Glomerular Structure in the Normal Human Kidney: Differences between Living and Cadaver Donors

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Abstract. Glomerular structure has been studied in tissues derived from normal living and cadaver kidney donors. Values obtained in such subjects were considered interchangeable and have been used to define the normal parameters when evaluating the effects of various renal diseases. The present study evaluated glomerular structure by light and electron microscopy in 83 living and 53 cadaver kidney donors. Glomerular basement membrane (GBM) width (356 ± 52 versus 329 ± 45 nm), glomerular volume (1.64 ± 0.47 versus 1.33 ± 0.39 × 10^6 μm^3), and mesangial matrix volume per glomerulus (0.15 ± 0.05 versus 0.12 ± 0.04 × 10^6 μm^3) were significantly greater in cadaver compared with living kidney donors, respectively. It is hypothesized that glomerular and extracellular matrix swelling is associated with the cadaver kidney preservation process. This study suggests that the normal values for glomerular structure should be derived only from living kidney donor tissues.

The study of normal human kidneys provides the values needed to interpret the effects of disease on renal structure. Unbiased morphometric techniques permit a more accurate estimation of glomerular structure (1). We have previously described normal glomerular structural parameters in 118 kidney donors, including 98 living and 20 cadaver kidney donors (2). Age and gender influences on normal glomerular structure were observed; however, this study concluded that there were no differences in glomerular basement membrane (GBM) width or mesangial measurements between living and cadaver kidney donors. Evaluating a larger cadaver donor cohort and using newer morphometric methods, the present study found glomerular structural differences between living and cadaver donors.

Materials and Methods

Patients

We studied 136 normal adult kidney donors (18 to 65 yr old) that had a research kidney biopsy performed at the University of Minnesota either for studies of normal kidney structure or for baseline values for studies in which the transplant recipient agreed to a follow-up research biopsy protocol. Subjects, to be kidney donors, needed to have normal kidney function, normal BP, absence of clinically important gross renal structural defects, and absence of diabetes and of any known kidney disease. A patient survey of cadaver donors whose kidneys were used for transplantation at the University of Minnesota found that 93% were pronounced as brain dead due to events that were primarily related to brain injury (trauma 56%, cerebrovascular accidents 35%, and brain tumors 2%), while 7% were due to anoxia, and <1% due to other causes (Life Source Upper Midwest Organ Procurement, unpublished data). These studies were approved by the Committee for the Use of Human Subjects in Research of the University of Minnesota, and informed consent was obtained from the living donors or from the recipients of the cadaver kidneys. Thirty-one of the subjects have been previously reported (2), albeit evaluated by different morphometric methods.

Renal Structural Studies

Kidney biopsies were performed during kidney transplant surgery under direct vision using Vim-Silverman or Tru-Cut needle. Cadaver donor biopsies (n = 53) were obtained 30 to 60 min after the kidney was reperfused in the recipients, while living donors biopsies (n = 83) were obtained before the kidney was removed from the donor. Information regarding cold ischemia time was available in 31 cadaver donor subjects. All tissues were processed for light and electron microscopy by identical protocols.

Tissue Processing. Tissue for light microscopy was fixed in Zenker’s solution, embedded in paraffin, cut in 5-μm sections and stained with periodic acid-Schiff (PAS). Tissues for electron microscopy were processed as detailed elsewhere (3,4). Briefly, tissue was fixed in 2.5% glutaraldehyde and embedded in Polybed 812.

Electron Microscopy Measurements. Ultrathin sections were examined with a JEOL 100CX electron microscope (Tokyo, Japan). At least three non-sclerotic glomeruli per biopsy in the electron microscopy blocks was an entry criterion for this study. A calibration grid was photographed with each glomerulus. Ten to twenty evenly spaced micrographs were obtained at ×11,000 for measurement of GBM width and for mesangial composition. Micrographs at ×3900 were assembled into a montage of the entire glomerular profile for measurements of mesangial fractional volume [Vv(Mes/glom)] and surface density of the peripheral GBM [Sv(PGBM/glom)]. GBM width was estimated by the orthogonal intercept method (5) and Vv(Mes/glom) by point counting (3). The mesangial components were also estimated by point counting using a grid over the high-magnification micrographs, where points falling on mesangial matrix (MM) and mesangial cell (MC) were noted, and the fractional volumes of glomerulus occupied by MM and MC were calculated (6). Sv(PGBM/glom) was assessed using intercept counting (3). Mes/
glomeruli were measured from each biopsy. Every fourth section was viewed; as a new glomeruli appeared, they were numbered, and a grid of points was randomly superimposed over each new profile. The number of points hitting each profile was noted. GV was calculated as: \( GV \left( \mu m^3 \times 10^6 \right) = 20 \times \Sigma Pg \times \left( 5000/150 \right)^2 \), where 20 is the distance in \( \mu m \) between grid points, and 150 is the magnification. The mean GV was calculated from all glomeruli measured from a biopsy. There were no significant age or gender differences between the 39 subjects who did and the 97 subjects who did not have GV measured.

**Light Microscopy Measurements.** GV was measured by a single observer in the 39 subjects who had light microscopic tissue serially sectioned for estimate of glomerular volume by the Cavalieri method (7,8). An average of 8.5 (3 to 16) complete, non-occluded single glomeruli were measured from each biopsy. Every fourth 5-\( \mu m \) section was viewed; as a new glomeruli appeared, they were numbered, and a grid of points was randomly superimposed over each new profile. The number of points hitting each profile was noted. GV was calculated as: \( GV \left( \mu m^3 \times 10^6 \right) = 20 \times \Sigma Pg \times \left( 5000/150 \right)^2 \), where 20 is the distance in \( \mu m \) between grid points, and 150 is the magnification. The mean GV was calculated from all glomeruli measured from a biopsy. There were no significant age or gender differences between the 39 subjects who did and the 97 subjects who did not have GV measured.

**Statistical Analyses**

Results are presented as mean ± SD. Unpaired t tests were used to compare continuous variables and \( \chi^2 \) to compare gender distribution between cadaver and living kidney donors. Comparisons of Mes/glom, MM/glom, MC/glom, and S/glom between cadaver and living kidney donors were only performed in the 39 subjects where GV had been estimated. Pearson correlation coefficient was used to evaluate the relationship between cold ischemia time and glomerular structural parameters in cadaver kidney donors. Multiple linear regression analyses were performed to evaluate the effects of kidney age, kidney gender, and tissue source on glomerular structural parameters. Values for \( P < 0.05 \) were considered statistically significant.

**Results**

There were no significant differences between cadaver and living donors for age (35.4 ± 14.1 years) versus 40.0 ± 14.1 years) or gender (52.8% males) versus 43.4% males), respectively. Vv(Mes/glom), Vv(MM/glom), and Vv(MC/glom), and Sv(PGBM/glom) were not significantly different between cadaver and living donors (Table 1). However, GBM width was about 8% greater in cadaver compared with living donors (Table 1). GV was increased in cadaver compared with living donors (Table 2). Consequently, absolute values for MM/glom and S/glom were also increased in cadaver donors (Table 2). Mes/glom and MC/glom were not different between the two groups.

There was no correlation between the cold ischemia time of cadaver donor subjects and Vv(Mes/glom) (\( r = 0.09, P = 0.614 \)). Vv(MM/glom) (\( r = -0.037, P = 0.844 \)). Vv(MC/glom) (\( r = 0.235, P = 0.203 \)). Sv(PGBM/glom) (\( r = 0.254, P = 0.168 \)). GBM width (\( r = 0.257, P = 0.162 \)). or GV (\( r = -0.09, P = 0.741 \)).

Multiple linear regression analysis revealed that 23% of GBM width variability was explained by kidney age (\( P = 0.006 \)), kidney gender (\( P < 0.001 \)), and source of tissue (\( P = 0.01 \)). Also, 12% of GV variability was explained by source of tissue (\( P = 0.032 \)) and independent of kidney age or gender. Source of tissue was the only independent predictor (\( r^2 = 0.14 \)) of MM/glom (\( P = 0.021 \)).

**Discussion**

Using a larger number of cadaver donor subjects than previously evaluated, this study demonstrates differences in glomerular structural parameters between cadaver and living kidney donors. The current study evaluated a much larger number of cadaver kidney donors (20 subjects in the previous paper (2) versus 53 in the current study). This increased number of patients provided us with 99% power to detect a 10% difference in glomerular structural parameters between living and cadaver kidney donors, compared with 75% power in the previous study (2).

The differences between the method used in the initial study (2) and the current method are minimal and restricted to the estimate of mesangial fractional volume. The present method includes three mesangial components to estimate the mesangial fractional volume, namely, mesangial matrix, mesangial cell, and mesangial glomerular basement membrane (3). The earlier method only included mesangial matrix and mesangial cell components (2). This change generates a systematic mean difference of 0.03 in the Vv(Mes/glom), independent of the patient grouping. The increase in GBM width observed in the present study was not detected in our earlier study (2), probably because of the smaller number of subjects in the earlier work. Numerically, GBM width in the earlier study was, on average, about 8% greater in cadaver than in living kidney donors, and this was similar to what was observed in the present study (2). The finding of increased mesangial matrix per glomerulus and surface per glomerulus are dependent on measurement of GV, which was not done in the earlier work (2).

Because the age and gender of subjects with GV estimates were not different from subjects without GV estimates, and because a significant difference in GV between living and

### Table 1. Glomerular structural characteristics of cadaver and living kidney donors

<table>
<thead>
<tr>
<th></th>
<th>Cadaver (n = 53)</th>
<th>Living (n = 83)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vv(Mes/glom)</td>
<td>0.19 ± 0.04</td>
<td>0.20 ± 0.03</td>
<td>NS</td>
</tr>
<tr>
<td>Vv(MM/glom)</td>
<td>0.09 ± 0.02</td>
<td>0.09 ± 0.02</td>
<td>NS</td>
</tr>
<tr>
<td>Vv(MC/glom)</td>
<td>0.08 ± 0.02</td>
<td>0.08 ± 0.02</td>
<td>NS</td>
</tr>
<tr>
<td>Sv(PGBM/glom)</td>
<td>0.130 ± 0.024</td>
<td>0.128 ± 0.018</td>
<td>NS</td>
</tr>
<tr>
<td>GBM width (μm)</td>
<td>356 ± 52</td>
<td>329 ± 45</td>
<td>0.002</td>
</tr>
</tbody>
</table>

* Data are mean ± SD. Vv(Mes/glom), fractional volume of mesangium; Vv(MM/glom), fractional volume of mesangial matrix; Vv(MC/glom), fractional volume of mesangial cells; Sv(PGBM/glom), surface density of peripheral glomerular basement membrane; GBM: glomerular basement membrane.
cadaver kidney donors was found despite the reduced cohort size, it is unlikely that this GV difference is spurious. We speculate that oxygen deprivation in the cadaver donors and/or in the subsequent kidney handling before transplantation could lead to decreased smooth muscle tone affecting glomerular arterioles and, perhaps, mesangial contractile mechanisms, resulting in the increase in GV noted in cadaver kidney transplant biopsies. However, the underlying mechanism whereby hypoxemia could lead to extracellular matrix swelling is unknown.

Nonetheless, the concept that extracellular matrix structures are stable in their volume within the glomerulus is challenged by the results of the present studies. Mesangial matrix volume per glomerulus was increased in the cadaver donors by approximately 25%. Given that both GBM width and GBM surface per glomerulus were increased, the volume of peripheral GBM per glomerulus was, necessarily, also increased in the cadaver donors. It is most improbable that alterations in glomerular extracellular matrix production or turnover are responsible for these changes. It is far more likely that ischemia/preservation/reperfusion variables in cadaver donors result in increases in the water content of these extracellular matrix structures, leading to the findings described here.

It is, however, possible that paraffin shrinkage may be different in tissues from cadaver versus living kidney donors, and we have not measured shrinkage in the two situations. Nonetheless, this is not likely, and would not explain the GBM data. Thus we cannot be certain that MM per glomerulus is increased in cadaver kidney donors. However, given the finding of increased GBM width in these subjects and the probability that this represents extracellular matrix swelling, it is likely that MM is also similarly altered. It is thus reasonable to be worried about the use of cadaver kidney tissue as a surrogate of normal. Based on these results, it is recommended that normative values for comparisons with kidney biopsies measurements in disease states be derived from kidney biopsies obtained from normal living donors before kidney removal. These results should also be considered in the design of animal experiments with renal structural endpoints. It may be difficult for some centers to obtain living donor kidney biopsies; therefore, it would be important that centers, such as ours, that have such materials make them available as a research resource to other investigators. Finally, due to differences in shrinkage rates that may occur with different fixation and embedding methods, it is crucial that tissues being compared be processed by identical protocols.

Acknowledgments
This work was supported by grants from National Institute of Health (DK 13083, DK 54638, and DK 51975) and National Center for Research Resources (M01-R000400). Dr. Caramori was a Research Fellow of the Juvenile Diabetes Foundation International (JDFI). We thank the transplant surgeons at the University of Minnesota for their acquisition of the donor kidney biopsy specimens. Mrs. Brenda Welsh from Life Source Upper Midwest Organ Procurement kindly provided the data on cause of death in kidney cadaver donors.

References

Table 2. Glomerular structural characteristics of cadaver and living kidney donors

<table>
<thead>
<tr>
<th></th>
<th>Cadaver (n = 17)</th>
<th>Living (n = 22)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>GV (× 10⁶ μm³)</td>
<td>1.64 ± 0.47</td>
<td>1.33 ± 0.39</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Mes/glom (× 10⁶ μm³)</td>
<td>0.33 ± 0.08</td>
<td>0.28 ± 0.14</td>
<td>NS</td>
</tr>
<tr>
<td>MM/glom (× 10⁶ μm³)</td>
<td>0.15 ± 0.05</td>
<td>0.12 ± 0.04</td>
<td>0.02</td>
</tr>
<tr>
<td>MC/glom (× 10⁶ μm³)</td>
<td>0.14 ± 0.04</td>
<td>0.12 ± 0.06</td>
<td>NS</td>
</tr>
<tr>
<td>S/glom (× 10⁶ μm³)</td>
<td>0.23 ± 0.08</td>
<td>0.17 ± 0.05</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

*Data are mean ± SD. GV, glomerular volume; Mes/glom, volume of mesangium per glomerulus; MM/glom, volume of mesangial matrix per glomerulus; MC/glom, volume of mesangial cells per glomerulus; S/glom, surface of peripheral glomerular basement membrane per glomerulus.