Segmental Glomerulosclerosis in Pima Indians with Type 2 Diabetes

Thesis submitted to the

University of Arizona College of Medicine – Phoenix

in partial fulfillment of the requirements for the degree of

Doctor of Medicine

Ruthanna Seidel

Class of 2012

Mentor: E. Jennifer Weil, MD

DEDICATION

Dedicated to the Pima Indians of the Gila River Indian Reservation, who inspired me to become a physician

ACKNOWLEDGEMENTS

Many thanks to Jennifer Weil, my mentor, who spent countless hours teaching me renal pathology and statistical analysis. Thanks also to Burt Feuerstein, who reviewed my proposal, and to Robert Nelson, who reviewed my thesis. Most of all, thanks to the Pima Indians who volunteered to participate in this study. It is because of them and other research subjects that we will eventually be able to prevent this terrible disease.

ABSTRACT

Diabetes mellitus is widespread in the United States, and diabetic kidney disease is one of the most common complications. There is increasing evidence that podocyte injury is the initial pathologic change in diabetic nephropathy, and podocytopathy often manifests on renal biopsy as segmental sclerosis in other kidney diseases. The purpose of this study was to determine if segmental sclerosis is widespread in diabetic kidney disease. This study examined 1142 glomeruli from 61 Pima Indians who had diabetes for at least ten years. 24 patients were normoalbuminuric, 20 were microalbuminuric, and 17 were macroalbuminuric. The presence of segmental sclerosis was noted, as was global sclerosis. Segmental sclerosis was present in less than 2% of glomeruli. All glomeruli showed evidence of diabetic nephropathy including mesangial hypercellularity and invasion of mesangium into capillary loops. These data suggest that segmental sclerosis is not present in significant amounts in diabetic kidney disease. Rather, pathologic changes in the glomeruli of diabetic patients occur in a more diffuse pattern.

LIST OF FIGURES AND TABLES

TABLE OF CONTENTS

Introduction and Background

Diabetes mellitus is increasingly common in the United States. In 2011, the National Institutes of Health estimated that 8.3 percent of the U.S. population had diabetes [1]. One of the most common and serious complications of diabetes is diabetic nephropathy. Diabetic kidney disease is usually marked initially by development of microalbuminuria, which is defined as urinary albumin excretion of 30-300 mg/day. As the disease progresses, macroalbumuria develops, which is defined as urinary albumin excretion over 300 mg/day. Over time, macroalbuminuria is followed by a slow decline in glomerular filtration rate and, eventually, end stage renal disease. In the general type 2 diabetic population, the incidence of microalbuminuria is 24.9% ten years after onset of diabetes, and the incidence of macroalbuminuria is 5.3% [2]. However, some populations are at very high risk of developing overt diabetic nephropathy. Pima Indians are one such high-risk population, with a rate of end-stage kidney disease 23 times that of the general population [3]. Up to half of these patients develop overt nephropathy within 20 years of the onset of diabetes [3]. Pima Indians also appear to progress from macroalbuminura to end-stage kidney disease more rapidly than the general diabetic

population, despite a younger age, lower blood pressure, and lower lipid levels at onset of nephropathy [4]. The extensive characterization of diabetes and its complications over the past 30 years in this high-risk population makes it ideal for studies examining early morphologic changes in diabetic kidney disease.

The morphologic changes that take place in the kidney as a result of diabetic nephropathy include thickening of the capillary basement membrane, diffuse increase in mesangial matrix, and glomerulosclerosis. The glomerulosclerosis seen in diabetic nephropathy typically occurs in a nodular pattern and within glomeruli [5, 6].

Focal segmental glomerulosclerosis (FSGS) is one of the causes of nondiabetic kidney disease and is responsible for up to 35 percent of cases of nephrotic syndrome in adults. In contrast to the diffuse sclerosis that takes place in diabetic nephropathy, FSGS first presents only in parts of the kidney (hence the name "focal") and only in parts of each glomerulus (hence the name "segmental"). At least 50 percent of individuals with FSGS develop widespread glomerulosclerosis within ten years [6].

Significance

Diabetic nephropathy is a gradually progressing disease, and early morphologic changes occur before albuminuria develops. However, early changes have not been extensively studied in the past due to a lack of kidney biopsy samples in the diabetic population that does not yet have overt diabetic nephropathy. Although a few studies have been conducted in humans as well as non-human primates, the sample sizes have been small (under 20 subjects) and the findings were inconclusive [7, 8]. Since the time period between the onset of diabetes and the onset of overt diabetic nephropathy is an ideal time period to prevent kidney disease, an understanding of the underlying morphologic changes is vitally important and will provide a foundation for further research on the prevention of diabetic kidney disease.

Subjects with type 2 diabetes and chronic kidney disease consistent with diabetic nephropathy are typically diagnosed without biopsy confirmation of diabetic changes in the kidney. When biopsies are performed in subjects with type 2 diabetes and kidney disease for research purposes, the incidence of renal diseases other than diabetic nephropathy is reportedly less than 5% [9]. However, a recent study examined kidney biopsies of 567 subjects with chronic kidney disease

and either type 1 or type 2 diabetes. Interestingly, only 68% of these subjects had diabetic nephropathy. Immune complex glomerulo-nephritis was present in 12% of subjects, and focal segmental glomerulosclerosis was present in 17% [10]. These new data suggest that other renal diseases may be significantly present in patients with diabetes and kidney disease.

Diabetic nephropathy is increasingly recognized as a disease of the glomerular podocyte, with loss of glomerular podocytes on kidney biopsies and an increase in urinary podocyte excretion [11]. Podocyte injury is also recognized to be the primary problem in FSGS [12]. Since both diseases affect the same cell, it is possible that early stages of these diseases may overlap. FSGS and diabetic kidney disease are different diseases, and this study did not attempt to diagnose FSGS in diabetic patients. Rather, the study aimed to identify similar structural lesions that might suggest a common pattern of injury in FSGS and diabetic kidney disease. If segmental glomerulosclerosis occurs in diabetic kidneys, this would provide more evidence that podocytopathy is the primary problem in diabetic kidney disease.

Hypothesis

The hypothesis tested by this study is that segmental glomerulosclerosis is present in biopsies of Native Americans known to have type 2 diabetes and various stages of kidney disease.

Subject Selection

This study was conducted as a part of a larger trial conducted by the National Institute of Diabetes and Digestive and Kidney Disorders (NIDDK) to examine whether combined treatment with an angiotensin-converting enzyme inhibitor (ACEi) and an angiotensin receptor blocker (ARB) is effective in slowing the development of chronic kidney disease in adults with diabetes mellitus type 2 (ClinicalTrials.gov number, NCT00340678). This study was approved by the review board of the NIDDK. As part of the larger trial, protocol kidney biopsies were obtained in order to assess the morphologic changes that occur in the renal tissue of diabetic individuals. The study contained 170 subjects, of which 122 consented to a kidney biopsy. Of these samples, 61 were available for analysis at the time of the study. The remainder is still being processed and will be evaluated at a later date.

Eligibility Criteria

Volunteers who met the following eligibility criteria were invited to participate in the trial:

- Aged 18-65 years.
- Diagnosis of type 2 diabetes for ≥5 years.

- Serum creatinine concentration <1.4 mg/dl.
- Serum potassium concentration ≤5.5 mEq/L.
- At least 2 of 3 weekly screening urinary albumin-to-creatinine ratios <300 mg/g. Half of those selected had normal urinary albumin excretion, and the other half had microalbuminuria. Microalbuminuria was defined as albumin-to-creatinine ratio >30 mg/g but <300 mg/g.
- Willingness, after receiving a thorough explanation of the study, to participate.

Exclusion Criteria

Subjects were excluded for the following reasons:

- Documentation of liver disorders, cardiovascular disease, cerebrovascular disease, peripheral vascular disease, pulmonary diseases, renal-urinary disorders (calculi, urinary tract obstruction, glomerulonephritis, chronic infection), gastrointestinal disorders (nausea, vomiting, diarrhea or anorexia sufficient to cause weight loss or wasting), systemic lupus erythematosus, cancer, amyloidosis, chronic infection, or hematocrit levels ≤30 percent in women or ≤35 percent in men.

- Renovascular or malignant hypertension; uncontrolled hypertension (systolic blood pressure ≥160 or diastolic ≥95 mm Hg) despite treatment with three antihypertensive drugs.
- Hematuria of unknown etiology.
- Currently receiving a drug regimen that included steroids, immunosuppressants, or investigational new drugs.
- Pregnancy.
- Evidence of inability to empty the bladder.
- Hypersensitivity to ACEi, ARBs, or iodine.
- Bleeding disorders, since kidney biopsies could not be performed safely in these individuals.
- Massive obesity with body mass index ≥45 kg/m². Kidney biopsies are more difficult and present greater hazards to persons with massive obesity.
- Non-diabetic renal disease.
- Conditions likely to interfere with informed consent or compliance with the protocol.

Rationale for Subject Selection

Biopsy samples were obtained from Pima Indians who were recruited for this study without preference regarding gender. Selection of this group for this study was based on 1) extensive clinical characterization of the population, 2) detailed knowledge of the onset and duration of type 2 diabetes in the Community, 3) high incidence and prevalence of diabetes and diabetic nephropathy, 4) previous detailed studies of the epidemiology and pathogenesis of diabetic nephropathy in the Community, 5) the young age at onset of type 2 diabetes and diabetic nephropathy, and 6) the very low prevalence of non-diabetic renal disease in those with diabetes. These factors made this population ideal for studying the pathogenesis of renal disease attributed to type 2 diabetes.

Given that diabetic nephropathy in Pima Indians is indistinguishable, both clinically and pathologically, from that in other populations, there is a high likelihood that the results of this study are not population-specific, but may be applicable to renal disease in other populations with type 2 diabetes.

Methods

Kidney biopsies were obtained in all subjects who had normal clotting parameters (platelet count, bleeding time, protime, and prothrombin time) and who were normotensive or had controlled hypertension (<160 mm Hg systolic and <95 mm Hg diastolic). The kidney biopsies were obtained five years after the commencement of the parent study. As previously described, all subjects enrolled in the parent study had a diagnosis of diabetes mellitus for at least 5 years at the time of enrollment. Kidney biopsy samples, then, were from individuals who are at least ten years past their initial diabetes diagnosis. Kidney biopsies were performed under ultrasound guidance with a 15-gauge Boston Scientific Easy Core® biopsy needle. One or two cores of tissue were fixed in buffered 4% glutaraldehyde and shipped to the Beckman Center for Electron Microscopy at Stanford University where the biopsy cores were processed. Tissue was dehydrated through a series of graded ethanols, and the fixed tissues embedded in Epon 812® prior to preparation for light and electron microscopy. Slides for light microscopy were prepared using a toluidine blue stain.

The pattern of glomerulosclerosis was assessed using light microscopy. Specifically, the presence or absence of segmental sclerosis as well as global sclerosis was noted. Sclerotic segments were identified based on the collapse of basement membrane and increase in mesangial matrix. The sclerosis had to be significantly more severe in a portion of the glomerulus in order to be classified as segmental sclerosis. Each glomerulus was classified as either exhibiting segmental sclerosis or not. Global sclerosis, defined as no open capillaries within the glomerulus, was also noted. The percentage of segmentally sclerosed glomeruli per subject was calculated, as was the percentage of globally sclerosed glomeruli per subject.

Clinical information was also obtained as part of the study. The subjects had an annual exam which included blood tests for serum creatinine, fasting plasma glucose, hemoglobin A1C. Height and weight measurements were taken and body mass index was calculated. Physiologic measures of kidney function were also obtained. Urinary albumin was measured by nephelometric immunoassay [13]. Urinary and serum creatinine were measured by a modification of the Jaffe reaction [14]. Albumin excretion was estimated by computing the urinary albumin-to-creatinine ratio (ACR) in an untimed urine

specimen. Glomerular filtration rate (GFR), which measures the filtration rate of the glomerulus and decreases as glomerular damage increases, was measured by the urinary clearance of non-radioactive iothalamate. GFR was expressed in ml/min, that is, not normalized to body surface area [15]. Renal plasma flow, which measures how much plasma is flowing to the glomeruli, was measured using para-aminohippuric acid. Filtration fraction, which is a measure of what percentage of plasma is filtered through the glomeruli, was calculated using these two values. For the purpose of this study, the examination information closest to the biopsy date was used for analysis.

At initial enrollment, all subjects either had normoalbuminuria or microalbuminuria. At the time of kidney biopsy five years later, some of the patients had progressed to macroalbuminuria. The patients were therefore split into three groups for further statistical analysis: normoalbuminuria, which was defined as ACR less than 30 mg/g; microalbuminuria, which was defined as ACR between 30 mg/g and 300 mg/g; and macroalbuminuria, which was defined as ACR 300 mg/g or greater.

The exact Wilcoxon two-sample test was used uniformly for comparisons of continuous variables between the three study groups,

as several variables were heavily skewed; the exact Fisher's test was used for dichotomous variables. Spearman correlations were calculated between structural and functional variables.

Results

A total of 61 subjects were included, with a total of 1142 glomeruli and an average glomeruli-per-subject of 18.7 (range, 5-58). Every glomerulus present on biopsy was evaluated. All glomeruli showed evidence of increased mesangial expansion consistent with diabetic nephropathy, even in the normoalbuminuric group (see figure 1). The results of the exact Wilcoxon test are shown in table 1, and the results of the Spearman correlation are shown in table 2.

Segmental sclerosis was present in at least 1 glomerulus in 12 of the 61 subjects (see figure 2), but in less than 2% of the total glomeruli examined. As expressed as a percentage of the glomeruli per subject, segmental sclerosis did not correlate with any other morphometric or physiologic variable. Global sclerosis was significantly and negatively correlated with glomerular filtration rate ($p=0.0107$) and renal plasma flow ($p=0.0476$) and positively with albumin-to-creatinine ratio ($p=0.0171$), fractional interstitial area ($p=0.0157$), age ($p =0.0084$), and duration of diabetes ($p=0.0011$).

Interestingly, albumin-to-creatinine ratio correlated positively with duration of diabetes ($p=0.0031$) but had no correlation with age. In the exact Wilcoxon test, there was no significant difference in age

between the normo-, micro-, and macroalbuminuria groups, but there was a significant increase in duration of diabetes between the micro- and macro-albuminuria group ($p=0.0324$), as well as between the normo- and macroalbuminuria groups ($p<.0001$). Glomerular filtration rate, however, did correlate negatively both with age ($p=0.0017$) and duration of diabetes ($p=0.0077$).

Fasting plasma glucose was not significantly different across the three groups. Hemoglobin A1C, however, was significantly higher in the macro- compared to the normoalbuminuria group ($p=0.0075$). Glomerular filtration rate was significantly higher in the microalbuminuria group compared to the normoalbuminuria group ($p=0.0064$) and significantly lower in the macroalbuminuria group compared to the microalbuminuria group ($p<.0001$). These findings are consistent with previous data that shows that hyperfiltration occurs initially in diabetes before kidney disease progresses and glomerular filtration rate falls [9].

Figure 1. A light micrograph of a glomerulus from a normoalbuminuric subject with diabetes duration of 11 years. Note the mesangial hypercellularity and mesangial invasion into capillary loops.

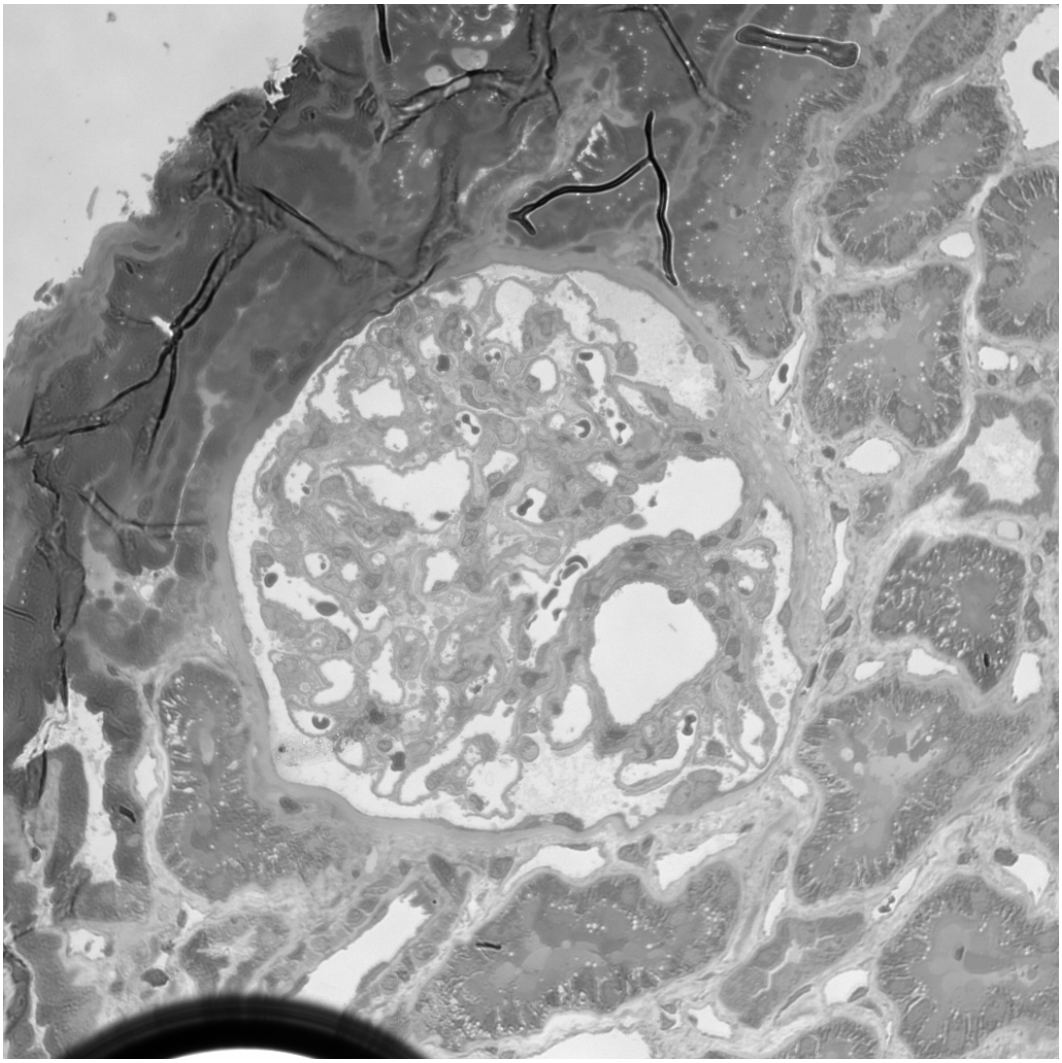

Figure 2. A light micrograph of a glomerulus showing evidence of segmental sclerosis. Note the increase in mesangial matrix in the lower half of the glomerulus compared to the upper half.

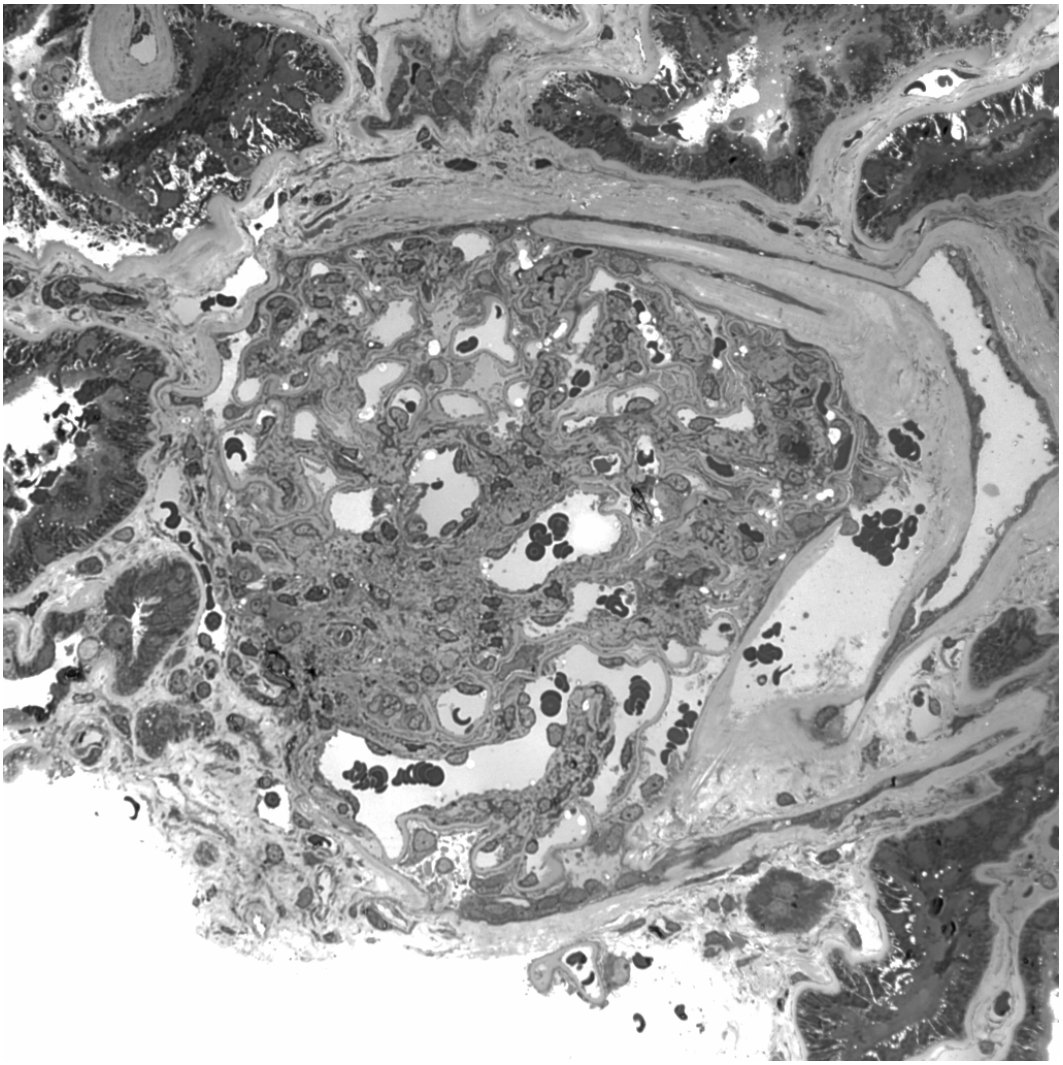

Table 1. Patient characteristics at biopsy from 61 Pima Indians with type 2 diabetes mellitus. *P* values are shown for [a]normo vs. micro-, [b]micro- vs. macro-, and [c]normo- vs. macroalbuminuric groups. *P* values less than 0.05 are shown in bold.

	Normo- (n=24)	Micro- (n=20)	Macro- (n=17)	*P* value		
Fraction female	18/24	14/20	9/17	[a]0.7456	[b]0.3277	[c]0.1877
Age (years)	48.4±11.6	47.0±8.7	47.1±7.6	[a]0.3588	[b]0.3646	[c]0.2957
Duration (years)	13.9±4.1	16.9±6.9	20.2±6.2	[a]0.0647	[b]**0.0324**	[c]**<.0001**
BMI (kg/m^2)	37.7±8.9	34.2±7.3	34.9±7.4	[a]0.0964	[b]0.3874	[c]0.1264
HbA1c (%)	8.1±2.0	9.2±2.2	9.6±2.0	[a]	[b]0.2321	[c]**0.0075**
FPG (mg/dL)	170.8±65.1	213.1±93.3	211.6±100.4	[a]0.0895	[b]0.4077	[c]
ACR (mg/g)	11.4 (4.8-25.8)	60.5 (34.0-194.3)	1031.7 (357.6-8869.6)	§	§	§
GFR (ml/min)	132.0±55.2	163.9±48.2	104.7±43.8	[a]**0.0064**	[b]**<.0001**	[c]**0.0272**
RPF (ml/min)	536.2±198.0	695.8±210.2	556.3±161.8	[a]**0.0028**	[b]**0.0165**	[c]0.3522
FF	0.25±0.03	0.24±0.04	0.19±0.12	[a]0.2758	[b]**<.0001**	[c]**<.0001**
%GS	9.1±11.1	12.0±11.6	29.6±33.1	[a]0.1405	[b]0.0815	[c]**0.0153**
%SS	0.92±2.2	1.8±4.1	2.0±5.0	[a]0.2136	[b]0.4373	[c]0.3135
MPGV (μ^3)	5473696.2± 1449615.7	6235675± 2008038.8	6907679.1± 3748522.9	[a]0.0776	[b]0.2033	[c]**0.0369**
MGGV (μ^3)	1422671.4± 1905551.01	2510112.4± 3665191.4	2693301.1± 2660071.0	[a]0.0526	[b]0.1998	[c]**0.0338**
FIA (%)	0.26±0.06	0.27±0.06	0.40±0.12	[a]0.3069	[b]**<.0001**	[c]**<.0001**

Data are means ± SD, median (range), or percent. BMI, body mass index; FPG, fasting plasma glucose; ACR, albumin-to-creatinine ratio; GFR, glomerular filtration rate; RPF, renal plasma flow; FF, filtration fraction; %GS, % globally sclerosis; %SS, % segmental sclerosis; MPGV, mean patent glomerular volume; MGGV, mean globally sclerosed glomerular volume; FIA, fractional interstitial area. §ACR values by group are different by definition.

Table 2. Spearman correlations (p-values) between physiologic and morphometric variables in 61 Pima Indians with type 2 diabetes mellitus. P values less than 0.05 are shown in bold.

	GFR	ACR	% GS	% SS	FIA	MPGV	MGGV	BMI	Age (years)	Dur	RPF	FF	HbA1C (%)	FPG
GFR	1.0	-0.12285 (0.3456)	**-0.32461 (0.0107)**	0.08088 (0.5355)	**-0.30465 (0.0190)**	**0.26171 (0.0416)**	**0.29722 (0.0200)**	0.05992 (0.6465)	**-0.39323 (0.0017)**	**-0.33818 (0.0077)**	**0.83273 (<.0001)**	**0.44490 (0.0003)**	**0.26056 (0.0425)**	**0.34880 (0.0059)**
ACR		1.0	**0.30443 (0.0171)**	-0.04568 (0.7267)	**0.43805 (0.0005)**	**0.25436 (0.0479)**	**0.29722 (0.0200)**	-0.15442 (0.2347)	-0.15362 (0.2372)	**0.37234 (0.0031)**	0.14532 (0.2638)	**-0.44553 (0.0003)**	**0.33896 (0.0075)**	0.22605 (0.0798)
% GS			1.0	-0.09954 (0.4453)	**0.31335 (0.0157)**	-0.08650 (0.5074)	**0.63647 (<.0001)**	0.10359 (0.4269)	**0.33438 (0.0084)**	**0.40736 (0.0011)**	**-0.25469 (0.0476)**	-0.20805 (0.1076)	-0.24518 (0.0568)	-0.25212 (0.0500)
% SS				1.0	-0.08170 (0.5384)	0.03337 (0.7985)	0.13905 (0.2852)	-0.05741 (0.6603)	0.01570 (0.9044)	-0.14545 (0.2634)	0.12214 (0.3484)	-0.09261 (0.4778)	0.10547 (0.4185)	-0.11716 (0.3685)
FIA					1.0	-0.03060 (0.8181)	**0.29552 (0.0231)**	-0.18180 (0.1682)	0.24977 (0.0564)	**0.41377 (0.0011)**	-0.07057 (0.5953)	**-0.49077 (<.0001)**	0.16381 (0.2151)	0.00102 (0.9939)
MPGV						1.0	-0.00252 (0.9846)	0.04469 (0.7324)	-0.20830 (0.1072)	0.00502 (0.9693)	**0.30465 (0.0170)**	-0.05685 (0.6634)	0.09535 (0.4648)	0.09335 (0.4743)
MGGV							1.0	-0.03046 (0.8158)	0.14834 (0.2539)	**0.29270 (0.0221)**	-0.12994 (0.3182)	-0.10238 (0.4324)	-0.03399 (0.7948)	-0.04698 (0.7192)
BMI								1.0	0.15394 (0.2362)	-0.05939 (0.6494)	0.02332 (0.8584)	-0.09519 (0.4656)	**-0.35666 (0.0048)**	**-0.25527 (0.0471)**
Age (years)									1.0	**0.38318 (0.0023)**	**-0.43099 (0.0005)**	-0.05738 (0.6605)	**-0.40268 (0.0013)**	**-0.45445 (0.0002)**
Dur										1.0	-0.19466 (0.1328)	**-0.31148 (0.0145)**	0.00918 (0.9440)	-0.04816 (0.7125)
RPF											1.0	-0.05309 (0.6845)	**0.25258 (0.0495)**	**0.29941 (0.0191)**
FF												1.0	0.09046 (0.4881)	0.15750 (0.2254)
HbA1C (%)													1.0	**0.80227 (<.0001)**
FPG														1.0

GFR, glomerular filtration rate (ml/min); ACR, albumin to creatinine ratio (mg/g); % GS, % global sclerosis; %SS, % segmental sclerosis; FIA, fractional interstitial area (%); MPGV, mean patent glomerular volume (□³); MGGV, mean globally sclerosed glomerular volume (μ³);BMI, body mass index (kg/m²); Dur, duration of diabetes (years); RPF, renal plasma flow (ml/min); FF, filtration fraction; FPG, fasting plasma glucose (mg/dL).

Discussion

The initial aim of this study was to evaluate whether segmental sclerosis is widely present in diabetic nephropathy. Less than 2% of glomeruli studied showed evidence of segmental sclerosis. Notably, all the glomeruli showed evidence of diabetic nephropathy, as manifested by mesangial expansion, even in the normoalbuminuric subjects.

The stain used for this study, toluidine blue, is not the ideal stain for identifying segmental sclerosis. The ideal stain is a periodic acid-Schiff (PAS) stain and methenamine silver stain, neither of which were available for this study. These stains enhance the sclerotic area and make identification easier. Due to this limitation, identification of segmental sclerosis was based more on architectural features, such as segmental increase in matrix.

Duration of diabetes was significantly longer in the macroalbuminuria versus the normoalbuminuria group, but the average age across the three groups was not significantly different. These data support previous findings that long duration of diabetes is a significant predictor of kidney disease [16].

Global sclerosis correlated negatively with glomerular filtration rate and renal plasma flow and positively with albumin-to-creatinine

ratio, fractional interstitial area, age, and duration of diabetes. These data suggest that global sclerosis is a marker of kidney disease progression. Previous studies have indicated that globally sclerosed glomeruli are eventually reabsorbed and disappear [9], so it is possible that global sclerosis was underestimated using this method.

Future Direction

The remaining biopsy samples will be evaluated once they become available, but it is unlikely that the results will be significantly different. Further studies should continue to examine podocytopathy in diabetic kidney disease, as this single negative study does not mean that podocyte injury does not occur in diabetic kidney disease. Loss of podocytes may still be very important in diabetic kidney disease, even if the areas of denuded basement membrane do not lead to segmental sclerosis as is the case in FSGS. The ideal stains for identification of segmental sclerosis, the PAS stain and the methenamine silver stain, may be useful in further research to better identify sclerotic segments.

Conclusions

Pathologic changes in glomeruli of diabetic patients occur before microalbuminuria develops. Changes visible on light microscopy include mesangial hypercellularity and invasion of mesangium into capillary loops. Segmental sclerosis is present only in a very small portion of diabetic glomeruli (<2%), and it is not possible to predict which patients will develop segmental sclerosis based on clinical characteristics.

References

1. U.S. Department of Health and Human Services, National Institutes of Health. National Diabetes Statistics 2011. NIH Publication No. 11-3892 Feb 2011.
2. Adler AI, Stevens RJ, Manley SE, Bilous RW. Development and progression of type 2 diabetes mellitus: The United Kingdom Prospective Diabetes Study (UKPDS 64). Kidney Int 2003; 63:225-32.
3. Nelson RG, Knowler WC, Pettitt DJ, Saad MF, Bennet PH. Diabetic kidney disease in Pima Indians. Diabetes Care 1993; 16:335-41.
4. Lemley KV. A basis for accelerated progression of diabetic nephropathy in Pima Indians. Kidney Int Suppl. 2003; 83:S38-42.
5. Adler S. Diabetic nephropathy: Linking histology, cell biology, and genetics. Kidney Int 2004; 66(5):2095-106.
6. Kumar V, Abbas AK, Fausto N. Robbins and Cotran pathologic basis of disease. 8th ed. Pennsylvania: Elsevier; 2009.
7. Moriya T, Tanaka K, Hosaka T, Hirasawa Y, Fujita Y. Renal structure as an indicator for development of albuminuria in

normo- and microalbuminuric type 2 diabetic patients. Diabetes Res Clin Pract. 2008 Dec; 82(3):298-304.

8. Thomson SE, McLennan SV, Kirwan PD, Heffernan SJ, Hennessy A, Yue DK, Twigg SM. Renal connective tissue growth factor correlates with glomerular basement membrane thickness and prospective albuminuria in a non-human primate model of diabetes: possible predictive marker for incipient diabetic nephropathy. J Diabetes Complications 2008 Jul-Aug; 22(4): 284-94.
9. Greenberg A, Cheung AK, Coffman TM, Falk RJ, Jennette JC. Primer on Kidney Diseases. 5th ed. Pennsylvania: Elsevier, 2009.
10. Haider DG, Peric S, Friedl A, Fuhrmann V, Wolzt M, Hörl WH, Soleiman A. Kidney biopsy in patients with diabetes mellitus. Clin Nephrol. 2011; 76(3):180-5.
11. Weil EJ, Lemley KV, Yee B, Lovato T, Richardson M, Myers BD, Nelson RG. Podocyte detachment in type 2 diabetic nephropathy. Am J Nephrol. 2011; 33 Suppl 1:21-4.
12. Rennke HG, Klein PS. Pathogenesis and significance of nonprimary focal and segmental glomerulosclerosis. Am J Kidney Dis. 1989; 13(6): 443.

13. Vasquez B, Flock EV, Savage PJ, Nagulesparan M, Bennion LJ, Baird HR, Bennett PH. Sustained reduction of proteinuria in type 2 (non-insulin-dependent) diabetes following diet-induced reduction of hyperglycemia. Diabetologia 1984; 26: 127-133.
14. Chasson AL, Grady HJ, Stanley MA. Determination of creatinine by means of automatic chemical analysis. Tech Bull Regist Med Technol 1960; 30: 207-212.
15. Delanaye P, Radermecker RP, Rorive M, Depas G, Krzesinski JM. Indexing glomerular filtration rate for body surface areas in obese patients is misleading: Concepts and example. Nephrol Dial Transplant 2005; 20: 2024-2028.
16. Pavkov ME, Bennett PH, Knowler WC, Krakoff J, Sievers ML, Nelson RG. Effect of youth-onset type 2 diabetes mellitus on incidence of end-stage renal disease and mortality in young and middle-aged Pima Indians. JAMA 2006;296(4):421.