for those variables by matching for age and duration of diabetes (Table 2). Thus, these risk factors do not differentiate between stroke and nonstroke patients.

Given the compared populations, this study demonstrated no association between smoking and hypercholesterolemia with stroke.

The absence of an association between smoking and stroke probably was because the narrow range of diabetic smokers made the statistical power low. Similarly, Lehto et al. reported a low prevalence of smokers among diabetic subjects and a lack of association between smoking and risk of stroke in such patients.

On the other hand, the relationship of serum cholesterol with the excess risk of stroke in subjects with NIDDM is not consistent. This circumstance could be only partly explained by the limitation of the cross-sectional design studies carried out. Recently, Davis et al. reported, in patients with newly diagnosed type 2 diabetes recruited to the United Kingdom Prospective Diabetes Study, that dyslipidemia was not significantly associated with stroke.

The implication of our findings is that proteinuria (≥20 and <200 µg/min) is an independent and strong risk factor for stroke in subjects with NIDDM. The significance of recognizing the relationship of proteinuria to stroke is that it is a modifiable and easily identifiable risk factor.

However, there is no direct proof that proteinuria is causative of macrovascular complications or that decreasing urinary protein excretion could delay or prevent stroke. Further research is necessary to support these hypotheses.

References


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