Large Glomerular Size in Pima Indians: Lack of Change With Diabetic Nephropathy

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ABSTRACT

The mean glomerular volume, glomerular fraction of cortical volume, and percentage of obsolescent glomeruli were calculated in kidney specimens from autopsies on 34 Pima Indians, of whom 15 had non-insulin dependent diabetes mellitus and kidney disease of diabetes mellitus. These values were compared with those of black, white, and non-Pima native American individuals without diabetes mellitus. Glomerular volume in the Pima Indians was similar in the diabetic and nondiabetic subjects and significantly greater than in the white subjects. Black and non-Pima native American individuals had glomerular volumes intermediate between white individuals and Pima Indians. The mean glomerular volume was not affected by the number of obsolescent glomeruli in diabetic Pima Indians. The glomerular volume fraction was greater in the Pimas than in the other groups. These data showed that glomerular volume in the Pima Indians was significantly greater than that in white subjects. There was no difference between diabetic and nondiabetics Pimas, and glomerular size was not correlated with the presence or degree of glomerulosclerosis in this population.

Key Words: Morphometry, kidney disease of diabetes mellitus, native Americans, glomerulosclerosis, non-insulin dependent diabetes mellitus

Native Americans of the Pima tribe, who live in the Gila River Indian Community of southern Arizona, have a very high prevalence of non-insulin dependent diabetes mellitus (NIDDM) (1). Nearly one half of the population over 34 yr of age have NIDDM. In addition, diabetic nephropathy is a frequent complication of NIDDM in this population. The cumulative incidence of overt diabetic nephropathy is 50% among Pima Indians who have had NIDDM for 20 yr (2). A long-term investigation of diabetes mellitus makes the study of diabetic nephropathy in this population unique in that the approximate time of onset of diabetes can be identified and the accompanying clinical complications have been well documented (1). Therefore, if the renal lesions in the Pima tribe resembled those in other groups, the findings would be of considerable general significance because diabetic nephropathy has become the single largest cause of ESRD in the United States population (3).

The glomerular size, expressed as mean volume of the glomeruli (Vg), glomerular fraction of cortical volume (Vv), and percentage of obsolescent glomeruli were calculated in a series of autopsies on diabetic and nondiabetic Pima Indians. The data were compared with those of other Americans, including white and black individuals as well as native Americans of other tribes in the local area. The relationships of body weight, body height, body mass index, kidney weight, and heart weight with variations in Vg were also investigated.

MATERIAL AND METHODS

Archival specimens from autopsies of 34 Pima Indians performed between 1968 and 1980 were examined. The patients were divided into three groups. The first group (group A) comprised 15 Pima Indians with NIDDM. All 15 NIDDM patients had clinical evidence of diabetic nephropathy. The average duration of NIDDM was 15 yr, with a range of 1.5 to 33...
yr. The second group (group B) comprised nine non-diabetic Pima Indians with no histologic evidence of glomerular lesions. The third group (group C) comprised 10 non-diabetic Pima Indians who had significant glomerular lesions, either primarily glomerular or due to vascular or interstitial disease. Early detection and clinical characterization of NIDDM in the Pima Indian population were achieved by the biennial survey program conducted by the National Institute of Diabetes and Digestive and Kidney Diseases.

The specimens from the Pima Indians were routine autopsy-derived kidney samples that had been fixed in 4% formaldehyde solution and embedded in paraffin. The blocks available for analysis were cut at 5 μm and stained with hematoxylin-eosin and the periodic acid–Schiff reaction. Lesions in diabetic patients were graded on a scale of 0 to 4, with [1+] denoting mild mesangial proliferation and/or basement membrane thickening, [2+] indicating moderate mesangial proliferation and/or basement membrane thickening, [3+] indicating more severe changes with sclerosis and nodular lesions, and [4+] indicating global sclerosis in most glomeruli. Extraglomerular lesions were not part of the grading criteria. The percentage of obsolescent glomeruli was calculated. Histologic evaluation was carried out separately by two pathologists (K. Schmidt, L.J. Striker) blinded to the ethnic background and diabetes status of the patients.

Additional groups of specimens were collected from forensic autopsies on (1) adult black and white Americans, age and sex matched with group A and B Pimas, ages 60 yr or younger, and (2) native Americans of other tribes. The causes of death in these subjects were primarily related to trauma. The kidney tissue was fixed and embedded as described above.

Morphometry

For morphometric analysis, the profile areas of 40 to 100 glomerular tufts (mean, 60) were measured on periodic acid-Schiff–stained histologic slides with a computer-assisted planimeter. All glomeruli encountered were included in the measurement, including superficial cortical and juxtamedullary glomeruli and obsolescent glomeruli.

The mean equatorial area of the glomeruli of each specimen was calculated by the method of DeHoff and Rhines (4) for mean size of particles of similar shape. First, the profile areas were divided into a number (N) of classes by the interval of 10% of the largest profile measured. The mean area of each class (A1, A2 . . . An) was calculated to determine the harmonic mean (Z) of the profile areas,

\[ Z = 1/N \left( \frac{1}{A_1} + \frac{1}{A_2} + \ldots + \frac{1}{A_n} \right) \]

The mean equatorial area (A\text{mean}) was estimated from the equation

\[ A_{\text{mean}} = k_2 \frac{k_4}{Z} \]

where \( k_2 \) and \( k_4 \) are dimensionless factors depending on the variable measured, the dimensional measure to be estimated, and the shape of the particles. The value of \( k_2 \) was set at 1 and that of \( k_4 \) was set at \( \pi/2 \), assuming that the glomeruli correspond to spheres or ellipsoids. The \( V_v \) was derived mathematically from the \( A_{\text{mean}} \). In four cases, the measurements were repeated to obtain the intraclass correlation coefficient (r) from the standard deviation (σ) of the samples (5). The formula for two replicates per observation is

\[ r_1 = \frac{(\sigma_w^2 - \sigma_{w'}^2)}{(\sigma_w^2 + \sigma_{w'}^2)} \]

which yielded an r1 value of 0.988.

The \( V_v \) of the glomeruli was calculated with the same planimeter over an area greater than 5.0 mm². Both superficial and juxtamedullary areas of the renal cortex were examined. Obsolescent glomeruli were included in the measurement.

Statistics

Correlation between variables was assessed by Pearson's coefficient. Comparisons of \( V_v \) and \( V_v \) values among matched groups were performed with one-way analysis of variance (ANOVA) followed by the Bonferroni adjustment.

RESULTS

Clinical Findings

The mean age (±SD) was 56 yr (±16) for group A, 54 (±15) for group B, and 38 (±18) for group C (Table 1). The causes of death were derived from the autopsy protocols. Uremia was the cause of death in 7 of 15 diabetic Pima Indians but was not a cause of death among the non-diabetic Pimas.

Group A and B Pima Indians ages 60 yr or less were matched for age and sex with black and white subjects (Table 2). The body mass index was greater in the Pimas, reflecting their relatively short stature and increased body weight (1). There was no significant difference in kidney or heart weights between the matched groups (ANOVA with Bonferroni adjustment).

Histologic Lesions in Pima Indians

Diabetic Subjects (Group A). All group A patients had glomerular lesions, including diffuse mesangial thickening, glomerular basement membrane thickening, capsular drops, nodular lesions, and glomerular obsolescence, with 13 of 15 scoring 2+ or more. Eight of 11 patients with NIDDM of more than 10

\[ A_{\text{mean}} = k_2 \frac{k_4}{Z} \]
TABLE 1. Age, sex, and cause of death in Pima Indians

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Cause of Death</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>69</td>
<td>F</td>
<td>Sepsis</td>
</tr>
<tr>
<td>2</td>
<td>40</td>
<td>F</td>
<td>Cholecystitis</td>
</tr>
<tr>
<td>3</td>
<td>69</td>
<td>M</td>
<td>Myocardial infarction</td>
</tr>
<tr>
<td>4</td>
<td>73</td>
<td>M</td>
<td>Cerebral thrombosis</td>
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<tr>
<td>5</td>
<td>30</td>
<td>M</td>
<td>Pneumonia</td>
</tr>
<tr>
<td>6</td>
<td>45</td>
<td>F</td>
<td>Uremia</td>
</tr>
<tr>
<td>7</td>
<td>35</td>
<td>M</td>
<td>Sepsis</td>
</tr>
<tr>
<td>8</td>
<td>60</td>
<td>F</td>
<td>Uremia</td>
</tr>
<tr>
<td>9</td>
<td>78</td>
<td>F</td>
<td>Uremia</td>
</tr>
<tr>
<td>10</td>
<td>78</td>
<td>M</td>
<td>Lung embolism</td>
</tr>
<tr>
<td>11</td>
<td>48</td>
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<td>14</td>
<td>71</td>
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<td>Uremia</td>
</tr>
<tr>
<td>15</td>
<td>40</td>
<td>F</td>
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Group B/Nondiabetic Patients without Glomerular Lesions

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</tr>
</thead>
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<tr>
<td>2</td>
<td>35</td>
<td>F</td>
<td>Cirrhosis</td>
</tr>
<tr>
<td>3</td>
<td>31</td>
<td>F</td>
<td>Subarachnoid hemorrhage</td>
</tr>
<tr>
<td>4</td>
<td>49</td>
<td>M</td>
<td>Pneumonia</td>
</tr>
<tr>
<td>5</td>
<td>63</td>
<td>M</td>
<td>Prostate cancer</td>
</tr>
<tr>
<td>6</td>
<td>84</td>
<td>M</td>
<td>Senile brain atrophy</td>
</tr>
<tr>
<td>7</td>
<td>51</td>
<td>M</td>
<td>Shock, pneumonia</td>
</tr>
<tr>
<td>8</td>
<td>47</td>
<td>F</td>
<td>Gangrene of small bowel</td>
</tr>
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<td>46</td>
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Group C/Nondiabetic Patients with Glomerular Lesions

<table>
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<tr>
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<td>31</td>
<td>M</td>
<td>Miliary tuberculosis</td>
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<tr>
<td>3</td>
<td>31</td>
<td>M</td>
<td>Cirrhosis</td>
</tr>
<tr>
<td>4</td>
<td>38</td>
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<td>Cirrhosis</td>
</tr>
<tr>
<td>5</td>
<td>37</td>
<td>M</td>
<td>Peritonitis</td>
</tr>
<tr>
<td>6</td>
<td>47</td>
<td>F</td>
<td>Small bowel infarct</td>
</tr>
<tr>
<td>7</td>
<td>84</td>
<td>M</td>
<td>Cirrhosis</td>
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<tr>
<td>8</td>
<td>45</td>
<td>M</td>
<td>Miliary tuberculosis</td>
</tr>
<tr>
<td>9</td>
<td>17</td>
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<td>Acute leukemia</td>
</tr>
<tr>
<td>10</td>
<td>30</td>
<td>M</td>
<td>Cirrhosis</td>
</tr>
</tbody>
</table>

years' duration had grade 3+ or 4+ lesions. Four patients with lesions ranging from grade 1+ to 3+ had NIDDM of less than 10 years' duration. Those patients with the most severe glomerular lesions also had subintimal hyaline deposits in the extraglomerular blood vessels.

Nondiabetic Subjects (Groups B and C). Seven nondiabetic patients died of cirrhosis or chronic liver failure. Four of this group had glomerulosclerosis and were included in group C. Two patients died of sepsis and were included in group C because of glomerular lesions that appeared secondary to diffuse intravascular coagulation. Four patients were included in group C because of membranoproliferative glomerulonephritis, multiple myeloma, and acute and chronic interstitial nephritis.

There were variable arteriosclerotic changes in the blood vessels of the patients older than 40 yr in all of the groups. These consisted of increased cellularity and thickness of the intima and media, hyalinosis in the afferent and efferent arterioles, and reduction of the vascular lumina. Mild-to-moderate lesions were seen in nine patients ranging in age from 38 to 78 yr. Severe arteriosclerotic lesions were seen in a 71-yr-old diabetic man and in a 49-yr-old nondiabetic man. Mild interstitial fibrosis and patchy tubular atrophy were associated with the arteriosclerotic lesions.

Morphometric Data

There was no difference in the mean $V_g$ (±SE) between the diabetic Pimas (group A) ($V_g = 3.41 ± 0.36 \times 10^6 \text{ mm}^3$) and the nondiabetic Pimas without renal lesions (group B) ($V_g = 3.41 ± 0.71 \times 10^6 \text{ mm}^3$). The $V_g$ values did not correlate with body mass index or with duration of disease in group A Pima Indians. Mean $V_g$ was higher (4.04 ± 0.65 $\times 10^6 \text{ mm}^3$) in group C Pima Indians.

The $V_g$ in the cohort of 14 Pima Indians ages 60 yr or less was 4.07 ± 0.40 $\times 10^6 \text{ mm}^3$. This value was
significantly greater ($P < 0.01$; ANOVA with Bonferroni adjustment) than that of the age- and sex-matched group of white individuals, in which the $V_g$ was $2.29 \pm 0.24 \times 10^6 \text{ mm}^3$. Age- and sex-matched black Americans had intermediate $V_g$ values (Figure 1). $V_g$ was $2.85 \pm 0.18 \times 10^6 \text{ mm}^3$ in the non-Pima native Americans.

The mean $V_v$ of the glomeruli in Groups A and B Pima Indians ages 60 yr or less was compared with age- and sex-matched black and white subjects (Fig. 2). The differences in $V_v$ among the Pima, black, and white individuals were not significant.

**Obsolescent Glomeruli**

Obsolescent glomeruli constituted 4% or fewer of the total number in group B Pima Indians and 8% or fewer in both the age- and sex-matched black and white individuals and the non-Pima native Americans. The number of obsolescent glomeruli ranged between 4 and 90% in group A Pima Indians. The percentage of obsolescent glomeruli correlated with the duration of diabetes in group A Pima Indians ($r = 0.62; P < 0.01$). Within group A diabetic Pima Indians, six subjects had 6% or less obsolescent glomeruli, with a mean $V_g$ of $3.77 \times 10^6 \text{ mm}^3$. The remaining nine subjects had 20 to 90% obsolescent glomeruli and a mean $V_g$ of $3.18 \times 10^6 \text{ mm}^3$, not significantly different from the above. There was no correlation between percentage of obsolescent glomeruli and $V_g$.

**DISCUSSION**

The $V_g$ of Pima Indians was significantly greater than that of a group of white subjects matched for age and sex. Furthermore, although the total number of glomeruli could not be estimated in the available archival specimens, the finding of both a high $V_g$ and a high glomerular $V_v$ in Pima Indians suggested that the number of glomeruli in this tribe was not decreased. The kidney specimens for Pima Indians, black and white subjects, and other local native Americans were processed and measured under the same conditions. We could not age and sex match the non-Pima native Americans with the Pima Indians because of the limited size of the forensic autopsy population available.

The black and white individuals and the non-Pima native Americans were examined because the published values for glomerular size (6-14) in paraffin-embedded material did not provide a consistent baseline for comparison (Figure 3). Although not all reports specified the ethnic composition of the source population, it was likely that the subjects were mainly white. The reported values for glomerular size vary widely. The main reasons for this variation lie in (1) selection of glomeruli for measurement; (2) tissue fixation and processing; and (3) measures and calculations of morphometric parameters. The choice of the morphometric parameters is not as important as the above three points (15).

Osterby and Gundersen (7) excluded partially or totally obsolescent glomeruli when comparing the mean areas of glomerular profiles from patients with insulin-dependent diabetes mellitus (IDDM) with those from normal controls. Others (9, 13) measured whole glomeruli, including Bowman's capsule, instead of glomerular tufts alone. Most studies, including ours, used paraffin-embedded specimens. Shrinkage due to paraffin embedding differs consid-
surface area, was not higher in diabetic than in nondiabetic subjects (20). Although the uncorrected GFR in diabetic Pima Indians was significantly higher than that in normal controls, the elevation was less than that reported in IDDM patients. Although blood pressure measurements were not available, the heart weights of the Pimas were similar to those of matched controls.

The pathogenesis of glomerular enlargement in diabetic nephropathy is obscure. Some have suggested that the glomerular hyperfiltration may be partly responsible for the increased glomerular size in IDDM. Glomerular enlargement was postulated to precede the development of sclerosis in IDDM patients (7). The findings in streptozotocin-induced diabetes mellitus in rats were not consistent. Hirose and coworkers found glomerular enlargement (21), but Mayhew and coworkers did not confirm these results in a different strain of rats (22). Glomerular enlargement was associated with sclerosis in other clinical and experimental settings, such as minimal change disease (14), oligomeganephronia (23), and the Goldblatt hypertension model (24).

Mauer and coworkers (25) and Ellis and coworkers (26) showed that glomerular enlargement in IDDM is associated with an expansion in the volume of the mesangium. Glomerular enlargement may result from hyperfiltration, an event that may also play a role in the initiation and progression of diabetic nephropathy (27, 28). Nonetheless, hyperfiltration and glomerular hypertrophy in otherwise healthy uninephrectomized subjects did not result in glomerulosclerosis, even after long periods of time (27, 28). Because of the variable incidence of nephropathy in normal kidneys transplanted into IDDM patients, Mauer and coworkers (29) hypothesized that susceptibility to develop diabetic lesions resides, at least in part, within the kidney allograft itself.

The clinical diagnosis of diabetic nephropathy was confirmed histologically in all of the Pima Indians with NIDDM. Data on white patients suggested that ESRD was more common in IDDM than in NIDDM. Renal failure has been recognized as the cause of death in 30 to 60% of patients with IDDM, but only in 5% or less of patients with NIDDM (2). This discrepancy may be a result of the age of the population, rather than representing a difference in susceptibility to diabetic nephropathy between these two forms of diabetes mellitus. The mean age of onset of NIDDM in the general population is such that these patients may not have sufficient time to develop nephropathy (30). Pima Indians, who have a relatively young (35 to 44 yr) peak age of onset of NIDDM, are affected by diabetic nephropathy at a rate similar to that of IDDM in the general population (2). In this respect, Pima Indians present a unique opportunity to study the natural history of the development of NIDDM nephropathy in a population with an age of onset of the

**Figure 3.** Values of glomerular size, normalized to $V_0$, from the literature on paraffin-embedded specimens. Numbers along the x axis are from the reference list. The dotted column represents the $V_0$ for white subjects from this study.
Large Glomerular Size in Pima Indians

disease comparable with that typical for IDDM patients.

The lesions in the extraglomerular vessels that are common in IDDM were milder and appeared later in the course of the kidney disease of NIDDM in Pima Indians. This difference could depend on the peculiar epidemiologic characteristics of diabetes mellitus or on a distinct pattern of damage of NIDDM in this tribe.

In two separate autopsy-based studies by Cohen (8) and by Kasiske and Umen (11), the glomeruli of massively obese patients from the general population of the United States were enlarged and mildly hypercellular, with variable widening of mesangial regions and vascular dilation. The body mass index in Pima Indians was greater than that in age- and sex-matched black and white subjects. This was because of the body habitus of this tribe. Nonetheless, their mean body mass index was 30.6 kg/m², a value considerably lower than that of 46 kg/m² in the patients studied by Kasiske and Umen (11). In addition, the mean Vg in Pima Indians did not correlate with body mass index. These considerations suggested that obesity alone was unlikely to have accounted for the increased Vg observed in Pima Indians.

Although Kamenetzky and coworkers (31) found no increased prevalence of renal disease other than that related to NIDDM in a study of 105 autopsies on Pima Indians, we found that renal lesions were present in 10 of 19 patients in the group of nondiabetic Pima Indians in this series.

In conclusion, we showed that Pima Indians have glomeruli of larger size than those of white subjects that did not increase in size with diabetes mellitus. There was an increase in the number of obsolescent glomeruli with the duration of diabetes in group A Pimas. Glomerular size did not decrease in subjects with an increase in the number of obsolescent glomeruli. The large size of the glomeruli in Pima Indians was independent of obesity or heart weight. We postulated that it constituted a genetic trait of this population. A familial predisposition to developing renal disease has been recently recognized in diabetic Pima Indians on clinical grounds (32).

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