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Renal structural-functional relationships in early diabetes mellitus

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Abstract. To define the earliest renal morphological changes in patients with type I diabetes, we studied renal function and morphometric analysis of renal biopsies in 59 patients with diabetes for 5–12 years and normal blood pressure, normal creatinine clearance (C_{Cr}), and negative dipstick urinary protein. Arteriolar hyalinization and intimal fibrous thickening were noted in 43%. Glomerular basement membrane thickness and fractional mesangial volume were increased in 51% and 56%, respectively. The pre-pubertal and post-pubertal years of diabetes were associated with similar degrees of renal structural changes, but during the pre-pubertal years normal urinary albumin excretion (UAE) was seen. Principal factor analysis of morphometric structural parameters yielded four clusters of variables: "glomerular size" correlated with patient age, C_{Cr}, and UAE; "peripheral capillary decrease" correlated with glycosylated hemoglobin, diastolic blood pressure, glomerular filtration rate, and UAE; "mesangial increase" correlated with UAE; and "interstitial scarring" correlated with diastolic blood pressure. This study provides unique documentation of renal structural abnormalities which precede clinically evident renal functional abnormalities and documents that these early structural abnormalities are present in the pre-pubertal years of diabetes as well as postpuberty, and are associated with each other in constellations that correspond to postulated mechanisms in diabetic nephropathy.

Key words: Glomerular basement membrane – Mesangium – Puberty – Urinary albumin excretion – Glomerular volume

Introduction

After 20 years of type I diabetes mellitus, approximately 30% of patients develop renal insufficiency [1–4], and diabetic nephropathy is the single most common cause of renal failure in adults [5]. The natural history of diabetic nephropathy is characterized by normal renal function for many years. Early and subtle functional abnormalities include increased glomerular filtration rate (GFR) [6], microalbuminuria [6,7], and increased renal size [6]. Clinical nephropathy, defined by albuminuria >250 mg/24 h, hypertension, and decline in GFR most often occurs after 15–17 years of diabetes [4]. Advanced renal structural lesions are characterized by mesangial expansion, decline of glomerular capillary filtration surface area, and marked hyalinosis of afferent and efferent arterioles [8–10]. Tubular atrophy and interstitial fibrosis also accompany clinical nephropathy. However, renal structural changes early in the course of diabetes have not been systematically studied.

Current concepts about the processes that lead to glomerular scarring in diabetic nephropathy include the postulates that glomerular enlargement results in increased glomerular capillary tension which enhances glomerular injury and increased synthesis of extracellular matrix due to altered cytokine levels and decreased matrix degradation resulting in mesangial expansion, thickened glomerular basement membrane (GBM), arteriolar hyalinosis, and interstitial scarring. Studies of glomerular histomorphometry have defined the presence of each of these structural changes but have failed to identify a parameter or constellation of parameters which consistently predicts development of progressive glomerular sclerosis. The current study was designed to document renal histomorphology as well as renal function in patients early in the course of diabetes and prior to the onset of clinical nephropathy.

Patients and methods

Fifty-nine patients with type I diabetes mellitus were studied. All consecutive type I diabetic patients presenting to the diabetes clinics in our three institutions who had diabetes for 5–12 years and normal blood pressure for age were invited to be screened with urinalysis and 24-h urine for creatinine clearance (C_{Cr}). They were invited to enter the study if the screened patients met the following entry criteria for the

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study: type I diabetes mellitus for 5–12 years, $C_{Cr} > 75$ ml/min per 1.73 m^2, negative urine protein on urinalysis, and systolic and diastolic blood pressure less than 95th percentile for age and sex. The protocol was approved by the institutional review boards of each of the participating institutions. In obtaining informed consent from the patient and family, the protocol procedures, especially the renal biopsy procedure, were explicitly described and possible complications delineated. Patients who may have increased risk at the time of renal biopsy (especially those with obesity, history of bleeding problems, or other concerns) were excluded from participation. After obtaining informed consent from the patient and parent, age, duration of diabetes, height, weight, Tanner stage, and medication history of each patient were recorded. Patients at Tanner stage I were considered pre-pubertal and patients of Tanner stage II or more were considered post-pubertal. In the post-pubertal patients, post-pubertal duration of diabetes was calculated as duration of diabetes from 11 years of age in girls and from 12 years of age in boys, as previously described [11, 12].

Auscultatory blood pressure was measured on at least four occasions throughout the screening and evaluation process and the values averaged; these were compared with the values for normal children determined by the Task Force on Blood Pressure in Children [13]. Three 24-h urines were collected while the patient was engaged in usual outpatient activities and within 1 month prior to the renal biopsy. C_{Cr} and urinary albumin excretion (UAE) were measured on each 24-h urine and values for the three samples were averaged. Blood was drawn for measurement of glycosylated hemoglobin. Each patient underwent a diethylene triaminopenta-acetic acid (^{99m}Tc-DTPA) clearance for measurement of GFR and ^{131}iodine (I) hippurate clearance for measurement of renal plasma flow (RPF), with collection of both blood and urine for four clearance periods.

Each patient underwent a percutaneous renal biopsy utilizing ultrasound guidance, midazolam sedation, and lidocaine anesthesia at the biopsy site. The biopsy needle was entered into the kidney no more than three times in order to obtain adequate tissue. The patient was monitored with frequent vital signs and hospitalized for less than 24 h following the biopsy procedure. Four of the patients were noted to have a small-to-moderate hematoma at the biopsy site in the kidney on ultrasound immediately post biopsy; none of the patients had clinical symptoms related to the biopsy-site hematoma and the hematoma resolved spontaneously without therapy in each patient. Mean hematocrit pre biopsy was 41.3% ± 1.7% and post biopsy 41.0% ± 2.4 (paired t = 0.69, NS). No other complication of the renal biopsy procedure was noted in any patient. The tissue obtained at renal biopsy was examined using both light and electron microscopy.

Clearance methods. The DTPA and hippurate clearances were completed under conditions of water diuresis initiated by an oral water load (1% of body weight, 1 l limit) and maintained with 150 ml of water taken orally every 30 min as previously described [14]. Baseline urine and blood samples were obtained after initiation of a stable diuresis; 200 uCi ^{99}Tc-DTPA was then given intravenously and 200 uCi ^{131}I hippurate was given subcutaneously in 1 ml (1:1,000) epinephrine in the opposite arm from the sampling site. After 60 min of equilibration, urine and blood were collected during four serial 30-min clearance periods. The coefficient of variation of the four clearance periods for DTPA clearance averaged 15.1% and was less than 30% in all patients.

Laboratory methods. Serum and urine creatinine were measured by the Beckman Creatinine Analyzer II (Beckman Instruments, Fullerton, Calif., USA). UAE was measured by radioimmunoassay with samples run in triplicate (Diagnostic Products Albumin Double Antibody kit, Los Angeles, Calif., USA). Normal albumin excretion was defined as less than 20 μg/min. Microalbuminuria was defined as UAE between 20 and 200 μg/min. The coefficent of variation between the three 24-h urine specimens measured for UAE averaged 27%. Total glycosylated hemoglobin was measured by the GlycoTest II GlyHb Assay Kit (Pierce, Rockford, Ill., USA); the normal range for glycosylated hemoglobin by this assay is 3.8%–6.0%. For the DTPA and hippurate clearances, the specific activity of ^{99}Tc and ^{131}I were determined on a gamma-scintillation spectrometer; DTPA and hippurate clearances were calculated as the mean of the four clearance periods.

Tissue methods. A portion of the renal biopsy tissue was processed by standard surgical pathology methods and embedded in glycomethacrolate for light microscopy. Light microscopy findings were qualitatively evaluated on periodic acid-Schiff-stained sections by one pathologist (W.P.R.) without knowledge of the patient's clinical status. Arteriolar injury was judged present if intimal fibrous thickening or hyalin deposition was seen in any arteriole in the biopsy specimen. The arteriolar lesions were semiquantitatively scored as mild (1+), moderate (2+), or severe (3+).

The remainder of the renal biopsy tissue was fixed in glutaraldehyde-paraformaldehyde fixative, post-fixed in osmium tetroxide, and embedded in Epon and Araldite mixture (EMS, Fort Washington, Pa., USA) for electron microscopy. Thick (1 μm) sections were cut from each block and stained with toluidine blue in order to randomly select for thin sectioning the center-most, non-sclerosed glomerulus that was at least one tubular diameter from the edge of the tissue [15]. Thin sections of 60- to 70-nm thickness were cut from each selected glomerulus and placed on Formvar-coated slot grids. Each thin section was stained with saturated uranyl acetate and lead citrate. Three glomeruli from each biopsy were studied by electron microscopy. Each glomerulus was examined on a Zeiss 902 transmission electron microscope. At an approximate final magnification of ×12,000, each glomerulus was entered randomly and approximately ten evenly spaced electron micrographs were taken systematically throughout [16]. A calibration grid with 28,800 lines/inch was photographed with each glomerulus to determine actual final magnification.

Morphometric methods. Glomerular volume (GV) was measured on the light microscopic sections using the stereological method of Weibel-Gomez [17–19]. To assure reliable measurement of GV, at least 15 glomerular profiles were present on the biopsy tissue for each patient [19] and all glomerular profiles present were measured. A mean of 22 ± 7 (± SD) glomerular profiles were measured on each biopsy. The remainder of the glomerular morphometric measurements were completed on the electron micrographs. GBM thickness was determined by the orthogonal intercept method [20, 21], with 515 ± 123 GBM measurements completed on each biopsy. Point counting was used to determine fractional mesangial volume (VvM) and fractional glomerular capillary luminal volume (VvL) [17, 20, 22]; a total of 2,526 ± 630 points were evaluated per biopsy. Intercept counting was used to determine the peripheral capillary filtering surface density (SvLE), the glomerular capillary luminal to mesangial interface (SvLM), and the mesangial to epithelial interface (SvME) [17, 20, 21, 23]; an average line length of 8,131 ± 2043 μm was examined per biopsy. GV was measured on all specimens by one researcher (P.H.L.) without knowledge of the clinical status of the patients. Glomerular basement width, fractional mesangial and capillary luminal volumes, and surface density measurements were completed on all patients by one researcher (E.N.E.) without knowledge of the clinical status of the patients.

After determining the GV and fractional structural parameters, per glomerulus total mesangial (TM) and total glomerular capillary luminal (TL) volumes, total peripheral capillary surface (SLE), total glomerular capillary luminal-mesangial surface (SLM), and total mesangial-epithelial surface (SME) were calculated as the product of the mean GV for each patient and the appropriate fractional parameter for each patient [9, 24].

Interstitium methods. Interstitial volume fraction of the renal cortex ($Vv_{int/cortex}$), the proportion of the renal cortex composed of intersitial tissue (i.e., not composed of glomeruli, tubules, arteries, arterioles, or veins at least the size of a tubule) was determined by point counting at least 20 cortical fields at an approximate final magnification of ×300 [25]. Each field was also scored qualitatively as normal or abnormal; abnormal was defined by the presence of globally sclerosed glomeruli, tubular atrophy, cellular infiltrates, or sectioning artifacts, and normal was defined as the absence of any these features. The percentage of fields that were abnormal (% AF) was then calculated. Only normal fields were used to determine a volume fraction for interstitium per normal areas of the cortex ($Vv_{int/N}$), only abnormal fields were used to determine a volume fraction for interstitium per abnormal areas of the

Table 1. Patient characteristics

	All patients $n = 59$		Pre-pubertal patients $n = 12$	Post-pubertal patients $n = 47$
	Mean ± SD	Range	Mean ± SD	Mean ± SD
Age (years)	14.7 ± 3.1	8 to 22	10.4 ± 1.4	15.3 ± 2.7
Duration of diabetes (years)	7.7 ± 2.0	5 to 12	6.8 ± 1.3	7.9 ± 2.1
Systolic blood pressure (mmHg)	114 ± 11	92 to 139	110 ± 10	115 ± 11
Diastolic blood pressure (mmHg)	66 ± 8	48 to 84	65 ± 4	66 ± 6
Glycosylated hemoglobin (%)	11.1 ± 4.1	7.0 to 20.2	10.3 ± 2.2	12.1 ± 3.4
C_{Cr} (ml/min per 1.73 m^2)	123 ± 37	78 to 169	130 ± 38	118 ± 23
GFR (ml/min per 1.73 m^2)	106 ± 47	60 to 238	103 ± 19	108 ± 51
RPF (ml/min per 1.73 m^2)	365 ± 135	198 to 679	397 ± 152	361 ± 130

GFR, Glomerular filtration rate; RPF, renal plasma flow; C_{Cr}, creatinine clearance

cortex ($Vv_{int/A}$), and all fields were used to determine the interstitial volume fraction per total cortex ($Vv_{int/T}$). As determined in the previous study [25], 20 fields for each biopsy were required for adequate tissue analysis; only 39 of the 59 percutaneous biopsies had adequate tissue for full interstitial analysis. The interstitial measurements were completed on all patients by one researcher (P.H.L.) without knowledge of the clinical status of the patients.

Normal morphometric measurements. The structural parameters measured in these diabetic patients were compared with previously reported values in normal patients (aged 17–65 years) who had renal biopsies performed at the time of living-related kidney donation [23, 26]. Similar morphometric techniques as described here were used in the evaluation of these previously reported biopsies in normal patients.

Results in our diabetic patients were also compared with values in 6 pediatric patients, aged 10–16 years, who underwent a clinically indicated percutaneous renal biopsy for evaluation of microscopic hematuria. These 6 pediatric patients had normal physical examination and blood pressure for age and sex and a normal laboratory assessment, including C_{Cr}, quantitative urinary protein excretion, urine calcium/creatinine ratio, serum complement, anti-nuclear antibody, and renal ultrasound. They also had normal renal morphology by light microscopy, immunofluorescence, and electron microscopy, as determined by the clinical renal pathology service at the University of Arkansas for Medical Sciences. The biopsy tissue from these 6 patients was processed and prepared for electron microscopy in an identical manner to the biopsy tissue from the diabetic subjects. Morphometric analysis was performed as described above for the diabetic subjects for determination of GBM thickness, VvM, and SvLE. GV could not be measured in these normal adolescents because adequate amounts of tissue were not available.

Statistical methods. Descriptive statistics were calculated for each variable and expressed as mean ± standard deviation unless otherwise stated. Pearson product correlation moment was performed to determine correlations among individual variables. Analysis of variance was used to compare results between groups. Significance was noted at the $P \leq 0.05$ level for all relationships. The normal range for adult and pediatric values was defined as two standard deviations above and below the mean. Since initial regression modelling was confounded by excessive variable colinearity and to ascertain whether there were naturally occurring clusters of variables, morphometric data were submitted to principal factor analysis, a technique that focuses only on variance shared by several variables. Varimax and oblique rotation were then used to obtain the best configuration of the clusters, termed factors. The factors could then be considered as latent variables, each of which represents a group of interrelated parameters. A score for each factor, or latent variable, was calculated for each patient. Factor scores were calculated in such a way that they formed a standard normal distribution for each factor with a mean of zero and standard deviation of one. Thus, a factor score represented the number of standard deviations from the mean for each patient. Factor scores were calculated for each patient and correlations between factors and clinical and functional parameters were determined.

Parameters included in the factor analysis were patient height and the morphometric parameters: GV, GBM thickness, fractional volumes of mesanigum and capillary lumen (VvM and VvL), relative capillary surface areas (SvLE, SvLM, SvME), total volumes and surface areas per glomerulus (TM, TL, SLE, SLM, SME), filtration area S (defined as the sum of SLE and SLM), and the interstitial variables ($Vv_{int/T}$, $Vv_{int/N}$, %AF, and tubular atrophy/glomerular sclerosis). Only the data on patients with all parameters available (including interstitial parameters) were included in the principal factor analysis reported here ($n = 39$ for principal factor analysis).

Results

Clinical characteristics of the patients

Demographic and clinical characteristics of the 59 diabetic patients are displayed in Table 1; 34 patients were male; the average duration of diabetes was 7.7 years and the average age of the patients was 15 years; 12 patients were Tanner I and were thus, pre-pubertal; the remaining 47 patients were post-pubertal. Conventional diabetes management consisted of two insulin injections daily in 47 patients; the remaining 13 patients received various conventional insulin regimens, most commonly three insulin injections daily with the evening intermediate-acting insulin at bedtime. Average C_{Cr}, GFR, and RPF were within the normal range. UAE ranged from 1 to 195 μg/min in all patients; UAE ranged from 1 to 16 μg/min in the pre-pubertal patients and from 1 to 195 μg/min in the post-pubertal patients. All pre-pubertal patients had UAE in the normal range, while 11 post-pubertal patients had microalbuminuria.

Light microscopy tissue evaluation

GBM was qualitatively thickened in many biopsies. Mesangial matrix expansion was noted in 83% of biopsies and ranged from mild and focal to moderate in amount. Arteriolar lesions were evident in 25 patients (43%). These lesions consisted of hyalin deposition within the arterioles and intimal fibrous thickening. Of the 25 patients with arteriolar lesions, 19 had mild, focal lesions (1+), 5 had moderate, prominent lesions (2+), and 1 had severe lesions (3+). Light microscopy was entirely normal in the 6 non-

Table 2. Morphological parameters in diabetic children, normal children, and normal adults

Morphological parameters	Diabetic children (n = 59) Mean ± SD	Normal children (n = 6)[b] Mean ± SD	Normal adults [23, 26] (n = 28)[a] Mean ± SD
GBM thickness (nm)	465 ± 93*	326 ±45	326 ±45 for females 373 ±42 for males
VvM (%)	16 ± 4	10 ± 2*	14 ± 4
SvLE (um^2/um^3)	0.140± 0.030	0.166± 0.026	0.142± 0.026
Fractional luminal (volume (%)	30 ± 6	30 ± 6	28 ± 5
GV ($\times 10^6$ um^3)	2.4 ± 1.0		
TM ($\times 10^6$ um^3)	0.39 ± 0.23		
Total peripheral capillary surface ($\times 10^3$ um^2)	328 ±126		
Total luminal volume ($\times 10^6$ um^3)	0.72 ± 0.35		

GBM, Glomerular basement membrane; GV, glomerular volume; VvM, fractional mesangial volume; SvLE, peripheral capillary surface density; TM, total mesangial volume

* $P < 0.05$ compared with other two groups (by Student-Newman-Keuls test)

[a] n = 118 for GBM and VvM in normal adults; n = 28 for other structural parameters in normal adults

[b] Tissue from only a small number of normal children is available (n = 6) and thus comparisons based on age groups within childhood are not possible

Table 3. Bivariate correlations among morphometric parameters in diabetic children

Correlations		Pearson correlation coefficient	P value
Basement membrane parameters			
GBM vs.	SvLE	–0.617	0.0001
	TM	0.600	0.0001
	VvM	0.481	0.0002
	GV	0.419	0.0012
	TL	0.348	0.0095
Surface area parameters			
SvLE vs.	GV	–0.318	0.0161
	GBM	–0.617	0.0001
	TM	–0.439	0.0001
	VvM	–0.495	0.0001
SvLM vs.	TM	0.411	0.0018
	VvM	0.589	0.0001
Interstitial parameters			
$Vv_{int/T}$ vs.	$Vv_{int/N}$	0.913	0.0001
	Tubular atrophy/ glomerular sclerosis	0.521	0.0005
%AF vs.	Tubular atrophy/ glomerular sclerosis	0.455	0.0028

GBM, Glomerular basement membrane width; TL, total capillary luminal volume; SvLM, glomerular capillary luminal to mesangial surface density; $Vv_{int/T}$, interstitial volume fraction per total cortex; $Vv_{int/N}$, interstitial volume fraction per normal cortex; AF%, percentage of abnormal fields

Table 4. Significant correlations between clinical and morphological parameters in diabetic children

Correlations		Pearson correlation coefficient	P value
Volume parameters			
GV vs.	Age	0.433	0.0008
	Height	0.440	0.0006
	UAE	0.348	0.0080
	C_{Cr}	0.344	0.0088
	Post-pubertal duration	0.492	0.0001
VvL vs.	GFR	0.311	0.0282
TL vs.	Age	0.375	0.0047
	Height	0.394	0.0029
	C_{Cr}	0.294	0.0292
	Post-pubertal duration	0.387	0.0070
TM vs.	UAE	0.401	0.0021
	Age	0.303	0.0246
	C_{Cr}	0.278	0.0400
	Post-pubertal duration	0.493	0.0001
Basement membrane parameters			
GBM vs.	glycosylated hemoglobin	0.310	0.0191
	UAE	0.271	0.0413
	Post-pubertal duration	0.581	0.0000
Surface area parameters			
SvME vs.	glycosylated hemoglobin	–0.357	0.0065
	C_{Cr}	0.294	0.0265
S vs.	Age	0.417	0.0012
	Height	0.322	0.0146
	Post-pubertal duration	0.334	0.0220
Interstitial parameters			
$Vv_{int/T}$ vs.	diastolic blood pressure	0.493	0.0010
$Vv_{int/N}$ vs.	diastolic blood pressure	0.357	0.0237
%AF vs.	age	0.411	0.0075

UAE, urinary albumin excretion

diabetic pediatric patients biopsied for microscopic hematuria.

Quantitative morphometric evaluation

Results from morphometric analyses of glomeruli from the diabetic patients and the 6 pediatric patients with hematuria, as well as values for normal adult controls are listed in Table 2. All but one of the morphometric parameters were similar between normal adults and the pediatric patients with hematuria; however, relative mesangial volume was decreased in pediatric patients with hematuria compared with normal adults ($P < 0.05$).

Table 5. Significant correlations between factors and clinical and functional parameters in diabetic children

Factor	Clinical and functional parameters	Pearson coefficient	*P* value
"Glomerular size" vs.	Age	0.474	0.0023
	C_{Cr}	0.413	0.0089
	UAE	0.388	0.0146
"Peripheral capillary vs. decrease"	Diastolic blood pressure	0.360	0.0243
	Glycosylated hemoglobin	0.342	0.0332
	UAE	0.325	0.0435
	GFR	–0.300	0.0453
"Interstitial scarring vs."	Diastolic blood pressure	0.406	0.0104
"Mesangial increase" vs.	UAE	0.331	0.0394

In 30 of the 59 diabetic patients, the GBM was thickened compared with normal adults or children. Fractional mesangial volume was increased compared with normal children in 56%. In the diabetic patients, 14 significant correlations were identified among the morphological parameters (Table 3). Twenty-five correlations between functional and structural parameters were significant at the $P < 0.05$ level (Table 4).

It has been suggested that post-pubertal duration of diabetes correlates better than overall duration of diabetes with the clinical complications of diabetes and thus, in the 47 post-pubertal patients, correlations between post-pubertal duration of diabetes and the renal structural parameters were determined. Post-pubertal duration of diabetes correlated with GBM width, GV, total mesangial volume, total capillary luminal volume, and filtration surface area (Table 4), but not with any other structural parameter. Furthermore, the clinical and structural parameters in the pre-pubertal and post-pubertal patients were compared. Only UAE was different between these two groups, averaging 5 ± 4 μg/min in the pre-pubertal patients and 23 ± 39 μg/min in the post-pubertal patients ($P = 0.01$). When considering only the 12 pre-pubertal patients, fractional mesangial volume was increased in 7 patients (58%) compared with normal children, despite the fact that all of these pre-pubertal diabetic patients had normal UAE.

Of the post-pubertal patients, 36 had normal UAE and 11 had microalbuminuria which ranged from 27 to 195 μg/min. When the post-pubertal patients with microalbuminuria were compared with the post-pubertal patients with normal UAE, weight was the only clinical parameter which was different (71 ± 20 vs. 56 ± 14 kg, $P < 0.01$). For the functional and structural renal parameters, post-pubertal diabetic patients with microalbuminuria had greater values for C_{Cr} (130 ± 23 vs. 115 ± 21 ml/min per 1.73 m^2, $P < 0.05$), GV (3.3 ± 1.5 vs. $2.3 \pm 0.5 \times 10^6$ um^3, $P < 0.01$), total mesangial volume (0.57 ± 0.37 vs. $0.37 \pm 0.14 \times 10^6$ um^3, $P < 0.05$), total capillary luminal volume (0.97 ± 0.49 vs. $0.71 \pm 0.27 \times 10^6$ um^3, $P < 0.05$), and filtration surface area (442 ± 181 vs. $316 \pm 82 \times 10^3$ um^2, $P < 0.01$) than post-pubertal patients with normal UAE.

Because increased relative mesangial volume has been proposed as an important marker of early nephropathy, diabetic patients with and without increased relative mesangial volume were compared. These groups did not differ in average age, duration of diabetes, body size, blood pressure, C_{Cr}, GFR, RPF, UAE, or glycosylated hemoglobin. However, GBM thickness was greater (525 ± 103 nm vs. 440 ± 80 nm, $P < 0.01$) and peripheral capillary filtering surface density less (0.120 ± 0.019 um^2/um^3 vs. 0.148 ± 0.026 um^2/um^3, $P < 0.01$) in patients with increased fractional mesangial volumes. In addition, total mesangial volume, luminal-mesangial capillary surface, and mesangial-epithelial surface were increased in patients with larger fractional mesangial volumes.

Since interstitial expansion may also be an indicator of early renal involvement in type I diabetes, the patients were classified as having normal $Vv_{int/cortex}$ (≤ 0.11) or elevated $Vv_{int/cortex}$ (> 0.11). Of the 39 patients with adequate samples for determination of $V_{int/cortex}$, 18 were classified as elevated. Patients with and without interstitial expansion did not differ in age, duration of diabetes, blood pressure, C_{Cr}, UAE, glycosylated hemoglobin, or any structural parameter studied.

Factor analysis

Principal factor analysis of all structural parameters and patient height identified four factors. The "glomerular size" factor included total peripheral capillary surface, total glomerular capillary luminal volume, GV, total mesangial volume, and patient height. Thus, increased "glomerular size" was associated with increased GV, luminal and mesangial volumes, and was observed in taller patients. The "peripheral capillary decrease" factor included decreased peripheral capillary filtering surface density and fractional capillary luminal volume, and increased GBM thickness. Patients with high "peripheral capillary decrease" had relatively small capillaries with diminished peripheral area and increased GBM thickness. The "interstitial scarring" factor included the interstitial volume fraction of the total cortex ($Vv_{int/T}$), the intersitial volume fraction of the normal renal cortex ($Vv_{int/N}$), and the presence of tubular atrophy and/or glomerular sclerosis; patients with high "interstitial scarring" scores had greater than average interstitial scarring. The "mesangial increase" factor included fractional mesangial volume and relative areas of mesangial interfaces with capillary and with epithelial cells. Patients with high scores for "mesangial increase" had mesangial expansion.

These four identified factors were correlated with several clinical and functional parameters (Table 5). Specifically, "glomerular size" was directly correlated with age, C_{Cr}, and UAE. "Peripheral capillary decrease" was correlated directly with diastolic blood pressure, glycosylated hemoglobin, and UAE, and inversely with GFR. "Interstitial scarring" was positively correlated with diastolic blood pressure. "Mesangial increase" was also correlated with UAE.

Discussion

The current study provides a unique view of a large number of patients with diabetes of short duration and without the

usual signs of clinical diabetic nephropathy. Our children did not manifest hypertension, proteinuria, or impaired glomerular filtration or have longstanding diabetes, as has been true of previously reported patients. Nonetheless, many of them had evidence of scarring of arterioles, thickening of GBM, and expansion of the mesangium, which were noted on light microscopy. Quantitative analysis revealed enlarged glomeruli with thickened GBM and increased fractional mesangial volume. Tubulointerstitial lesions were also present.

The histological lesions of established diabetic nephropathy include enlarged glomeruli, diffuse mesangial expansion, thickening of the GBM, nodular glomerular sclerosis, and arteriolar hyalinosis [27–29]. GBM width has been noted to be increased after as little as 2–5 years of diabetes [27]. Diffuse glomerular sclerosis is associated with clinical nephropathy, as defined by hypertension, proteinuria, and diminished GFR [28]. In prior studies, the association between mesangial expansion and clinical nephropathy was noted [10] and data were interpreted as evidence that loss of capillary filtration area from impingement of the mesangium was the cause of decreasing GFR [9]. Mesangial expansion and GBM thickening appear to progressively increase over time after transplantation of normal kidneys into diabetic patients [30]. Our study extends these previous observations in longstanding diabetes and confirms that mesangial expansion occurs early in the course of diabetes and, in contrast to previous work, may even occur in pre-pubertal patients.

Previous studies have indicated that the pre-pubertal years of diabetes may not contribute to the risk of diabetic complications [11, 31, 32], as pre-pubertal diabetic children have a decreased prelevance of microalbuminuria [11, 31, 33] and diabetic retinopathy [11, 32]. We confirm the decreased prevalence of microalbuminuria prior to puberty, as all of our pre-pubertal diabetic patients had normal UAE. However, despite normal albumin excretion and only pre-pubertal years of diabetes, over half of these patients had mesangial expansion. Thus, the pre-pubertal years of diabetes may not be associated with the development of microalbuminuria, but renal structural changes appear to proceed unabated without regard to pubertal status.

In our post-pubertal patients, 23% had microalbuminuria which is similar to previously reported prevalence values of microalbuminuria in post-pubertal adolescents of 20%–37% [31, 33]. The post-pubertal patients with microalbuminuria, despite similar age, duration of diabetes, and diabetes control as post-pubertal patients with normal UAE, tended to be heavier in weight, have greater C_{Cr}, and have larger glomeruli with increased mesangium, capillary lumen, and filtration surface. Whether this group of post-pubertal adolescents represents a sub-group at particular risk of end-stage renal disease cannot be determined from this study and careful follow-up will be needed.

The numerous parameters measured in our diabetic patients are associated to form constellations which are mutually independent: the factors “glomerular size,” “peripheral capillary decrease,” “interstitial scarring,” and “mesangial increase.” Each of these four factors correspond to a current hypothesis regarding the risk for progression of renal injury in diabetes. Our data suggest that, when evaluated early in the disease process, diabetic patients with the largest glomeruli, as reflected by increased “glomerular size” factor, may be at increased risk for diabetic nephropathy. We postulate this because “glomerular size” correlates with increased UAE, an indicator of glomerular dysfunction, and with increased C_{Cr}, a possible indicator of hyperfiltration. In our post-pubertal patients, GV also correlates with the post-pubertal duration of diabetes. Large GV has also been noted in long-term diabetic patients with nephropathy [29].

It has been previously demonstrated that the glomerular peripheral capillary area correlates with GFR in patients with diabetes for less than 5 years [34] and in long-term diabetic patients [9]. Relative peripheral capillary surface area has been shown to correlate with mesangial expansion, and GV appears to influence the effects of mesangial expansion on peripheral capillary surface in long-term diabetic patients [9, 10]. Thus, it has previously been postulated that it is the impingement of the expanding mesangium on the peripheral capillary surface, mediated by GV, which eventually compromises GFR [35]. However, we demonstrate here that in early diabetes the diminished peripheral capillary may be a separate and independent parameter in the progression of diabetic nephropathy, since the factor “peripheral capillary decrease,” encompassing measures of both peripheral capillary area and capillary luminal volume, correlates independently with diastolic blood pressure, glycosylated hemoglobin, UAE, and GFR.

Several studies have shown correlations between interstitial expansion and renal function in type I diabetes mellitus [10, 25, 36, 37]. While mesangial and interstitial lesions are both advanced in patients with overt nephropathy, one or the other may predominate earlier in the course of diabetes. Furthermore, these lesions correlate independently with renal function in patients with longstanding diabetes [25]. In the present study, the expansion of the interstitium seen early in the course of diabetes, as demonstrated by the factor “interstitial scarring,” correlates with diastolic blood pressure, indicating that early changes in blood pressure may be a significant indicator of diabetic nephropathy.

Two final points deserve mention. The use of an invasive procedure such as renal biopsy in any study must be carefully considered. However, percutaneous renal biopsy is the mainstay of the diagnostic and prognostic renal evaluation in many types of renal diseases, such as nephrotic syndrome where the incidence of renal failure is much less than in diabetes mellitus. Renal biopsies have been performed for over 30 years and there is a large experience with this technique in both adults [38–43] and children [44–47], with a low incidence of serious complications, such as prolonged gross hematuria, hematoma formation, transfusion requirement, or surgical intervention, when carefully performed [48–50]. Thus, percutaneous renal biopsy in children is a safe and reasonable procedure when performed by an experienced pediatric nephrologist using standard techniques under a study protocol approved by the Institutional Review Boards of the participating institutions, as was done in this study. Fur-

thermore, in the 59 diabetic patients who underwent renal biopsy for this study, none had serious complications of the biopsy procedure and none required a prolonged hospital stay.

The majority of data available regarding normal human glomerular structure are derived from studies of adult renal cortex [23, 26]. In order to provide parameters for direct comparison with the diabetic patients in this study, we performed morphometric studies of clinically indicated renal biopsies of children biopsied for hematuria and without evident structural or other clinical abnormalities. The availability of tissue from only a small number of these normal adolescents is unavoidable, but not ideal, and precludes further comparisons based on age within the adolescent group; likewise, similarities of renal structure between these normal adolescents and larger numbers of normal adults is indicated, as was done here. These normal children had GBM thickness and peripheral capillary filtering surface density comparable to adults, but decreased fractional mesangial volume compared with adults.

The current study uniquely documents abnormalities of renal structure that precede the functional abnormalities of overt diabetic nephropathy. These early structural abnormalities include arteriolar hyalinization, glomerular hypertrophy, GBM thickening, and mesangial and interstitial expansion. The pre-pubertal years of diabetes, as well as the post-pubertal years, are associated with these renal structural abnormalities despite normal UAE. Additionally, we used morphometric measurements to define clusters of variables, termed the factors “glomerular size,” “mesangial increase,” “interstitial scarring,” and “peripheral capillary decrease,” which correspond to current hypotheses regarding the risk for progression of renal injury in diabetes. Long-term follow-up of this cohort of diabetic patients may permit us to determine the importance of early structural abnormalities to progression of diabetic nephropathy.

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Literature abstract

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Effects of recombinant interleukin-2 and revaccination for hepatitis B in previously vaccinated, non-responder, chronic uraemic patients

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Background. Growing evidence suggests that it is possible to seroconvert chronic renal failure patients who are absolute non-responders to hepatitis B vaccine by means of either additional booster vaccine doses or associated IL-2 administration or both. We have studied the possibilities of hepatitis B seroconversion by revaccination and its dependence on vaccine dose, and the effects of a concurrent low-dose rHuIL-2 regime.

Methods. Forty known absolute non-responders with chronic renal failure were entered into a complete revaccination protocol. Patients were randomly assigned to two dosage groups of either 20 or 40 μg hepatitis B vaccine administered at 0, 1, 2 and 6 months. Further randomly selected patients from each dosage group were given 500 000 U of rHuIL-2 in the same deltoid area 4 h after vaccine administration.

Results. Sixty-seven per cent of patients revaccinated with 40 μg attained antibody protecting levels compared to only 20% of those receiving doses of 20 μg ($P < 0.025$). When compared with initial values, the Th-CD4/CD25 cell count was significantly reduced immediately after HuR-IL2 administration ($P < 0.0003$) and significantly increased 1 month after the last dose was given ($P < 0.0003$). A definite rHuIL-2 effect on HBV antibody synthesis could not be demonstrated, nor was erythropoietin found to enhance seroconversion.

Conclusion. From these results we suggest that more intense and frequent antigenic stimulation as obtained by revaccination using four doses of 40 μg may effectively reduce the pool of hepatitis B vaccine non-responders in chronic renal failure patients.