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Enlargement of the juxtaglomerular apparatus in insulin-dependent diabetes mellitus patients with microalbuminuria

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Abstract Kidney biopsies from 15 insulin-dependent diabetes mellitus (IDDM) patients with microalbuminuria were investigated to obtain quantitative data on the juxtaglomerular apparatus. The IDDM patients were young and normotensive with a mean duration of microalbuminuria of 2 years. Eight healthy kidney donors served as controls. Measurements taken by light microscopy, using 1-µm serial sections of epon blocks, included volumes of the juxtaglomerular apparatus and of glomeruli, areas of the macula densa and luminal area of the juxtaglomerular (afferent and efferent) arterioles at the level of the glomerular vascular pole. The volume of the juxtaglomerular apparatus was significantly larger in the IDDM group than in controls [6.08 (2.96–18.8) 10^4 µm^3 vs 3.48 (1.84–5.21) 10^4 µm^3, P=0.003, median and (range)], as was the volume of the juxtaglomerular apparatus relative to glomerular volume [1.89(1.28–4.21)% vs 1.48 (1.13–1.71)%, P=0.004]. The area of the macula densa was also larger in the IDDM patients (1370 µm^2 vs 937 µm^2, P=0.03). Luminal areas of the afferent and efferent arterioles and the ratio between them did not differ significantly between the two groups. In conclusion, the juxtaglomerular apparatus is enlarged more than would be expected from the glomerular hypertrophy in IDDM patients with microalbuminuria.

Key words IDDM · Microalbuminuria · Diabetic glomerulopathy · Juxtaglomerular apparatus pathology · Stereology

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Introduction

The juxtaglomerular apparatus (JGA) has two main functions: the synthesis and secretion of renin and direct control of glomerular haemodynamics via the tubulo-glomerular feedback (TGF) [22]. In insulin-dependent diabetes mellitus (IDDM) these functions are of special interest, as they may be involved in the development and progression of diabetic nephropathy.

The finding of expanded extracellular volume and sodium retention in IDDM originally led to the suggestion that the renin–angiotensin system was depressed in IDDM [5]. However, angiotensin-converting-enzyme inhibitors (ACE inhibitors) lower albumin excretion significantly in incipient diabetic nephropathy [25], which suggests activity of the renin–angiotensin system as a factor in the development of albuminuria in diabetes. Studies in IDDM patients have revealed normal or low plasma renin levels in most cases [3, 5]. In view of the volume and sodium retention in IDDM, plasma renin may be inappropriately high [3] and the activity of the renin–angiotensin system in the kidney may not be reflected in the plasma renin activity [7, 8]. In experimental diabetes there is some evidence that this local renin–angiotensin system is hyperfunctioning [1].

The TGF cannot be studied directly in patients. However, in studies in experimental diabetes and in models of hyperglycaemia in which microperfusion techniques are used, single nephron TGF has been found to be depressed [4, 9]. Increased renal perfusion and glomerular filtration rate, (GFR) are common features in early stages of IDDM [24] and may be caused by an abnormal setting of the TGF [26].

Few studies have dealt with the structure of the JGA in IDDM [2, 18, 19]. Two earlier studies on diabetic patients have described enlargement of the JGA [2, 18], but unbiased quantitative methods were not applied in either and the variables measured and patient groups were not clearly defined.

The present study was performed to obtain quantitative data on the JGA in young IDDM patients in the mi-

croalbuminuric phase of the diabetic nephropathy, and to investigate possible correlations between structural and clinical variables.

Subjects and methods

Subjects

Kidney biopsies were available from 8 controls and 15 young IDDM patients with microalbuminuria. Informed consent has been obtained from all subjects, and the biopsies were approved by the local ethics committees.

The IDDM patients from three departments of paediatrics were all more than 14 years old and had had diabetes for over 5 years. All patients had had a prepubertal onset. Albumin excretion rate (AER) was within the range defined as microalbuminuria: 15–200 µg/min in at least two out of three timed overnight urine samples collected consecutively in the last year before the biopsy was taken. Microalbuminuria had been present for 1–7 years, with a mean duration of 2 years. None of the patients had received antihypertensive drugs or a low-protein or low-salt diet

The controls were living, nondiabetic kidney donors. Thorough clinical investigations were made prior to nephrectomy to ensure normal renal function.

Table 1 presents clinical data. Blood pressures were obtained by sphygmomanometer after rest, and the means of two measurements taken before the biopsy are given. GFR was measured at the time of the biopsy, using either continuous inulin clearance (IDDM patients) or CrEDTA clearance (controls). Timed overnight AER was obtained in the IDDM patients by an immunoturbidimetric method [23], and the values in Table 1 are the means of the last two measurements prior to biopsy. HbA_{1C} was analysed by high-pressure liquid chromatography (Auto-A, Kyoto-Daiichi, Kagaku, Japan) with reference level 4–6%. The current HbA_{1C} is presented in Table 1.

Methods

In the IDDM patients needle biopsies taken with ultrasound guidance were performed with an 18-G needle (PrecisionCut AB, BD, New Jersey, USA). Surgical wedge biopsies were taken from the controls during transplantation. The specimens were fixed in 2% glutaraldehyde in buffer and mailed to the laboratory in Aarhus for dehydration and embedding in epon.

The blocks were sectioned systematically in 1-µm-thick sections. Examination levels were every 5th or 10th section. The average distance, *T*, between the levels was determined by a technique described in a previous work [17]. All variables except the mesangial volume fraction and basement membrane thickness were obtained by light microscopy (Fig. 1). Glomerular volume, V (Glomerulus): the glomerulus was defined as the minimal circumscribed polygon enclosing the capillary tuft [11]. Every new glomerulus that appeared was used, making sampling independent of size. At 292× magnification the areas, A (Glomerulus), of glomerular profiles were determined by point counting, using a point distance corresponding to 34 µm and 10 µm between levels. The glomerular volume was then calculated as V (Glomerulus)=T×ΣA (Glomerulus) (Cavalieri's principle [6]). If some levels of the glomerulus were missing, V (Glomerulus) was obtained by the maximal profile area method [15] assuming spherical glomeruli.

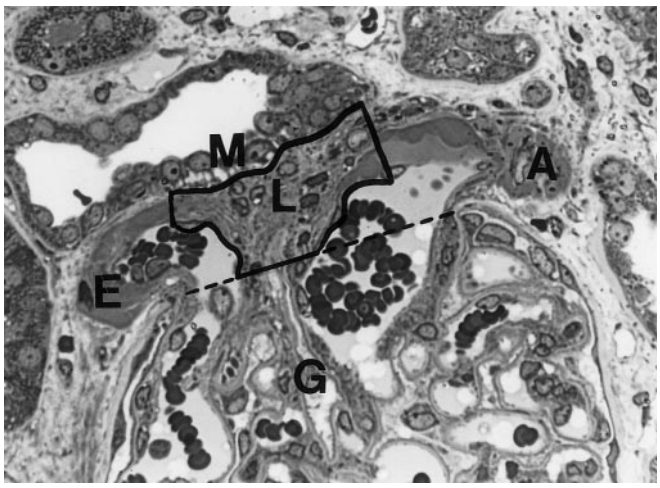

Fig. 1 Light micrograph of a 1-µm section, showing a profile of the juxtaglomerular apparatus: afferent arteriole (*A*), efferent arteriole (*E*), glomerulus (*G*), lacis cell field (*L*), macula densa (*M*). The measured area of the profile of the juxtaglomerular apparatus is outlined as the lacis cells and the part of the arteriolar walls bordering these. The level of reflection of the Bowman's capsule is indicated by the *broken line*. Luminal widths of the arterioles are also measured along the *broken line*. ×210

The following parameters were obtained from measurements at 5-µm intervals between levels. The microscopical image was projected onto a computer screen together with a point grid at a total magnification of 1440×.

JGA volume, V (JGA): an operational definition of the JGA was used in this study, namely the lacis cell field plus the part of the wall of the juxtaglumerular arterioles adjacent to the lacis cells (Fig. 1), which meant measuring a structure that could be clearly identified on any section regardless of the sectioning angle, taking advantage of the possibility of following the region in sequential sections. The limit towards the glomerulus was at the level of reflection of Bowman's capsule. V (JGA) was estimated by Cavalieri's method [6] using every new JGA that appeared in the tissue. The point distance in the grid was 15 µm. V (JGA) as a percentage of glomerular volume, V (JGA)/V(Glomerulus)%, was found for each nephron. The minimal accepted number of JGAs per biopsy was 5; the median number of JGAs per case was 10 (range 5–17). The number of examined sections on each JGA varied between 5 and 25 (median 14). The total number of points hitting one JGA was also very variable; the median was 50 (range 18–234). The variation due to measurement errors is likely to be negligible relative to intra- and interbiopsy variation in JGAs.

Table 1 Clinical data [median (range)]

	Controls	IDDM
Number	8	15
Sex (F/M)	3F/5M	9F/6M
Age (years)	30 (20–34)	18 (14–23)[a]
BMI (kg/m^2)	23.7 (19.6–26.4)	24.6 (19.6–31.2)
Diabetes duration (years)		10 (6–16)
Blood pressure (mm Hg)	118 (110–130)/70 (60–85)	123 (105–140)/80 (65–95)
GFR (ml/min per 1.73 m^2)	108 (82–144)	117 (87–176)
AER (µg/min)	Dipstick negative	26.5 (19–160)
HbA_{1C} (%)		8.6 (5.7–11.5)

[a] P=0.003 vs controls

The area of the macula densa, A (Macula densa), was defined as the projected plane (i.e. without curvature) interface between the macula densa and lacis cells and juxtaglomerular arterioles. An approximate estimate was obtained as the sum of lengths on all the sections through the macula densa multiplied by the mean distance between the levels.

The arterioles were identified as afferent or efferent by following their course in the serial sections to larger arteries or to capillaries. The arteriolar luminal spaces are likely to change more during fixation than solid structures, and the lumina were therefore measured at the level of reflection of Bowman's capsule (Fig. 1), being the best defined and also the least variable point of the arteriolar lumen as the walls were probably held in distension by the structures surrounding them here. The luminal areas of the afferent and efferent arterioles, A (Afferent) and A (Efferent), were estimated as the sum of luminal length on all the sections multiplied by the mean distance between the levels. For each pair of arterioles belonging to the same nephron the ratio of the areas, A (Afferent)/A (Efferent), was calculated.

The sectioning plane was not perpendicular to the areas measured, and the curvature in the plane of sectioning was disregarded in the measurement of A (Macula densa); this means that the real areas were underestimated, but relative to the measured area the errors were probably small and affected the groups similarly. In particular, the problem of the sectioning angle disappeared in the ratio of afferent to efferent arterioles.

Mesangial volume fraction, V_v (mesangium/glomerulus), and basement membrane thickness (BMT) were estimated by electron microscopy using standard stereological methods [15].

The Mann-Whitney's test was used to test differences between the two groups. Statistical significance was defined as $P<0.05$. Correlations between variables were tested with Spearman's rank correlation. Two variables were considered significantly correlated at $P<0.05$; the correlation coefficient, ρ, is also given.

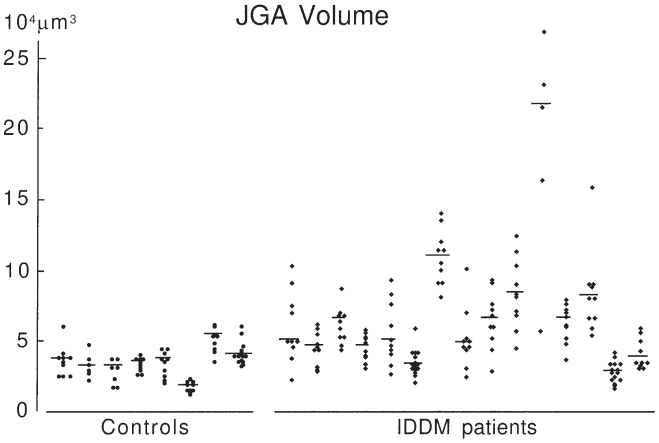

Fig. 2 Volumes of all the individual JGAs, with the median value for each biopsy indicated

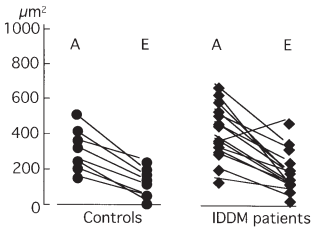

Fig. 3 Mean luminal areas of the afferent (*A*) and efferent (*E*) arterioles in biopsies. The *lines* connect results from individual biopsies

Results

The results are shown in Table 2. V (JGA) and V (JGA)/V (Glomerulus)% were significantly elevated in the IDDM patients. One IDDM patient showed strikingly high values in V (JGA) (Fig. 2), but statistical significance was independent of this and the results from this subject are included. V (Glomerulus) was not significantly elevated in the IDDM patients (P=0.093 vs controls).

The coefficient of variation (CV) in V (JGA) within biopsies was 0.17–0.44 (median 0.26) in the IDDM patients and 0.15–0.30 (median 0.24) in controls (Fig. 2). With the number of JGAs used in this study the coefficients of error within biopsies were 0.05–0.20 (median 0.10) in IDDM patients and 0.05–0.11 (median 0.075) in controls. CV among subjects was 0.57 in the IDDM group and 0.28 in controls.

There was a significant elevation of A (Macula densa) in the IDDM group compared with the controls (Table 2).

A (Afferent) and A (Efferent) did not differ significantly between IDDM patients and controls (P=0.11 and P=0.06 respectively; Fig. 3). The luminal area of the afferent arteriole relative to that of its corresponding efferent arteriole, A (Afferent)/A (Efferent), also did not differ significantly between the two groups (P=0.37).

Mesangial volume fraction of the glomerulus, V_v (mesangium/glomerulus), was not increased in the IDDM patients (P=0.55), whereas the difference in basement membrane thickness was highly significant (Table 2).

Correlations between structural parameters in the IDDM group were studied using mean values from biopsies. Positive associations were seen between JGA volume and luminal areas of both the afferent and efferent arterioles (ρ=0.63, P=0.014 and ρ=0.74, P=0.002 respectively). A positive correlation between JGA volume and glomerular volume was also noted (ρ=0.73, P=0.002).

Table 2 Structural data [median (range)]

	Controls	IDDM
JGA volume (10^4 µm³)	3.48 (1.84–5.21)	6.08 (2.96–18.8)[a]
V (Glomerulus) (10^4 µm³)	243 (150–366)	307 (189–592)
JGA volume/V (Glomerulus) (%)	1.48 (1.13–1.71)	1.89 (1.28–4.21)[b]
A (Macula densa) (µm²)	937 (542–1865)	1370 (959–3355)[c]
A (Afferent) (µm²)	307 (172–534)	397 (172–686)
A (Efferent) (µm²)	128 (26–246)	197 (68–490)
A (Afferent)/A (Efferent)	3.22 (1.63–10.1)	2.92 (1.04–6.29)
V_v (Mesangium/glomerulus) (%)	19 (17–21)	19 (13–29)
Basement membrane thickness (nm)	362 (326–442)	580 (440–820)[d]

[a] P=0.0027 vs controls
[b] P=0.0041 vs controls
[c] P=0.033 vs controls
[d] P=0.0001 vs controls

There was no correlation between the severity of glomerulopathy, expressed as the mesangial volume fraction and basement membrane thickness, and any of the parameters pertaining to the JGA. No correlations were noted between the structural parameters describing the JGA and clinical parameters [age, duration of diabetes, body mass index (BMI), GFR, AER, HbA_{1C} and blood pressure]. However, there was a positive correlation between diabetes duration and the mesangial volume fraction of the glomerulus (ρ=0.68, P=0.006).

Discussion

The JGA is important in the direct control of glomerular haemodynamics and in the regulation and secretion of renin; both mechanisms have been reported to be abnormal in IDDM [7, 9]. Little information exists on the structure of the JGA in diabetic renal disease [2, 18].

This study provides proof that the JGA is enlarged in incipient diabetic nephropathy: the average JGA volume in IDDM patients was almost double that in controls. Although a positive correlation was noted between JGA volume and glomerular volume in the IDDM patients this was not just an expression of general renal and glomerular hypertrophy in incipient nephropathy [16], the ratio between the volume of the JGA and that of its corresponding glomerulus was also increased. Further, glomerular volume was not significantly increased in the present series of IDDM patients. The macula densa was also found to be enlarged, and there were several correlations between different structural entities, implying a parallel growth of the individual structural units of the JGA. The mesangial volume fraction of the glomerulus was not significantly increased in the present series of IDDM patients, which is in keeping with the early stage of glomerulopathy in these subjects.

It might be expected a priori that a constant higher glomerular filtration rate would be associated with enlargement of the JGA, but no significant correlations were seen between clinical and structural parameters. However, the ranges of the clinical variables in the IDDM patients were narrow and correlations may still exist that would be detected if a larger IDDM group with broader ranges was studied.

Although the mean luminal areas of both afferent and efferent arterioles were larger in the IDDM patients than in controls, this difference was not statistically significant. The tendency corresponds to the recent demonstration of increased area of the whole vascular pole region in IDDM patients [17]. It is interesting that the ratio between the lumina of the afferent and efferent arterioles did not differ between the two groups. This is at variance with the results of short-term studies in experimental diabetes, showing dilatation of the afferent arteriole [10, 12]. However, animal studies may not be directly applicable to humans in this respect.

Variation in the volume of the juxtaglomerular apparatus was wide, both within biopsies and between subjects, and necessitated extensive sampling. However, coefficients of error were acceptably small, indicating that the number of JGAs sampled was sufficient for a reasonable estimate in regard to the particular biopsy.

In the IDDM group needle biopsies were performed, whereas wedge biopsies were taken during transplantation in the controls. Juxtamedullary nephrons may be under-represented in the wedge biopsies and, as juxtamedullary glomeruli are larger than superficial glomeruli [13, 20], a similar trend could exist for JGAs, although this suspicion has not been studied. However, the size of the JGA relative to that of its corresponding glomerulus was also greater in the IDDM patients, and therefore the problem of distribution seems to be of minor importance.

The IDDM patients were significantly younger than controls. Whether this could have affected the result is doubtful, and there was no trend for decreasing JGA size with increasing age in both groups. BMI was the same in the two groups. Therefore, if body size has any effect on the structural parameters it seems unimportant in the present series.

The mechanisms leading to enlargement of the JGA are not known, but some possibilities may be suggested: in the microalbuminuric phase of diabetic renal disease the mesangial volume fraction of the glomerulus tends to be slightly increased [14]. Some of the JGA volume consists of lacis cells, also termed the extraglomerular mesangium [21], and it could be that the same mechanisms governing the increase in the size of the intraglomerular mesangium also cause enlargement of the lacis cell field and hence of the JGA. However, the mesangial volume fraction of the glomerulus was not enlarged in the early stage of incipient diabetic nephropathy studied here and it would not explain the enlargement of the macula densa.

In streptozotocin diabetic rats an enlarged macula densa area has been found in one study [19] and it was speculated to be a structural reflection of a decrease in the TGF in the diabetic state. An increase in macula densa area was also found in our series, and it might be due to a compensatory growth caused by an abnormal TGF in diabetic patients.

In a recent work by Paulsen [18] it was noted that in microalbuminuric IDDM patients increased numbers of juxtaglomerular cells corresponded to increased levels of renin in the blood. Neither cell numbers nor plasma levels of renin were determined in our series. Some of the enlargement of the JGA could be due to hypertrophy or hyperplasia of renin-secreting cells in the afferent arteriole, in accordance with studies in experimental diabetes showing increased renin levels in the kidney [1].

In conclusion, the present study documents an increased size of the JGA in the microalbuminuric stage of diabetic nephropathy. This may well be a morphological counterpart of functional aberrations of this important structure.

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