Kidney International, Vol. 32 (1987), pp. 930–932

TECHNICAL NOTE

Estimation of mean glomerular volume in patients with insulin-dependent diabetes mellitus

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The measurement of glomerular volume may be important for the understanding of structural-functional relationships in renal disease. For example, glomerular volume is often increased in diabetic patients, and such increases probably occur soon after the onset of the disease [1, 2]. Furthermore, glomerular filtration rate in insulin-dependent diabetic patients is highly correlated with capillary filtration surface per glomerulus, and it is necessary to have an estimate of mean glomerular volume in order to calculate this surface [3]. Glomerular volume has been estimated by a number of different techniques [1, 2, 4–6], and it has generally been accepted that this mean volume should be estimated from tuft profiles at least 100 μm apart. The recent development of computer-linked digitizer tablets and of new estimators of mean particle volume [7] prompted us to explore the relative efficiency of these different methods by investigating the effects of varying sample size and sampling interval on the estimation of mean glomerular volume in renal biopsies from insulin-dependent diabetic patients.

Methods

Percutaneous renal biopsies were obtained using a Franklin modification of the Vim Silverman needle [6]. Biopsy specimens were placed in Zenker's fixative and embedded in paraffin according to standard techniques. Serial sections of 2 to 3 μm thickness were cut, and sequential strips of three to six sections were collected on numbered slides so that it was possible to estimate approximate distances between sections on different slides. We examined slides stained with periodic acid Schiff's reagent and hematoxylin and eosin in eight biopsies from insulin-dependent diabetic patients. There were at least 39 glomerular profiles in each of these eight biopsies based on counting profiles on sections that were 100 μm apart. Mean glomerular volume (MGV) was then estimated using the following techniques.

Mean glomerular volume determined by point counting

Specimens were examined using a Zeiss microscope with a drawing tube attachment under which was placed a tesselation of points so that they appeared superimposed on the section image. The number of points covered by each glomerular tuft profile were counted together with the number of sampled glomerular profiles. Because of the skewed distribution of values [1, 2], data were logarithmically transformed and geometric mean glomerular area was calculated from the formula:

$$\bar{A}_{(Glom)} = \text{antilog}_{10}\left[\sum_i (\log_{10} Pi) \cdot \frac{k^2}{N}\right]$$

where P was the number of points falling on each glomerulus; k was the distance between the points in μm; and N was the number of glomerular profiles sampled.

Mean glomerular tuft volume was then calculated from:

$$MGV = 1.25 \times [\bar{A}_{(Glom)}]^{3/2}$$

as described by Hirose et al [5].

Mean glomerular volume determined with a digitizer tablet

Individual glomerular tuft areas were also measured using a Summagraphics BitPad One digitizer tablet (Summagraphics Corp., Fairfield, Connecticut, USA) interfaced with a Terak 8510 computer (Terak Corp., Scottsdale, Arizona, USA). The area of each glomerular profile was traced using the tablet's cursor; mean glomerular area for all profiles was then calculated and MGV estimated as for point counting.

Glomerular volume determined by intercept lengths

An estimate of the volume-weighted MGV was calculated from the formula:

$$\bar{V}_V = \frac{\pi}{3} \times \overline{l_o^3}$$

where V_V is the volume-weighted mean glomerular volume; and $\overline{lo^3}$ is the average of the third power of the observed point sampled intercept lengths across the glomerular tuft using the method of Gundersen and Jensen [7]. These lengths were measured on serial sections of 10 glomerular profiles from each biopsy at ×167 using a 60 mm lo^3 ruler incorporating 15 classes, and designed according to Gundersen et al [7]. At least 62 such lengths were measured in each biopsy.

Statistical methods

In order to compare the methodologies of point counting and the digitizer tablet, MGV was estimated under different condi-

Received for publication December 29, 1986
and in revised form July 13, 1987

Table 1. Mean glomerular volume ($\times 10^6$ μm^3) calculated from the digitizer tablet and point counting at lower magnification (167) of profiles >120 μm, 60 to 100 μm and <30 μm apart, and from serial sections of 10 profiles using the lo^3 ruler.

Patient number	Profiles >120 μm		Profiles 60–100 μm		Profiles <30 μm	Intercept Ruler
	Digitizer	Point counts	Digitizer	Point counts	Point counts	$\pi/3 \cdot lo^3$
1	1.00	1.04	1.88	1.92	1.66	1.54
2	1.63	1.77	2.03	1.90	1.57	1.92
3	0.90	1.09	0.99	0.88	1.04	0.98
4	1.98	1.72	1.99	1.70	1.77	2.40
5	2.28	2.29	2.44	2.43	2.07	2.57
6	2.17	2.04	2.14	1.92	1.76	1.55
7	1.69	1.52	1.55	1.67	1.57	1.24
8	2.26	2.60	2.60	2.79	1.88	1.97
Range of CV	47–107%	50–86%	68–110%	52–82%	57–92%	59–87%

Renal biopsies were obtained from patients with insulin-dependent diabetes mellitus.

tions: firstly, from sections 60 to 100 μm and >120 μm apart and at two different magnifications ×167 and ×666; and secondly, by random sampling of profiles 60 to 100 μm apart in order to obtain population sizes of 10 to 20 and 20 to 30 tuft profiles. MGV was also calculated by point counting of at least 50 tuft profiles less than 30 μm apart at ×167.

Tests for analysis of variance (ANOVA) of MGV were performed in the following order: firstly, on values obtained from point counting and the digitizer tablet on profiles 60 to 100 μm and >120 μm apart at both magnifications. A second ANOVA was performed on MGV obtained from different numbers of profiles 60 to 100 μm apart, using point counting and the digitizer tablet at both magnifications. Finally, a third ANOVA was performed on MGV calculated from point counting at ×167 on (1) profiles greater than 120 μm apart; (2) 20 to 30 profiles 60 to 100 μm apart; (3) on greater than 50 profiles less than 30 μm apart; and (4) from serial sections of 10 glomeruli using the lo^3 ruler.

Results are expressed as the geometric mean, glomerular tuft volume for each of the biopsies using each of the techniques. The range of the coefficients of variation (derived from the tolerance factor) for each of the techniques is also given. Because of the number of multiple comparisons, statistical significance was accepted at the 1% level using two-tailed P values.

Results

There was a tendency for calculated MGV using the digitizer tablet to be higher than that calculated from point counting, but the first ANOVA failed to show a significant effect of either technique at either ×167 (Table 1), or ×666 (data not shown). Furthermore, there was no significant effect of varying the sampling distance from 60 to 100 μm to greater than 120 μm, or of varying the magnification at which the measurements were undertaken. The second ANOVA also failed to show a significant effect of varying the number of sampled profiles on sections 60 to 100 μm apart.

Since point counting at the lower magnification was by far the quickest technique, it was decided to use only this method to estimate mean glomerular volume from profiles less than 30 μm apart. The ANOVA on values obtained from ×167 magnification point counting on profiles greater than 120 μm, 60 to 100 μm and less than 30 μm apart failed to show an effect of varying the distance between sections (Table 1). There were also no significant differences between the values obtained from point counting or from the lo^3 ruler at this magnification (Table 1). The range of coefficient of variations was wide for all techniques, but similar for point counting, digitizer tablet and lo^3 ruler, and at both magnifications.

Discussion

Morphometric evaluation of renal biopsy material has proven very useful in investigating structural and functional relationships in diabetic nephropathy [8, 9] and other renal diseases [10]. Glomerular volume is known to change in diabetes [1, 2, 5]; thus estimates of volume, surface or length densities of glomerular structures provide incomplete information without an estimate of the reference volume [11], in this case the glomerular tuft. Previous studies have used differing techniques, magnifications, and sample sizes in the calculation of mean glomerular volume, with reported values of up to 4×10^6 μm^3 [1–6]. There is no agreement as to which methodology should be regarded as providing the reference volume to which other estimates should be compared. From previous studies in diabetes, it has generally been accepted that mean glomerular volume should only be estimated from tissue sections >100 μm apart in order to ensure sampling each glomerulus only once [6]. However, mean glomerular volumes have been reported as high as 2.8×10^6 μm^3 in insulin-dependent diabetic patients [3], which would imply a diameter, and thus sampling interval, of approximately 120 μm. Patients studied here had mean volumes less than 2.80×10^6 μm^3; we therefore considered that studying sections greater than 120 μm apart would provide an adequate separation of glomerular profiles for this study, and thus provide a reference value similar to that reported in other studies to act as a comparison. In glomerular profiles thus sampled, the mean glomerular volume did not significantly differ using either point counting or a digitizer tablet at either low or high magnifications. This was also the case if profiles 60 to 100 μm apart were measured, and mean glomerular volume was also independent of the sample size at this distance. Moreover, there were no significant differences between volumes obtained from profiles greater than 120 μm, 60 to 100 μm and <30 μm apart, measured by point counting at the lower magnification.

The use of the lo^3 ruler provides a precise and unbiased estimate of particle volume [7] and may well give the most accurate estimate of MGV. However, the methodology was time consuming because of the necessity to identify two or

more section profiles from the same glomerulus, and as the results obtained for MGV did not differ significantly from those obtained by point counting, it was felt to be less efficient. Point counting at the lower magnification was by far the quickest, simplest and cheapest method of all of those reported here, and we therefore feel it to be the technique of choice in the estimation of MGV.

The large coefficients of variation for MGV in these biopsies may have several contributing components. Glomerular volumes have been reported to vary considerably within the normal human kidney, with larger glomeruli in the juxtamedullary cortex [12]. In addition, diabetics with established nephropathy are known to have a larger MGV than those with newly diagnosed diabetes [1, 2], although it is not known if this enlargement is uniform throughout the total glomerular population. Weibel has shown that over 75% of randomly sampled cross-sectional profiles of spherical particles will be within 80% of the true diameter [13]. Thus it is unlikely that any sampling bias within individual glomeruli contributes significantly to the observed variation in MGV. The biopsies were obtained from patients with a wide range of glomerular pathology and, although it is beyond the scope of this paper to explore any renal functional correlates of MGV, there was no signficant correlation between the variation of MGV and individual urinary albumin excretion rates, and thus clinical severity of diabetic nephropathy. Previous studies have not reported individual CV values for mean glomerular volume [1–3, 11, 12] and we feel that the wide range of coefficients of variation for our mean glomerular volume-values most probably reflects a combination of both biological and pathological variation. It is therefore not surprising that more precise measurements of glomerular area using a digitizer did not result in a significantly different variation [14]. We conclude that mean glomerular volume has an intrinsic biological variability and that estimation of this important parameter from point counting at low magnification of approximately 50 profiles in sections less than 30 μm apart gives values indistinguishable from those obtained by more time consuming measures (such as digitizer tablets) or by measuring profiles from individual glomeruli only once from sections greater than 120 μm apart.

Acknowledgments

RWB is funded by a post-doctoral research fellowship from the Juvenile Diabetes Foundation (International) and is also the recipient of a travel grant from the Wellcome Foundation.

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