


Collapsing glomerulopathy superimposed on diabetic nephropathy: insights into etiology of an under-recognized, severe pattern of glomerular injury

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ABSTRACT

Background. Collapsing glomerulopathy (CG) represents severe podocyte injury with massive proteinuria, rapid progression and relative resistance to therapy. It is associated with multiple etiologies, including obliterative arteriopathy in transplants. However, its association with diabetic nephropathy (DN) has not been reported.

Methods. Renal biopsies performed in diabetic patients for either increasing proteinuria or deteriorating renal function, or both, were retrospectively reviewed. The clinicopathologic features and immunohistochemical staining of podocytes were analyzed.

Results. Of 534 patients with DN, 26 human immunodeficiency virus (HIV)-negative patients were found to have CG superimposed on DN (5% DN cases). At the time of biopsy, their mean serum creatinine was 3.8 mg/dL and proteinuria was 9.8 g/24 h. Renal biopsy showed CG in 2–30% (mean 16% of glomeruli), with segmental (2%) and global (33%) glomerulosclerosis. DN classification was Class IV-12, III-8, IIb-4 and IIa-2. Vascular sclerosis was moderate (44%) and severe (56%). Extensive arteriolar hyalinosis with >50% luminal stenosis was seen in 85% of cases. Markers of podocyte differentiation were lost, consistent with other types of CG. Cytokeratin was focally positive in 70% and VEGF overexpressed in 43%. Follow-up on 17 patients: 13 developed end-stage renal disease (ESRD) in 7 months from the time of biopsy. The development to ESRD in these patients was more rapid than diabetic controls without CG (P = 0.005). The remaining four, 5–24 months follow-up, had an increase in creatinine with stable proteinuria.
Conclusions. CG contributes to an increased level or new onset of proteinuria in DN which may be intractable. CG in DN with advanced vascular hyalinosis is presumably due to ischemic podocyte injury and is of prognostic significance.

Keywords: collapsing glomerulopathy, diabetic nephropathy, proteinuria, pathology

INTRODUCTION

Diabetic nephropathy (DN) is the leading cause of end-stage renal disease (ESRD) in the USA and many countries in the world [1]. The clinical course of proteinuric DN is well understood, and 35–45% of both type 1 and 2 diabetic patients develop ESRD in 20–35 years [1]. However, some diabetic patients, particularly newly diagnosed type 2 diabetics, present with nephrotic-range proteinuria or nephrotic syndrome and deteriorating renal function without any other manifestations of diabetic complications. Renal biopsy in such patients showed a non-diabetic glomerular lesion in 10–12% [2–4]. Such non-diabetic lesions occurred either alone or superimposed on DN, which included idiopathic membranous glomerulonephritis (GN), focal segmental glomerulosclerosis (FSGS), acute post-streptococcal GN, chronic immune complex-trapping GN, minimal-change disease, lupus nephritis and chronic GN. The development of collapsing glomerulopathy (CG) in DN as a cause of proteinuria has not been definitively established.

CG is an increasingly recognized, severe pattern of glomerular injury with a rapid clinical course, including massive proteinuria and relative resistance to standard treatment. The pathological appearance is characterized by segmental or global collapse of the glomerular capillary tuft with wrinkling and retraction of the capillary walls, overlaid by epithelial cell proliferation in the Bowman space and frequently accompanied by tubulointerstitial disease [5–7]. CG is regarded as a podocytopathy [8]. It is predominantly seen in the primary form in patients of African descent [9]. Also, it has been found in association with numerous etiologies, including viral infections (HIV, CMV, Parvovirus B19 and HCV) [6, 9], drugs (pamidronate, all forms of interferons) [10, 11], autoimmune diseases (SLE and mixed connective tissue disease) [12], and in the setting of renal transplantation [13–15]. The primary etiology of CG has been elusive but multiple triggers have been proposed such as an immune-mediated or ischemic injury to the podocyte.

Mature podocytes have a characteristic phenotype in the normal adult glomerulus, expressing transcription factor WT-1 (Wilms’ tumor protein 1) as well as podocyte proteins such as podocin, synaptopodin, podocalyxin, nephrin and GLEPP-1 (glomerular epithelial protein 1) [16]. In CG, WT-1, GLEPP-1, synaptopodin, podocalyxin and cyclin inhibitors p21 and p27 are not present in the proliferative/hyperplastic epithelial cells (pseudosclerotic) [8, 9, 17].

In this study, we provide evidence that CG is superimposed on DN, and that severe obliteratorive microvascular disease in diabetic patients, due to arteriolar hyalinosis and comorbid hypertensive disease, may cause ischemic podocyte injury as well as a collapsing pattern of glomerular pathology similar to that seen in the transplant setting and may correlate with significant proteinuria.

METHODS

Native renal biopsies, processed using standard techniques for light, immunofluorescence (IF) and electron microscopy, from 2003 to 2011 were reviewed from patients with biopsy-proven DN and CG. None of these glomeruli showed evidence of an immune complex-mediated disease. We used the definition of CG suggested by the Columbia Classification, which indicates that a single segmental or global lesion is sufficient to define the pattern of glomerular injury, although in our reports a descriptive terminology such as ‘features of collapsing glomerulopathy’ was used in many of the cases rather than the term ‘CG’ or ‘collapsing FSGS’ [5]. Collapsing features of glomerular injury are defined by light microscopy as at least one glomerulus with global or segmental collapse of the capillary tuft, wrinkling of the glomerular basement membrane (GBM) and hyperplasia and/or hypertrophy of the overlying epithelial cells usually containing intracytoplasmic protein droplets and vacuolization [5]. Vascular sclerosis was evaluated in the small arteries and arterioles and semi-quantitatively scored as mild (<25% luminal narrowing), moderate (25–50% narrowing) or severe (>50% narrowing). Hyalinosis was scored as present or not present. Demographic, clinical, laboratory and serologic findings were obtained and correlated with renal biopsy findings.

Immunohistochemical stains on paraffin-embedded sections were performed using the Bond Max Autostainer (Leica Microsystems, IL) to further characterize the pattern of expression of podocyte proteins in these glomerular lesions. The primary antibodies used were mouse monoclonal antibody against β-dystroglycan (Clone 43DAG1/8D5, NovoCasta, 1:50 dilution), mouse monoclonal antibody against synaptopodin (Clone G1D4, Progen Biotechnik,1:10 dilution), rabbit anti-human podocin (Clone PODO 11-A, Alpha Diagnostics, 20 µg/ml dilution), polyclonal anti-serum against WT-1 (C19, Santa Cruz Biotechnology), monoclonal pan-cytokeratin (AE1/AE3, Millipore MAB3412) and anti-VEGF (VG1, Dako, 1:50 dilution). The sections were deparaffinized and endogenous peroxidase was inactivated. Antigen retrieval was accomplished using the Bond Epitope Retrieval Solution 2 (ER2) at 99–100°C for 20 min (Leica Microsystems). The sections were then incubated sequentially with the primary antibody for 25 min, post-primary for 15 min and polymer for 25 min ending with colorimetric development with diamobenzidine for 10 min (Bond Polymer Define Detection; Leica Microsystems).

A control group was selected, matched according to class of DN, using biopsies from patients with DN but not showing lesions of CG for clinical, immunohistochemical and follow-up comparison.

Statistical analyses were performed using a Student’s T-test for all variables except for gender and presence of hypertension, for which χ2 testing was utilized.
RESULTS

Between January 2003 and December 2011, in our center, there were 4264 native kidney biopsies, of which 534 (12.5%) were diagnosed with DN. A superimposed disease was additionally present in 262 cases, or 49% of those with DN. The superimposed diseases were most commonly acute interstitial nephritis (15%), a podocytopathy (i.e. minimal-change disease, FSGS or CG) (17%) or post-infectious GN (9%). Rarely, other diseases were present including crescentic GN, Bence Jones cast nephropathy or membranous GN.

CG accounted for 11% of the cases (n = 30) of superimposed disease, 5.6% of all cases with DN. Four cases were excluded from the study because the patients were positive for human immunodeficiency virus (HIV, n = 3) and/or hepatitis C virus (HCV) on interferon therapy (n = 2). There remained 26 patients, age ranging in age from 26 to 80 years. Of these 26 patients, 22 (85%) had type 2 diabetes, while the remaining 4 (15%) had type 1 diabetes. Patients with type 1 diabetes were younger, all under the age of 40 (mean 29 year) at the time of biopsy, while all the type 2 diabetic patients were over the age of 40 (mean 49.5 year). The duration of diabetes ranged from new onset to >20 years (mean 14 years). Fifty-six percent of the patients were on insulin therapy at the time of biopsy. Patient ethnicity varied: 11 were Hispanics, 8 Caucasians and 7 African of descent. Hypertension, defined as blood pressure ≥140/90 mmHg, was present in 80%, and a total of 86% were on anti-hypertensive medications.

Serum creatinine levels were elevated in all but two patients, ranging from 1.1 to 10.4 mg/dL (mean 3.6 mg/dL). All patients except for two (2 and 2.9 g/24 h) had nephrotic-range proteinuria, mean 9.5 g/24 h (range 2–29). None of the patients were positive for HIV. Three had positive serology for hepatitis C antibody.

The biopsies were performed in all patients for new onset or recent rise in proteinuria. The cases were divided into classes of DN according to Tervaert et al, Table 1 [18]. There were 3 cases of Class IIA (mild diffuse mesangial expansion), 4 Class IIB (severe diffuse mesangial expansion), 7 Class III (nodular mesangial lesions) and 12 Class IV (>50% global glomerulosclerosis) (Table 1). There was a direct correlation between the class of DN and higher level of presenting creatinine.

Pathological findings

The pathologic findings are summarized in Table 2. With the exception of one case, all biopsies had >10 glomeruli. The median percentages of globally sclerosed glomeruli increased along with increased class of DN. Segmental to global CG was focal, ranging from a median 5–15% of glomeruli, with the exception of one case. The features of CG in this setting were partly distorted by the diabetic mesangial expansion. That is, total capillary wall collapse was impeded by the thickening and mesangial expansion, leaving capillary wall wrinkling and epithelial cell proliferation as the more defining features (Figure 1). In addition, the collapsing lesions had a more chronic appearance in this setting with a greater degree of underlying sclerosing change present. On EM, foot process effacement was extensive, but this varied somewhat based on the sampling of affected glomeruli. IF microscopy did not reveal deposits in any of the cases.

Vascular disease was prominent with moderate-to-severe arteriosclerosis and hyaline arteriolosclerosis seen in nearly all cases (Table 3). Hyalinosis was present in 25 of 26 patients showing extensive or circumferential distribution with >50% luminal stenosis in more than half of the arterioles seen in 85% of cases (Figure 2).

Markers of mature podocytes were absent in the glomeruli with CG, in the segments with collapse for 74% of cases for WT-1, 100% synaptotodin and podocin, and 82% β-dystroglycan (Figure 3). The background glomeruli, those without collapse, had preservation of staining for all four podocyte markers. There was subjectively less intense WT-1 and synaptotodin and comparable podocin and β-dystroglycan staining in the non-collapsing glomeruli compared with normal control glomeruli. VEGF over-expression was seen in 43% of cases in glomeruli without collapse, but was lost in areas of glomerular collapse (Figure 3).

Cytokeratin AE1/AE3 (CK), a marker for parietal epithelial cells, was variably present in the hyperplastic epithelial cells of the collapsed glomeruli in 70% of cases. In glomeruli which expressed CK, there was a mixture of CK+ and CK− epithelial cells with ~68% of cells showing positivity. The glomeruli without collapse were negative for CK staining in visceral and positive for CK in parietal epithelial cells.

FOLLOW-UP

Follow-up was available for 17 patients (Table 4). Thirteen developed renal failure (ESRD) on average 6.9 months from the time of biopsy. The remaining four patients, with 5 months to 2 years follow-up, had stable yet elevated creatinine (1.2–3.6 mg/dL) and proteinuria (0.3–8.8 g/24 h) at last follow-up. One patient was treated with prednisone, while the rest were managed strictly for diabetic and hypertensive symptoms.

CONTROL GROUP

A control group of diabetic patients with renal biopsies showing DN without CG during the same time period was retrospectively evaluated (Tables 5 and Supplementary Table S1a). The outcomes for the patients with CG were statistically worse as a group, with 78% of CG cases going to ESRD compared with 40% of controls, despite having significantly longer follow-up time in the control population.

DISCUSSION

Herein, we summarize a cohort of 26 patients with long-standing diabetes and biopsy-proven DN with advanced vascular disease and arteriolar hyalinosis, who additionally show glomerular features of focal or diffuse CG. The biopsies were...
performed mainly for new onset or recent increase in proteinuria and all but two were in the nephrotic range. These patients had worse outcomes compared with matched controls with 13 of 17 developing ESRD on average 7 months from the time of biopsy. We postulate that ischemic podocyte injury secondary to severe obliterative microvascular disease, such as arteriolosclerosis and hyalinosis, may have played a role in the development of collapsing glomerular lesions.

Diabetic disease affects all compartments of the kidney. The glomeruli display increased accumulation of extracellular matrix characterized by mesangial expansion and glomerular capillary basement membrane thickening. CG in DN, depending on the class of DN, has both wrinkling and retraction of the capillary walls (a classic feature of CG) with increased and sometimes nodular mesangial matrix accumulation. This leads to a unique glomerular morphology, in that the mesangial matrix expansion prevents complete ‘collapse’. There are also more sclerosing glomerular features associated with the CG in this setting, possibly due to the longer duration of the disease. However ultimately, the defining characteristic remains as the

### Table 1. Presenting creatinine and proteinuria levels at the time of biopsy in patients with CG in the setting of DN, by class of DN

<table>
<thead>
<tr>
<th>Class</th>
<th>Creatinine (mg/dL)</th>
<th>Proteinuria (g/24 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class IIa (3 patients)</td>
<td>1.1–6.5 (median–1.4)</td>
<td>7–14 (median–13.1)</td>
</tr>
<tr>
<td>Class IIb (4 patients)</td>
<td>1.3–10 (median–2.2)</td>
<td>3.5–10.5 (median–4.5)</td>
</tr>
<tr>
<td>Class III (7 patients)</td>
<td>1.2–3.6 (median–2.9)</td>
<td>2.9–18 (median–9.5)</td>
</tr>
<tr>
<td>Class IV (12 patients)</td>
<td>3–10.4 (median–3.8)</td>
<td>2–29 (median–9.2)</td>
</tr>
</tbody>
</table>

### Table 2. Pathologic findings on biopsy in patients with CG in the setting of DN, by class of DN

<table>
<thead>
<tr>
<th>Class</th>
<th>Total glomeruli</th>
<th>Global sclerosis (%)</th>
<th>Segmental sclerosis (%)</th>
<th>Glomeruli with collapsing features (%)</th>
<th>Tubular atrophy/interstitial fibrosis (%)</th>
<th>Foot process effacement (%)</th>
<th>GBM thickness (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class IIa (3 cases)</td>
<td>24–51 (26)*</td>
<td>0–15 (8)</td>
<td>0–15 (2)</td>
<td>4–8 (5)</td>
<td>0–50 (15)</td>
<td>20–100 (80)</td>
<td>600–800 (750)</td>
</tr>
<tr>
<td>Class IIb (4 cases)</td>
<td>14–59 (40)</td>
<td>14–49 (19)</td>
<td>0</td>
<td>5–19 (14)</td>
<td>30–100 (75)</td>
<td>100</td>
<td>800–1250 (1000)</td>
</tr>
<tr>
<td>Class III (7 cases)</td>
<td>17–42 (26)</td>
<td>15–35 (22)</td>
<td>0–8 (4)</td>
<td>2–22 (8)</td>
<td>20–90 (70)</td>
<td>20–90 (70)</td>
<td>660–1150 (750)</td>
</tr>
<tr>
<td>Class IV (12 cases)</td>
<td>5–67 (36)</td>
<td>50–80 (70)</td>
<td>0–23 (3)</td>
<td>3–100 (15)</td>
<td>35–100 (75)</td>
<td>15–100 (80)</td>
<td>600–1250 (800)</td>
</tr>
</tbody>
</table>

*Mean values are in parentheses.

### Table 3. Vascular pathology seen on biopsy in patients with CG in the setting of diabetes, by class of DN

<table>
<thead>
<tr>
<th>Class</th>
<th>Arteries: mild</th>
<th>Moderate</th>
<th>Severe</th>
<th>Arterioles: mild</th>
<th>Moderate</th>
<th>Severe</th>
<th>Intimal hyalinosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class IIa (3 patients)</td>
<td>2</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>2/3</td>
</tr>
<tr>
<td>Class IIb (4 patients)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>4/4</td>
</tr>
<tr>
<td>Class III (7 patients)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>7/7</td>
</tr>
<tr>
<td>Class IV (12 patients)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>12/12</td>
</tr>
</tbody>
</table>

Vascular sclerosis in the arteries and arterioles was scored as mild (<25% luminal narrowing), moderate (25–50%) or severe (>50%). Hyalinosis was present or not.

#### FIGURE 1: DN showing Class IIa and III glomerular lesions with CG (A. methenamine silver 40×, B. Periodic acid Schiff, 40×).
epithelial cell proliferation, or pseudocrescent formation within the urinary space with marked changes in the phenotype. The CG glomeruli in the setting of diabetes appear to be similar to other described CG etiologies. Markers of mature podocytes were variably absent in glomeruli in segments of collapse: 100% of cases for synaptopodin and podocin, 74% for WT1 and 82% for β-dystroglycan. These epithelial markers are preserved in the glomeruli not affected by CG. Previous reports in renal transplants have suggested a reactive, localized process causing dedifferentiation and injury only, or more

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Table 4. Follow-up data of patients with CG in the setting of diabetes, by class of DN

<table>
<thead>
<tr>
<th>Class</th>
<th>Follow-up period (months)</th>
<th>Renal failure</th>
<th>Median time period to end stage renal failure</th>
<th>Stable creatinine and proteinuria</th>
<th>Last follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ia</td>
<td>6–24</td>
<td>1</td>
<td>Time of biopsy</td>
<td>2</td>
<td>6 and 24 months</td>
</tr>
<tr>
<td>Ib</td>
<td>2–12</td>
<td>2</td>
<td>2 months</td>
<td>1</td>
<td>12 months</td>
</tr>
<tr>
<td>III</td>
<td>5–15</td>
<td>3</td>
<td>15 months</td>
<td>1</td>
<td>5 months</td>
</tr>
<tr>
<td>IV</td>
<td>1–36</td>
<td>7</td>
<td>7 months</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Follow-up was available on 17 of the 26 patients, as indicated. Thirteen of 17 had end-stage renal failure from the time of biopsy to 15 months. The remaining four had persistent proteinuria at last follow-up, 5–24 months following biopsy.
reported forms of FSGS [20, 21]. CG glomeruli, differing immunohistochemically from other CK positivity has been documented in the epithelial cells of the visceral and parietal epithelial cells in these lesions. Mixed and -negative cells possibly indicating participation of both protein resorption droplets, display a mixture of CK-positive trophic epithelial cells, many of which contain prominent ent with previous reports of CG, the hyperplastic and hyper-signi

The CG cases show signi

addition, Canaud development of these glomerular features in the allograft. In vascular toxicity or other vascular insults leading to ischemia in the observation in these cases was moderate-to-severe hyalinosis regions of severe obliterative vascular changes. A common glomerular features in a zonal distribution corresponding to the 7 days to 6 years after transplantation which showed collapsing glomerular features in a zonal distribution corresponding to the regions of severe obliterative vascular changes. A common observation in these cases was moderate-to-severe hyalinosis from cyclosporine-induced arteriolopathy with extensive hyalinosis, thrombotic microangiopathy, acute rejection or recurrent DN. The authors postulate a role for cyclosporine-induced vascular toxicity or other vascular insults leading to ischemia in the development of these glomerular features in the allograft. In addition, Canaud et al. [19] reported three biopsies taken from renal allografts immediately adjacent to the areas of infarction, which demonstrated typical features of CG including characteristic loss of markers of epithelial differentiation.

If podocyte ischemia plays a role in inducing the podocytopathy and subsequently leading to collapsing glomerular lesions, this could also potentially be a causative factor in native kidney disease with significant vascular disease. In fact, significant microvascular disease in the form of cholesterol atheroembolic disease in the native kidney has been associated with the cellular variant of FSGS, another form of podocyte injury morphologically similar to CG [22]. A recent study of FSGS lesions in IgA nephropathy reported 11 cases of CG in the setting of IgA [23]. These cases also displayed significant vascular sclerosis with evidence of thrombotic microangiopathy in 90%, implicating a role for ischemic glomerular/podocyte injury.

Renal lesions in diabetes, especially type 2 diabetes, may be heterogeneous. Gambara et al. [24] described different patterns of renal injury in diabetic patients which led to renal dysfunction, a subset of which were due to significant vascular sclerosis. Fioretto et al. [25] also describes a subset of diabetic patients with purely vascular sclerosis and subsequent tubulointerstitial and glomerular scarring without typical diabetic glomerular lesions. In diabetes and DN, renal dysfunction may result primarily from progressive vascular sclerosis which may be a cause or consequence of concomitant hypertension and metabolic factors of obesity in this patient population. Vascular compromise may lead to glomerular and podocyte ischemia in the diabetic kidney in a similar mechanism as in other secondary forms of CG seen in native and transplant kidneys (schematically depicted, Figure 4). In the present cohort, arteriolar hyalinosis was prominent in 25 of 26 biopsies and moderate-to-severe small arterial sclerosis was prominent in 24 of 26 cases with luminal narrowing.

Podocyte marker protein expression varied considerably between the cases of diabetes with and without collapsing glomerular lesions. Typical DN cases had preserved expression of WT-1 in 78% compared with 30% in DN-CG. Podocin and synaptopodin were present in 44 and 71% of DN and

<table>
<thead>
<tr>
<th>N</th>
<th>CG cases</th>
<th>Controls</th>
<th>P-value</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>26</td>
<td>20</td>
<td></td>
</tr>
</tbody>
</table>

Clinical
DN classification
II
III
IV
Age (mean)
Ethnicity
Gender
Hypertension
Diabetic type
Creatinine (mg/dL)
Proteinuria (g/24 h)
Path
Global glomerulosclerosis
Interstitial fibrosis
Vascular Sclerosis (0–3)
GBM thickness (nm)
FP effacement
Follow-up
ESRD?
mean time to ESRD (months)
mean f/u, non-ESRD (months)

De novo CG has been recognized in the transplant setting since the late 1990s. Meehan et al. [13] and subsequently Stokes [14] reported five and six cases, respectively, of HIV-negative patients of varying ethnic backgrounds. Proteinuria was seen in all cases except one, ranging from 1.8 to 11.8 g/24 h. Nadasdy et al. [15] reported three allograft nephrectomies removed from 7 days to 6 years after transplantation which showed collapsing glomerular features in a zonal distribution corresponding to the regions of severe obliterative vascular changes. A common observation in these cases was moderate-to-severe hyalinosis from cyclosporine-induced arteriolopathy with extensive hyalinosis, thrombotic microangiopathy, acute rejection or recurrent DN. The authors postulate a role for cyclosporine-induced vascular toxicity or other vascular insults leading to ischemia in the development of these glomerular features in the allograft. In addition, Canaud et al. [19] reported three biopsies taken from renal allografts immediately adjacent to the areas of infarction, which demonstrated typical features of CG including characteristic loss of markers of epithelial differentiation.

If podocyte ischemia plays a role in inducing the podocytopathy and subsequently leading to collapsing glomerular lesions, this could also potentially be a causative factor in native kidney disease with significant vascular disease. In fact,
were entirely lost in the collapsing glomeruli of DN-CG. β-Dystroglycan was preserved in all cases of DN, but was only seen in 18% of DN-CG. Such varied expression and loss of podocyte markers are indicative of podocyte injury in DN and probably aggravated by ischemia.

VEGF is produced by podocytes which play a key role in endothelial function within the glomerulus. In diabetes, VEGF mRNA is increased in the podocytes. High glucose levels stimulate this expression under experimental conditions [26]. The over-expression of VEGF may also play a role in the development and progression of proteinuria in DN, since anti-VEGF therapy is known to reduce proteinuria in rodent models [26]. Over-expression of certain VEGF isoforms also leads to CG in the mouse model [27]. Increased immunohistochemical expression of VEGF was seen in 44% of the glomeruli in our cases with collapsing glomerular features, which was only present in 11% (1 of 9) of matched DN controls.

Significant microvascular compromise, however, by itself may not be sufficient to cause the collapsing lesion. In transplant kidney biopsies, severe ischemia may be seen with advanced vascular calcineurin inhibitor toxicity or chronic vascular rejection, yet CG is not always identified. The spectrum of ischemic glomerular disease and podocyte injury may depend on the degree and duration of micro- and macrovascular occlusion. Global ischemic glomerular collapse without CG features characterized by isolated wrinkling and retraction of the capillary walls and no epithelial cell proliferation and sclerosis has been associated with arteriolar occlusion and can be seen in cases with advanced vascular disease in DN [25]. Different thresholds of vascular occlusion may determine total glomerular obsolescence versus CG features. Of course, other as yet undetermined or modifying factors may make this threshold different in each individual patient.

Diabetes frequently displays advanced vascular hyalnosis, but not manifest apparent CG. We believe that CG in the setting of advanced DN is more common than currently observed, and may be under-recognized. An earlier report describes cellular epithelial proliferation in the urinary space in DN as glomerular ‘crescents’, presumably related to injury of the basement membranes which correlated with severity of vascular disease and renal insufficiency [28]. ‘Prominent parietal epithelium’ has been reported by Gaffney in a variety of conditions including all cases examined with DN, which he believed to develop in the setting of ischemia [29]. However, CG is focal, involving 5–15% of glomeruli in our cases and could be missed on biopsy. Since the presentation of proteinuria is a shared manifestation of the underlying diabetic disease process, it may be clinically overlooked without a biopsy by the treating nephrologist.

This study has a few limitations. In a retrospective analysis, there was no uniformity in treatment, and some patients had other conditions which may contribute to renal disease, such as hypertension in 88% (on anti-hypertensive medications) and hepatitis C virus infection. Selection of control cases was also a limitation. Diabetic patients were not frequently biopsied in our institution, unless they had atypical or suspected secondary disease processes. This made case controls with adequate follow-up difficult to identify. The controls, used to evaluate outcomes, were matched according to class of DN on biopsy; however, they did differ significantly in other parameters such as foot process effacement and level of proteinuria at presentation. This is a potentially expected and explainable finding since the CG should have higher proteinuria and more foot process effacement, but there was overlap in the range of proteinuria between the control and CG groups. The possibility of focal or localized collapsing glomeruli in the control cases, potentially missed by the biopsy needle, could be considered as a cause of increased proteinuria.

In summary, severe podocyte injury and CG contribute to the increased level or new onset of proteinuria in DN. Significant hyalnic vascular disease, commonly observed in DN, could contribute to ischemic insult to podocytes, which does not appear to be related to age, gender, ethnicity or insulin dependence. Recognition of active or chronic features of CG in DN could potentially be of prognostic significance and offers a pathogenic explanation for some intractable proteinuric states.

**SUPPLEMENTARY DATA**

Supplementary data are available online at http://ndt.oxfordjournals.org.

**ACKNOWLEDGEMENTS**

The authors would thank doctors Satish Arora, Pat Audia, Nazifa Banu, Premila Bhat, Larissa Chaplia, Odler Jean-Louie,
Joshua Kaplan, Moyna Kapoor, Jeffrey Kozlowski, Helena Krol, Manuel Moquete, Nader Shabibi, Kevin Sperling and Albert Tartini for providing the clinical history and follow-up for the patients in the study. This project has been presented in abstract form at the 2012 American Society of Nephrology annual meeting, San Diego.

CONFLICT OF INTEREST STATEMENT

None declared.

REFERENCES


Received for publication: 11.6.2013; Accepted in revised form: 20.8.2013