Virchows Archiv A Pathol Anat (1993) 423:201–207

Virchows Archiv A
Pathological Anatomy and Histopathology

Three-dimensional analysis of increased vasculature around the glomerular vascular pole in diabetic nephropathy

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Received March 23, 1993 / Received after revision July 10, 1993 / Accepted July 12, 1993

Abstract. In diabetic nephropathy, several small vessels are frequently observed around the glomerular vascular pole in addition to the usual afferent and efferent arterioles. To elucidate the morphology of these abnormal small vessels, a three-dimensional study was performed by using computer-aided reconstruction techniques. In the present study, the renal tissue samples of 21 biopsy and 73 autopsy cases of diabetic glomerulonephropathy were examined. In addition to ordinary light microscopic observations, three series of serial sections from one autopsy and two biopsy cases were analysed. Five glomeruli with increased numbers of vessels around the vascular pole were reconstructed three-dimensionally. The vasculature in and around the glomerulus was analysed in detail by rotating and viewing in different planes via computer-generated three-dimensional images. These vessels anastomose to the lobular structure of the intraglomerular capillary network, mainly to the afferent branches through the widened vascular hilus. The distal end of the vessels anastomoses to the peritubular capillary. The increased vasculature is interpreted as neoangiogenesis resulting from diabetes, which may have a functional role in facilitating efferent blood flow from the glomerulus.

Key words: Diabetic nephropathy – Glomerular vascular pole – Neoangiogenesis – Computer graphics – Three-dimensional reconstruction

Introduction

While progress in therapy for diabetes mellitus has resulted in the elongation of survival periods of diabetic patients, the incidence of diabetic microangiopathy, including retinopathy and nephropathy is increasing. In diabetic retinopathy, neoangiogenesis with formation of a collateral circulation has long been observed (Ballantyne and Loewenstein 1943). In diabetic nephropathy, nodular and diffuse lesions, as well as exudative lesions are well-known (Kimmelstiel and Wilson 1936; Spühler and Zollinger 1943; Laufer and Stein 1959) but there have been no reports on the increase of new vessels in and around the glomerulus. We have studied the changes of glomerular vasculature in diabetic nephropathy and confirmed the phenomenon of increased vascularity around the vascular pole area, in addition to the usual afferent and efferent arterioles. It was frequently noticed that these newly formed small vessels in this region anastomosed to the glomerular capillary network.

To elucidate the morphological characteristics of these vessels as well as the relationship between diabetic glomerular lesions and vascular changes, we observed and analysed the three-dimensional structure of these changes by using computer-aided reconstruction technique from serial sections. These were applied together with conventional two-dimensional observations of histological findings in biopsy and autopsy subjects from diabetic patients with glomerulopathy.

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Materials and methods

We examined the renal tissues of 21 biopsy and 73 autopsy cases with diabetic nephropathy which were examined at the Department of Pathology of Nippon Medical School. We also examined the kidneys of 17 autopsy cases of non-diabetic patients who have been hypertensive for a duration of more than 10 years and 28 biopsy cases of membranoproliferative glomerulonephritis with nodular accentuation, as controls. The distribution of age and the sex of these patients are shown in Table 1.

For two-dimensional observations the renal tissues were fixed with 20% formalin solution, processed by the routine method and made into 2 μm thick paraffin sections. Haematoxylin-eosin (HE), periodic acid Schiff (PAS), Masson trichrome (Masson) and periodic acid methenamine silver-HE (PAM) stainings were performed on these sections. The specimens were observed by light microscopy. Small portions of the cortex of these kidneys were fixed in 2.5% glutaraldehyde solution in phosphate buffer (pH 7.4) and

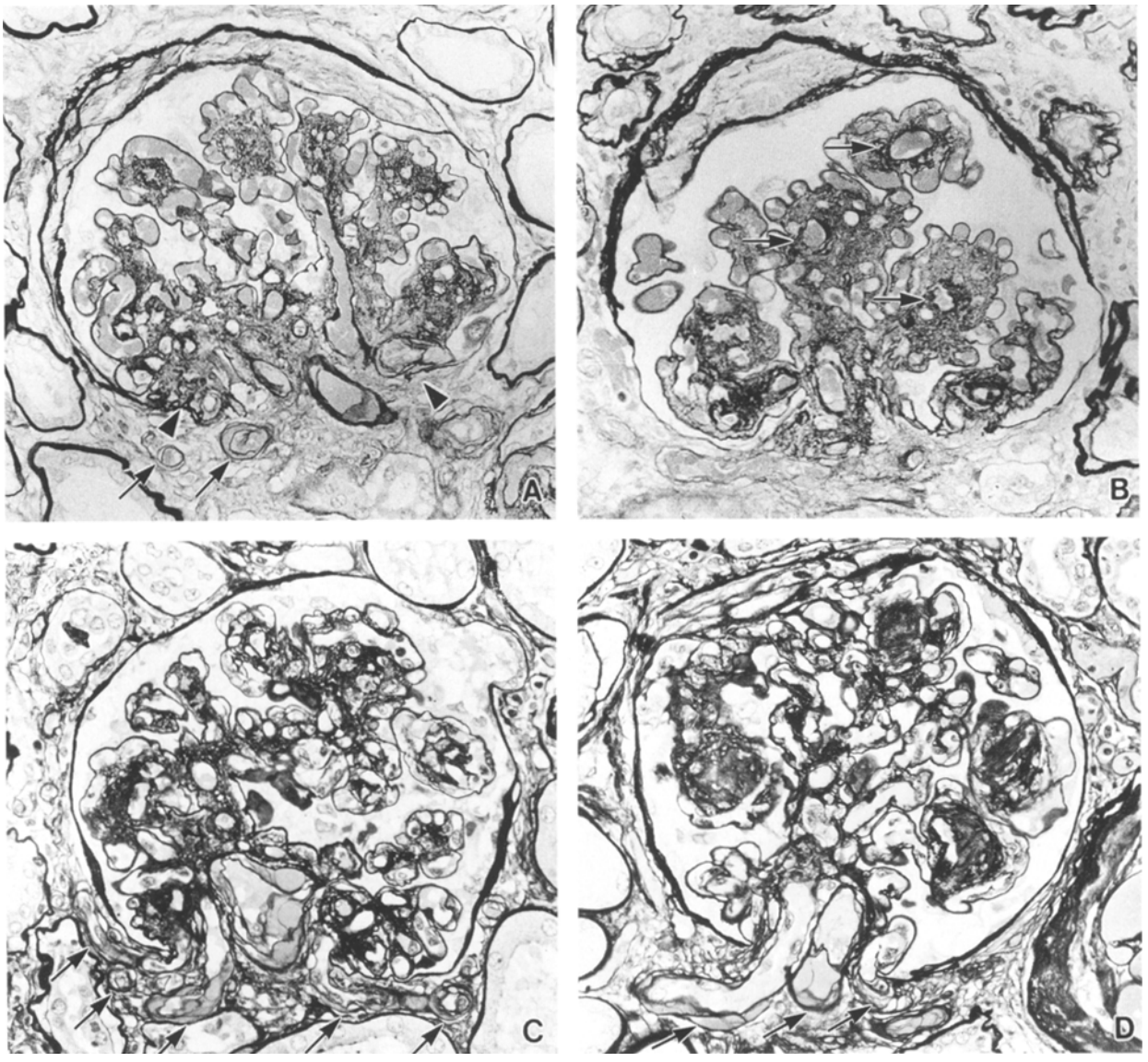

Fig. 1 A–D. Variable features of glomerulus from an autopsy case of diabetic nephropathy. **A** *Arrowheads* indicate the widening of vascular hilus. Hyalinized small vessels (*arrows*) increase in the vascular pole area. **B** Centralization of vascular lumens (*arrows*) within nodularized mesangium with increased mesangial matrix. **C.** Increased small vessels (*arrows*) of which three apparently anastomose to intraglomerular capillaries. **D** Three vessels with arteriolar characteristic at the vascular pole (*arrows*). PAM staining, $\times 400$

Fig. 2. A One section of 339 plastic embedded serial sections from a biopsy case with nodular glomerular sclerosis (methyl-blue stain). Note the narrow lumen of the efferent arteriole. **B–D** Three-dimensional reconstruction of the same glomerulus as **A** from the serial sections (the most peripheral capillaries are omitted). Several additional small vessels other than the afferent and efferent arterioles anastomosing to glomerular capillaries are apparently present in B (*arrows*). **C** shows the feature of the reconstructed glomerulus cut at the 180th section. The colour indicates light-blue for basement membrane, red for afferent arteriole and its branches, yellow for efferent arteriole and its branches and green for other vessels anastomosing to glomerular capillaries. The dark blue areas, one of which is marked with an *asterisk*, are artificial gaps in the three-dimensional reconstructions due to minor tracing errors. **D** is the feature of the glomerulus cut at the 224th section in serial sections. An *arrow* indicates the anastomosis of an increased vessel and the main branch of the efferent arteriole. (*a:* afferent and *e:* efferent arterioles)

Fig. 3 A–F. A computer-generated image of the main branches of the glomerular capillaries reconstructed by 248 plastic embedded serial sections from an autopsy case of diabetic nephropathy (the most peripheral capillaries are omitted). **A** shows increased small vessels at the vascular pole area (bottom) and anastomosed glomerular lobular units with different colours respectively. (*a:* afferent and *e:* efferent arterioles) **B–E** demonstrate each lobular unit by cutting off the intraglomerular capillary anastomoses. Each lobular unit anastomoses to the afferent arteriole in the lobule **B**, the efferent arteriole in the lobule **E** and other excess vessels in the lobules **C**, **D** and **F**. The cut points are indicated by numbers. (For example, the cut point No. 1 in lobulus **B** corresponds to No. 1 in lobule **F**.)

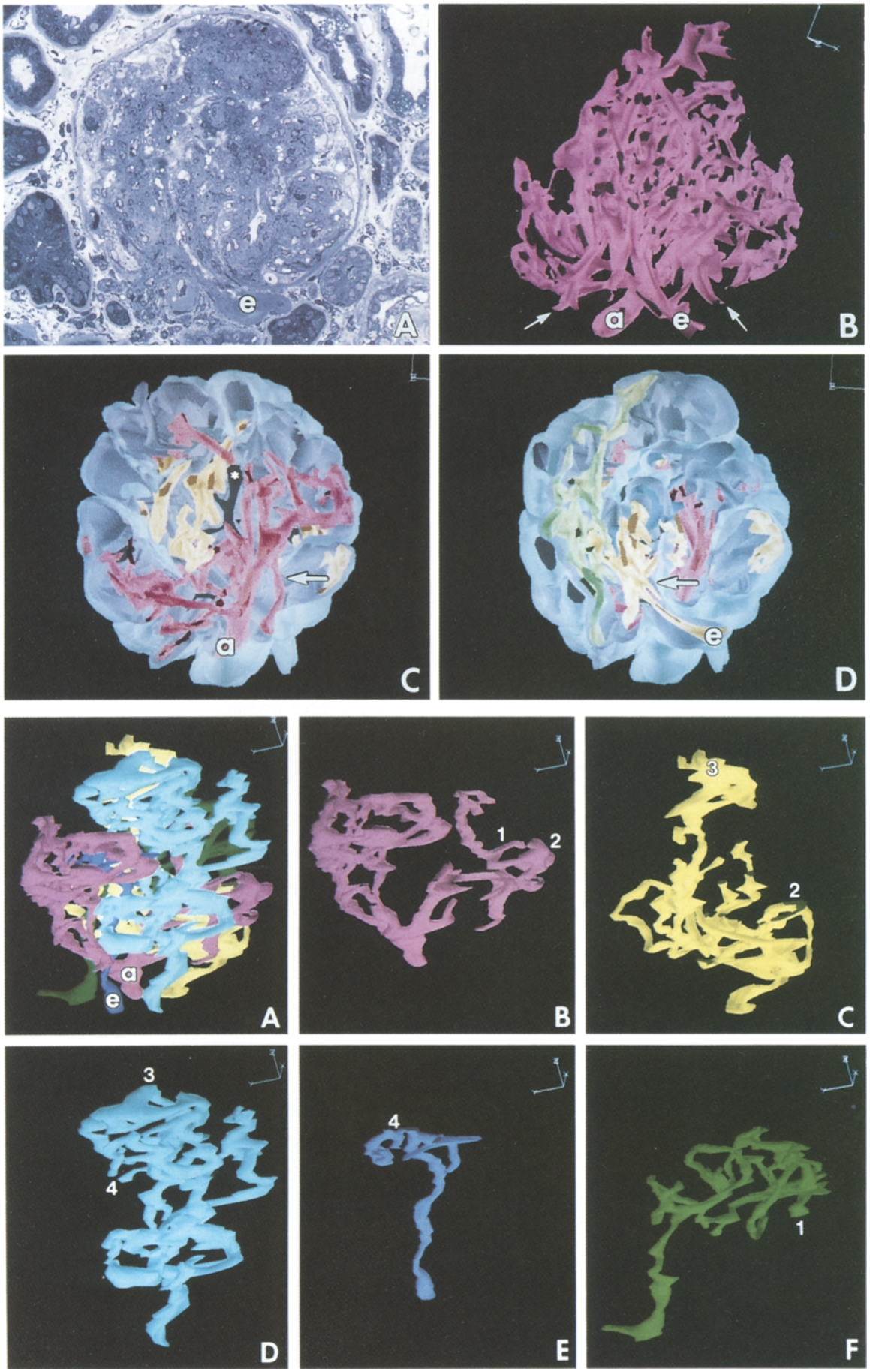
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Table 1. Age distribution of diabetic and control cases

Age	~40	41~50	51~60	61~70	71~	total
Biopsy cases						
Male	2	6	4	2	2	16
Female	–	1	1	2	1	5
Autopsy cases						
Male	–	4	9	12	26	51
Female	–	1	2	8	11	22

Control:

Hypertension (Autopsy)

Age	~60	61~70	71~	total
Male	1	2	7	10
Female	4	2	1	7

Membranoproliferative glomerulonephritis (Biopsy)

Age	5~20	21~30	31~40	41~	total
Male	8	2	2	6	18
Female	7	–	1	2	10

post-fixed with 1% osmium tetroxide in phosphate buffer, dehydrated and embedded in epoxy resin (Epok 812), and several series of 0.7 μm-thick serial sections were prepared. After methyl-blue staining, each section was observed by light microscopy.

For three-dimensional observations one case of diabetic glomerulonephropathy was chosen from the autopsy patients and small pieces of the renal cortical tissue were fixed in 0.25% glutaraldehyde and postfixed in osmium tetroxide, then embedded in Epok 812. One series of 500 pieces of 0.7 μm-thick serial sections was prepared. Two cases of diabetic glomerulonephropathy from the biopsy materials were prepared for Epok embedding and two series of serial sections of 500 and 300 respectively were prepared. These sections were stained with methyl-blue. In each series, every second section of one glomerulus was targeted to be microscopically photographed at a magnification of ×400. The photographs were enlarged into 25×30 cm prints and every feature of each glomerulus was traced onto tracing-paper. The information of the traced image was automatically input into a NEC PC9801-RA computer through an image-scanner by using application software for three-dimensional reconstruction, named "TRI SYSTEM (Ratoc Co. Tokyo)". In addition to these plastic-embedded 2 μm thick serial sections, 200 paraffin-embedded serial sections with a PAM stain were prepared, and photographs by light microscopy magnifying 200 times were taken in order to examine the details of extraglomerular vascular distribution around the vascular pole area.

The input graphical information from the serial sections was processed and reconstructed into three-dimensional glomerular architecture which included the small vessels around the vascular pole area. Five sets of three-dimensional glomerular images were reconstructed. The vasculature in and around the glomerulus was analysed in detail by rotating and viewing in different planes in the reconstructed three dimensional images. In order to examine the relationship between the distribution of vessels and the surrounding tissue, three-dimensional reconstruction at a magnification of ×100 was also performed.

In addition to these 5 reconstructed glomeruli, the other glomeruli in the serially sectioned specimens were carefully examined in each individual slide, in order to confirm the relationship between extraglomerular vascular structures and glomerular capillaries.

Results

We described the three main glomerular lesions (diffuse, nodular and exudative) of diabetic glomerulopathy as slight, moderate and marked. The "slight" change of the diffuse lesion was defined as a slight increase of PAS or PAM positive substance in the glomerular stalk region, occupying under $^1/_3$ of the glomerular area. We defined "moderate" when the increase of PAS or PAM positive material in the glomerular stalk region was between $^1/_3$ and $^2/_3$ of the glomerular area. We defined "marked" when the increase of PAS or PAM substance was diffuse in the stalk region. In the nodular lesions, there were fairly large nodules with lamellar structure and scanty cellularity, and rather small nodules with increased cellularity. The exudative lesions showed PAS positive homogenous substance in the subendo-mesangial region with occasional microfatty droplets.

These well-known diabetic glomerular lesions were frequently observed in all cases. In addition, the widening of the vascular hilus, the increase of the mesangial matrix from the vascular pole area to the glomerular stalk region, dilatation of the intraglomerular capillaries and centralization of the vascular lumen within the nodularized mesangium were observed (Fig. 1 A, B). In the

Table 2. Increased vascularity and type of diabetic lesion

	Number of cases[a]		Duration of diabetes (years)[b]		
	Biopsy	Autopsy	~5	6~10	11~
iv/total number	10/21 (48%)	58/73 (81%)	9/9	10/10	21/23
iv/wide vp	5/11 (46%)	58/66 (88%)	9/9	10/10	21/21
iv/DL mild	1/3 (33%)	8/16 (50%)	2/2	3/3	9/9
iv/DL moderate	3/7 (43%)	23/23 (100%)	4/4	3/3	7/7
iv/DL severe	6/9 (67%)	24/24 (100%)	3/3	3/3	7/7
iv/NL	1/3 (33%)	22/22 (100%)	1/1	3/3	2/2
iv/EL	3/6 (50%)	13/13 (100%)	–	3/3	2/2

iv, Increased vascularity; wide vp, widening of vascular pole; DL, diffuse lesion; NL, nodular lesion; EL, exudative lesion

[a] The cases with incomplete records on the onset of diabetes were not included in this table

[b] The shortest case history of DM was 2 years (1 case) and the longest case history of DM was 44 years (1 case): one case with a 14-year history of DM showed no definite neoangiogenesis

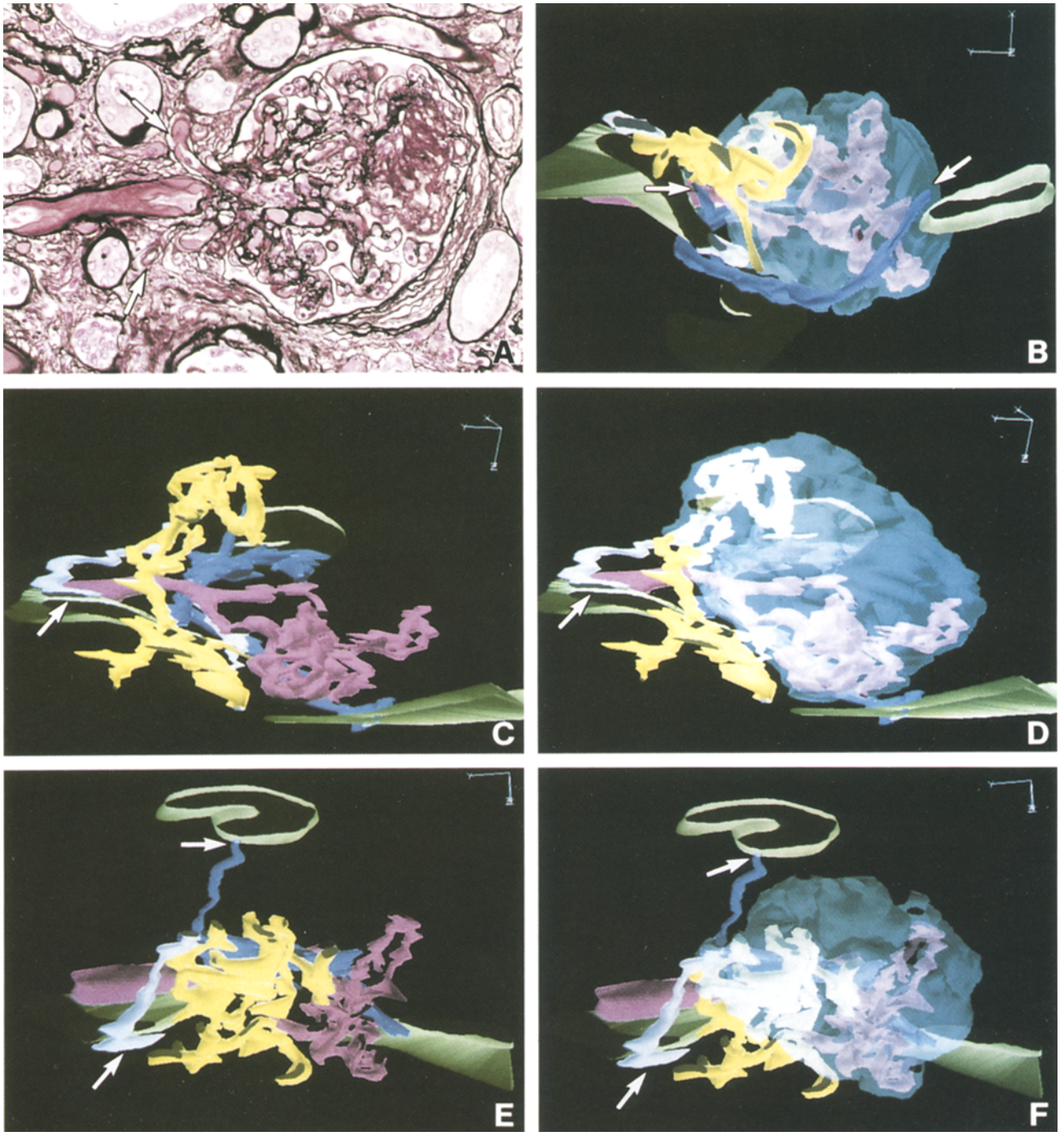

Fig. 4. **A** shows one section of 130 paraffin-embedded serial sections with 2 μm thickness in PAM staining. The *arrow* indicates newly formed vessels. **B–F** are three-dimensional computer images of the same glomerulus as **A**. Pale-blue colour is assigned to the basement membrane, green to the tubule, red to the afferent arteriole and anastomosed capillaries, blue to the efferent arteriole, and yellow and white to the other vessels. The vessels branched out from the efferent arteriole run to the proximal tubule as shown in **B** (*arrows*). The vessels other than the afferent and efferent arterioles run also to the vicinity of the tubules as shown in **C–F** (*arrows*). **C** and **E** are the images with the basement membrane erased from the images of **D** and **F**. **E** and **F** are the 90° rotated images of **C** and **D** along the *x*-axis

vicinitiy of the glomerular vascular pole, we often recognized small vessels other than the usual afferent and efferent arterioles, most of which were markedly hyalinized (Fig. 1 A, C, D). Under careful observation, more than three additional vessels were recognized around the vascular pole area of one glomerulus, anastomosing with the intraglomerular capillary network (Fig. 1 C, D). The relationship between the type of the glomerular lesions, the frequency of these small vessels and the patient's diabetic history is shown in Table 2.

Approximately 90% of diabetic autopsy cases exibited a marked widening of the vascular hilus of the glomerulus (shown as Fig. 1A). There was a tendency that the more severe the degree of diabetic glomerular lesions, the more the degree of the hilar widening increased. Around the widened vascular hilar area, an increased number of small vessels were noted frequently and the degree of hyalinization of these vessels tended to be prominent. Even when there were less severe diabetic glomerular lesions, an increase of small vessels around the vascular hilus was observed. While the frequency of these small vessels was less than that of the autopsy cases, basically similar results were obtained in the biopsy cases of diabetic patients.

In all the control cases, which were composed of 17 hypertension and 28 membranoproliferative glomerulonephritis with nodular accentuation cases, no excess of small vessels was present.

When examining the serial sections, we observed several small vessels around the vascular pole area in each of the different dimensions. After exiting the glomerulus, these small vessels continued to show arteriolar characteristics and gradually transformed into peritubular capillaries. We also observed that the lumen of the efferent arteriole became very narrow in every glomerulus examined, as shown in Fig. 2A.

The computer-generated three-dimensional reconstruction revealed the existence of increased vascularization around the vascular pole area of the glomerulus. In addition to the usual afferent and efferent arterioles, there appeared to be at least three more vessels (Fig. 2B and 3A). By rotating or cutting the three-dimensional images of the reconstructed glomerulus of Fig. 2, the anastomoses of these vessels with glomerular capillaries were revealed at the main branches of afferent and occasionally efferent arterioles and rarely at more peripheral capillaries (Fig. 2C, D).

Careful comparative observations of each serial section and three-dimensional images revealed that one vessel always anastomosed to one lobular unit of the glomerular capillary network. Between each lobular unit there were only two or three anastomosed capillaries. We assigned different colours to each unit of the capillary lobule having vascular pole anastomosis and then we viewed computer images of each lobule respectively by cutting off the intraglomerular capillary anastomoses (Fig. 3A–F). Most of the excess vessels anastomosed in the glomerulus at a point at least after second or third branchings of the afferent arteriole (Fig. 3B–F), and occasionally to the proximal branches of the efferent arteriole (Fig. 2D). The narrowing of the efferent arteriole was also confirmed in the reconstructed three-dimensional images (Fig. 2B, D). In order to examine the extraglomerular distribution of the excess vessels, we analysed the three-dimensional images under low magnification. It was confirmed that these vessels branched out and anastomosed to the peritubular capillaries (Fig. 4A–E).

Discussion

In diabetic nephropathy, several small vessels with mural hyalinization are frequently recognized around the glomerular vascular hilus in addition to the usual afferent and efferent arterioles. The existence of these two arterioles which enter into and exit from the glomerulus is well established. There are some reports of the occasional occurrence of double efferent arterioles in the human glomerulus as well as in various animals (Shonyo and Mann 1944; Smith 1956; Okano et al. 1959; Moffat and Fourman 1963). However, the existence of true double arterioles of the glomerulus is quite a rare phenomenon, as revealed by scanning electron microscopic observations (Murakami et al. 1971). In the present study of diabetic nephropathy, 72% of the examined autopsy and biopsy cases have multiple small vessels around the glomerular vascular pole, which anastomose to the intraglomerular capillary network. Computer image analysis of the three-dimensional reconstructions showed that these vessels anastomose to the intraglomerular capillary of the independent lobular unit and most of them connect at a point after two or three branchings from the afferent arteriole. The opposite end of the vessels outside the glomerulus anastomoses to the peritubular capillary. It is evident that these small vessels are not duplicated efferent arterioles but vessels newly formed under the unusual condition of diabetic nephropathy. These vessels probably compensate for the decreased function of efferent arteriole as they exit from the glomerular capillaries. The increased vasculature represents a type of diabetic microangiopathy.

To date there has been no report in the literature on the analysis of these small vessels around the glomerular vascular pole in diabetic nephropathy. Because it is almost impossible to study the lesion sequentially in human cases, a definite interpretation of the pathogenesis is difficult. In the cases of diabetic nephropathy we examined, the existence of small vessels around the glomerular vascular pole was observed not only in cases with severe glomerular lesions but also in those with slight glomerular lesions. This indicates that the pathogenesis of these vascular changes is not clearly related to the severity of the glomerular lesions. These unusual vessels were not observed in cases of non-diabetic hypertension or in cases of membrano-proliferative glomerulonephritis with lobular changes.

The history of diabetes mellitus in this study ranged from 2 to 44 years, but no relationship was found between the duration of the disease and the degree of neoangiogenesis in the vascular pole area. Indeed, one case with only a 2 year history of diabetes exhibited definite neoangiogenesis. This finding suggests that the early appearance of vasomotor disturbances of the glomerular capillary network might be one of the causative factors in the neoangiogenesis. There was also a case with a 14 year history of diabetes with a mild glomerular lesion that exhibited no definite neoangiogenesis. The formation of these new vessels seems to have no relationship to the duration of diabetes but may relate to the status of the therapeutic control of diabetes.

It is well-known that diabetic retinopathy frequently shows increased vascularization and the pathogenesis is thought to be ischaemic (Ashton 1961; Wise 1961; Merin 1978). However, it is unlikely that the increase of vessels in diabetic nephropathy is due to glomerular ischaemia, since ischaemic glomerulus usually shows findings of hypoperfusion or collapse of the capillary network. On the contrary, dilatation of the afferent arteriole and the main branches of intraglomerular capillaries were frequently observed in this study; this was in contrast to the narrowing of the efferent arteriole. These findings can be interpreted as a morphological expression of intraglomerular hypertension in diabetic nephropathy. It is probable that the aetiology of neoangiogenesis in diabetic retinopathy and in diabetic nephropathy is different.

The vascular lesions in the efferent arteriole, which are at the point of exit of the glomerulus, characterize diabetic nephropathy. It is well-established that sclerosis of the efferent arteriole is a characteristic finding in diabetic nephropathy (Bell 1953). There is a report on tubular reabsorption rates in diabetic children, whose kidneys show dilatation of the afferent arteriole and contraction of the efferent arteriole, ascribed to the elevated GFR and activated intrarenal renin-angiotensin system (Ditzel and Brochner-Mortensen 1983). The sclerotic change or constriction of the efferent arteriole might result in glomerular hypertension, and a continuation of this abnormality might lead to neoangiogenesis around the vascular pole. It is possible that this phenomenon is a form of collateral circulation to maintain the normal level of blood flow in the glomerulus.

In conclusion, in the case of diabetic nephropathy, several small vessels other than the usual afferent and efferent arterioles are frequently present in the glomerular vascular pole area. The computer generated three-dimensional reconstructions of the glomerulus revealed that these vessels anastomose to the intraglomerular capillary network through the glomerular hilus, and at the distal end to the peritubular capillary. The present study suggests that these vessels have a functional role to facilitate efferent blood flow from the glomerulus.

Acknowledgements. We would like to express our deep appreciation to Mr. T. Arai, Ms. M. Kataoka, Mr. H. Matsunami and Ms. K. Wakamatsu for their excellent technical assistance in preparing the serial sections used in this study. We also like to express our gratitude to Mr. T. Iizuka of RATOC Co. Ltd. for his co-operation in generating the three-dimensional computer images.

References

Ashton N (1961) Neovascularization in ocular disease. Trans Ophthalmol Soc UK 81:145–161

Ballantyne AJ, Loewenstein A (1943) Diseases of the retina, I. Pathology of diabetic retionopathy. Trans Ophthalmol Soc UK 63:95

Bell ET (1953) Renal vascular disease in diabetes mellitus. Diabetes 2:376–389

Ditzel J, Brochner-Mortensen J (1983) Tubular resorption rates as related to elevated glomerular filtration in diabetic children. Diabetes 32 [Suppl 2]:28–33

Kimmelstiel P, Wilson C (1936) Intercapillary lesions in glomeruli of kidneys. Am J Pathol 12:83–97

Laufer A, Stein O (1959) The exudative lesion in diabetic glomerulosclerosis. Am J Clin Pathol 32:56–61

Merin S, Ber I, Ivry M (1978) Retinal ischemia (capillary nonperfusion) and retinal neovascularization in patients with diabetic retinopathy. Ophthalmologica 177:140–145

Moffat DB, Fourman J (1963) The vascular pattern of the rat kidney. J Anat 97:543–553

Murakami T, Miyoshi M, Fujita T (1971) Glomerular vessels of the rat kidney with special reference to double efferent arterioles. A scanning electron microscope study of corrosion casts. Arch Histol Jpn 33:179–198

Okano HY, Ohta T, Fujimoto T, Ohtani Y (1959) Cubical anatomy of several ducts and vessels by injection method of acrylic resin. VI. On the vascular system of the kidney in *Macacus cynomolgos*. Okajimas Fol Anat Jpn 34:27–41

Shonyo ES, Mann FC (1944) An experimental investigation of renal circulation. Arch Pathol 38:287–296

Smith JP (1956) Anatomical features of the human renal glomerular efferent vessel. J Anat 90:290–292

Spühler O, Zollinger HU (1943) Die Diabetische Glomerulosklerose. Dtsch Arch Klin Med 190:321–379

Wise GN (1961) Factors influencing retinal new vessel formation. Am J Ophthalmol 52:637–650