An autopsy study suggests that diabetic nephropathy is underdiagnosed

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The reported prevalence of diabetic nephropathy (DN) among patients with diabetes varies widely. Most studies use the presence of microalbuminuria for clinical onset of DN in the absence of a histopathologic evaluation. In this autopsy study, we collected and analyzed data from a cohort of patients with type 1 or 2 diabetes and determined the prevalence of histologically proven DN in patients with or without clinical manifestations of renal disease. We also examined the distribution among histopathologic classes with respect to clinical parameters. Renal tissue specimens from autopsies and clinical data were collected retrospectively from 168 patients with diabetes. The histopathologic classification for DN was scored as were interstitial and vascular parameters. In this cohort, 106 of 168 patients had histopathologic changes in the kidney characteristic of DN. Twenty of the 106 histologically proven DN cases did not present with DN-associated clinical manifestations within their lifetime. Glomerular and interstitial lesions were associated with renal function but not with proteinuria. We also found that underdiagnosed DN may encompass all histopathologic classes except the sclerotic class. Thus, the prevalence of histologically proven DN was higher than previously appreciated, and we found a relatively high proportion of DN that was clinically underdiagnosed yet histologically proven, suggesting that DN lesions may develop before the onset of clinical findings.

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Diabetic nephropathy (DN) is 1 of the leading causes of end-stage renal disease,[1–3] which develops in approximately 10% to 30% of patients with diabetes mellitus. The reported prevalence of DN depends on the type of diabetes, its duration, and the patient's ethnicity. DN presents approximately 10 years after the onset of type 1 diabetes (T1D)[4]; in contrast, the time of onset of DN among patients with type 2 diabetes (T2D) is highly variable.[5] The prevalence of clinically diagnosed DN in T1D varies between 5% and 20%,[6–8] and the prevalence in T2D is between 25% and 35%, based on microalbuminuria or proteinuria.[5,7] Data on renal pathologic conditions in patients with DN is relatively limited, because a renal biopsy is performed only in cases in which the renal disease's manifestations cannot be explained sufficiently by the presence of clinically suspected DN.[9,10] Relatively few studies on DN have confirmed clinical manifestations of DN by renal biopsy.[11–14]

It is generally thought that the clinical onset of DN is characterized by microalbuminuria. However, some studies suggest that a reduction in glomerular filtration rate (GFR) may precede the development of microalbuminuria.[15–18] Relatively little is known about the number and severity of histologic lesions in the kidney before the clinical onset of DN. Thickening of the glomerular basement membrane and mesangial changes were described in 1985 by Mauer *et al.* as the earliest histologic manifestations of DN.[19] A recent study with normoalbuminuric patients with T1D showed that greater glomerular basement membrane width is an independent predictor for progression to DN.[20] Nodular sclerosis is often encountered in patients with substantial proteinuria. Interestingly, there is evidence that to some extent, lesions of DN in T1D may be reversible.[21,22]

A histopathologic classification for DN was launched in 2010.[23] The original study included a substudy on interobserver agreement among pathologists, showing good agreement for the evaluation of the classes. Over the years, several clinical validation studies appeared,[11,13,14] of which the most recent study[11] showed that the severity of glomerular and interstitial lesions is significantly associated with renal outcomes in patients with DN. In a smaller study, interstitial lesions—but not glomerular lesions—were determined to be a significant predictor of renal prognosis.[14] Studies that include patients with diabetes who underwent a renal biopsy may be subject to selection bias regarding the moment in time at which the biopsy was performed.[13,24] To avoid such a

selection bias, we obtained tissue samples from autopsy specimens rather than from biopsy samples.

In this autopsy study, we collected and analyzed data from a unique cohort of patients with T1D or T2D, and we determined the prevalence of histologically proven DN in patients with or without clinical manifestations of renal disease. Virtually none of the included patients underwent a renal biopsy during their lifetimes. The aim of this study was to investigate the prevalence of histopathologically proven DN in patients with diabetes with or without clinical signs of DN and its distribution over histopathologic classes. Furthermore, we investigated which clinical parameters were related to the histopathologic classes in patients with and those without clinical manifestations of DN.

RESULTS

The baseline characteristics of the 168 included patients are summarized in Table 1. The cohort contained 17 patients with T1D and 127 patients with T2D; in 24 cases, the type of diabetes was unclear. The mean age of the 168 patients was 69 years, and 55% of the cohort were men. The histopathologic examination revealed lesions that were consistent with DN in 106 patients. For 21 patients, the clinical data were insufficient for determining whether they had received a clinical diagnosis of DN. In 65 of 106 patients, the clinical diagnosis of DN was in accordance with the histologic lesions, that is, a clinical diagnosis of DN had been made before death and lesions consistent with DN were found at autopsy. In 20 of the 106 patients with histologic signs of DN at autopsy, no clinical diagnosis of DN was made before death, because neither microalbuminuria nor proteinuria had been observed during these patients' lifetimes. The 20 patients with underdiagnosed DN had multiple negative results on dipstick tests or no albuminuria (<30 mg/24 h) found in the 24-hour urine collection (or both) in the year before death (Table 2). Of these 20 patients, the histopathologic classification revealed that 7 patients had class I DN, 5 patients had class IIa DN, 3 patients had class IIb DN, and 5 patients had class III DN (Figure 1). Table 3 summarizes the characteristics of patients with diagnosed and underdiagnosed DN, as well as the matched control group. The decades of death varied among the underdiagnosed patients (1 patient died before 1990, 15 patients died between 1990 and 2000, and 4 patients died between 2000 and 2004), so the treatment of diabetes did not seem to influence this phenomenon. Eight patients were diagnosed clinically as having DN; however, no lesions consistent with DN were found in their renal tissues at autopsy.

Table 1 | Baseline characteristics of the cohort

Baseline characteristic	Percentage or mean (SEM)
Sex (% male)	54.8
Age, y	69.3 (0.96)
T1D (%)	10.1
Duration of diabetes, y	13.69 (1.27)
eGFR, ml/min per 1.73 m^2	53.14 (2.85)
Microalbuminuria or proteinuria (%)	49.4%
Creatinine serum, µmol/l	136.21 (11.23)
Hb, mmol/l	7.12 (0.13)
HbA1c, (% units)	8.2 (0.82)
Cholesterol, mmol/l	4.97 (0.24)
Death by CV event (%)	47.6
Systolic pressure, mm Hg	134 (2.63)
Diastolic pressure, mm Hg	75.6 (1.30)

CV, cardiovascular; eGFR, estimated glomerular filtration rate; Hb, hemoglobin; HbA1c, glycated hemoglobin; T1D, type 1 diabetes.

Histopathologic lesions

Lesions consistent with DN were found in 63% of the tissue specimens (106 of 168). Twenty-two of the samples had class I DN, which is characterized by a thickened glomerular basement membrane as determined by electron microscopy. The glomerular basement membrane width can change because of hypertension, but in this cohort there was no significant difference between patients with and those without hypertension between class I and class 0 ($P = 0.904$). Therefore, it was unlikely that increased glomerular basement membrane width was caused by hypertension. Thirty-three samples had class II DN, characterized by mesangial expansion; 21 of these samples were class IIa, and 12 samples were class IIb. Forty-five samples had class III DN, characterized by the presence of nodular sclerosis. The remaining 6 samples had lesions that were consistent with class IV DN, characterized by more than 50% of glomeruli with global sclerosis. We found that the percentage and range of glomeruli with mesangial sclerotic nodules was significantly lower in the underdiagnosed group compared with the diagnosed group. Overall, the mean percentage of nodules in the 45 cases with class III DN was 22.9% (range, 2.6%–67.6%). In the diagnosed and underdiagnosed groups, the mean percentage of nodular sclerosis was 24.4% (range, 5.6%–67.6%) and 10.9% (range, 3.0%–26.2%), respectively ($P = 0.031$).

In addition, we examined mesangial expansion in the underdiagnosed group. The 5 patients with class IIa DN had mesangial expansion in 40%, 51%, 51%, 57%, and 60% of glomeruli (mean, 51.8%). The 3 patients with class IIb DN had mesangial sclerosis in 92%, 93%, and 100% of glomeruli (mean, 95%). Glomerular lesions are associated with other histopathologic lesions in DN, including interstitial fibrosis and tubular atrophy (IFTA) ($P < 0.001$) and lesions such as arteriosclerosis, hyalinosis, capsular drops, and glomerular hyalinosis ($P = 0.238$, $P < 0.001$, $P = 0.004$, $P < 0.001$, respectively). IFTA, arteriosclerotic lesions in the arterioles, and hyalinosis were more prevalent among the patients with histologically proven DN than among the patients who had no DN lesions (i.e., class 0 DN) ($P = 0.007$, $P = 0.017$, and $P < 0.001$, respectively). There were significantly more instances of IFTA, arteriosclerotic lesions, and hyalinosis with more severe DN ($P < 0.001$ for both arteriosclerotic lesions and hyalinosis). In contrast, no significant correlation was found between the degree of arteriosclerosis and the histopathologic class of DN ($P = 0.238$). The number of capsular