Structural Alterations of the Glomerular Wall and Vessels in Early Stages of Diabetes Mellitus
(Light and Transmission Electron Microscopic Study)

Awad Allah R S[1], Dkhil M A[2] and Danfour M A[3]
(1) Department of Anatomy, Faculty of Medicine, Al-Azhar University, Egypt
(2) Department of Zoology and Entomology, Faculty of Science, Helwan University, Egypt;
(3) Faculty of Medicine, 7th of October University, Misurata, Libya

Abstract
Objective: The capillary changes at the initial stage of diabetes may show an angioarchitecture clearly different from those of later stages and/or very severe glomerular change. However, the onset of alterations in the early phases is unclear. This study attempts to determine the structural alterations of the glomerular wall and vessels in the early stage of diabetes. **Material and Methods:** Twenty-five adult rats were used in this study. They were divided into two groups: the first group of five was used as a control .The second group of 20 (the experimental group) was injected intraperitoneally by a single dose of streptozotocin to induce hyperglycemia. Rats were sacrificed after ten days, two months, and four months. Five rats at two months of age with hyperglycemia were treated with insulin for eight weeks. Renal tissues were prepared by routine technique for light and transmission electron microscopic evaluation. **Results:** By light microscopy after ten days of induced hyperglycemia, there were no structural modifications detected either in renal glomerular fine vessels or in the glomerular basement membrane of the glomerular capillaries. After two months, there was a moderate glomerular enlargement and dilatation of glomerular capillaries, afferent, and efferent arterioles. After four months, glomerular basement membrane thickening was the only structural alteration observed. Recovery of the glomerular alterations was observed after two months of treatment with insulin. **Conclusion:** In early stages of diabetes mellitus in rats, there was an increase in the diameter of glomerular vessels. In later stages of the disease, the reverse was seen, but insulin treatment had a positive role in reversing these changes in the study subjects.

Key Words: *Kidney, Histology, Glomerulus, Diabetes Mellitus*

Introduction

Diabetes mellitus has become a global scourge. Diabetic end-stage renal disease (ESRD) has imposed a severe economic burden on society as well [1, 2]. Diabetic ESRD, which is frequently accompanied by retinopathy with blindness, hypertension, coronary artery disease, and peripheral gangrene with subsequent amputation, devastates the life of these patients as well as their families [2].The precise mechanisms of the diabetic vascular complications are not fully understood. However, the vascular endothelium appears to be the initial target site of injury [3-6]. Treatment of diabetes has thus far revolved around controlling hyperglycemia to prevent these complications. To that end, prospective clinical trials have determined that tight glycemic control and concomitant blood pressure control significantly reduce the risk of microvascular complications. [7,8].

Congenital diabetes has been reported in many experimental animals, i.e. mice, rats and hamsters [9]. Glomerular lesions similar to those found in human diabetes have been studied in these experimental animals [I0-2]. Glomerular oedema, expansion of the glomerular capillary lumen, and enhanced glomerular perfusion were reported to be the major symptoms during the initial stage of the disease [12-15].

Glomerulosclerosis, diffuse sclerosis of mesangium, thickening of basal laminae, and proliferation of glomerular cells can all be observed in young KK mice (two to four months old) [15,16]. Abnormal glucose tolerance begins at four months of age in these animals [10]. Few detailed studies, however, have been directed to the initial alteration in capillaries in diabetic glomerular changes. The capillary changes at the initial stage may show an angioarchitecture clearly different from those at later stages and/or that which is noted in very severe glomerular changes. However, the onset of alterations at the early phases of diabetes is unclear. This study attempts to determine the functional and structural alterations of the glomerular wall and vessels in the early stages of diabetes.

Materials and Methods

Animals

Twenty five male adult albino rats (ten weeks old and 250-280 g body weight) were used. They were divided into two groups: the first group (five rats), with tail blood sugar level (130-160 mg %), was used as the control group to study the normal size and shape of the glomerulus and glomerular vessels. Twenty rats comprising the second group (20 rats) were injected intraperitoneally by a single dose of 55 mg streptozotocin/Kg of body weight as described previously [11,17]. Twenty four hours later, induction of diabetes was confirmed by measuring plasma glucose levels (above 400 mg%) using an Accu-check advance meter (Roche Diagnostics, Indianapolis, IN). Animals were divided into four subgroups, each with five rats. The first three subgroups were sacrificed by cervical dislocation after ten days, two months,

and four months. The last subgroup (two months of age) of hyperglycemic rats was treated three times a week with a low dose (1 - 1.5 IU) of insulin (Ultralente; Novo, Copenhagen, Denmark) for two months to evaluate reversibility of the glomerular alteration as described previously [3]. Animals were fed a standard commercial diet. The experiments were approved by the state authorities and followed Egyptian law on animal protection.

Histological studies

For light microscopy: small pieces of the kidney were quickly removed, then fixed in Carnoy's fixative fluid. Following fixation, specimens were dehydrated, embedded, and then sectioned to five microns thickness. For histological examinations, sections were stained with Ehrlich Haematoxylin and Eosin [18].

For transmission electron microscopy: renal tissues were fixed in 2.5% glutaraldehyde at 4 °C for two hours, rinsed with cacodylate buffer solution and post fixed in 2% osmium tetroxide. The specimens were dehydrated in graded concentrations of ethanol and embedded in epoxy resin. Ultra-thin sections stained with uranylacetat, lead citrate, and tannic acid were examined by light and transmission electron microscope (T.E.M)

Result

Light microscopy: After ten days of streptozotocin injection, kidney sections of hyperglycemic rats had mild enlargement of glomeruli and glomerular capillaries (Fig. 2) with no structural modification detected either in renal glomerular fine vessels or basement membrane in comparison to the control group (Fig. 1), which allowed us to conclude these rats suffered early disease effect.

Examination of kidney sections of hyperglycemic rats after two months of streptozotocin injection showed a moderate enlargement of glomeruli, dilatation of glomerular capillaries, and afferent and efferent arterioles (Fig. 3) with no structural modification either in renal glomerular fine vessels or in basement membrane in comparison to the control group (Fig. 1). So, this was determined to be the intermediate disease effect. After four months (late disease effect) hyperglycemic rats' glomerular basement membrane thickening was the only structural alteration observed (Fig. 4). Recovery of the glomerular alteration (glomerular enlargement, vessels dilatation and thickening of basement membrane) was observed after two months of insulin treatment. (Fig. 5).

Transmission electron microscopy: In hyperglycemic rats of ten days and two months of age , gradual enlargement of glomeruli and dilatation of glomerular capillaries were found (Fig. 6). Structural modification in the form of glomerular basement membrane thickening was observed in rat kidneys after four months of hyperglycemia (Fig. 7). Recovery of the glomerular alteration (glomerular enlargement, vessel dilatation and thickening of basement membrane) were observed after two months treatment with insulin for rats who had two months duration of diabetes mellitus (Fig. 8).

Discussion

The present study provided a clear demonstration of the increase in size of the glomerulus and dilatation of the glomerular fine vessels in rats with early diabetes. These findings have confirmed previous histological studies [15]. Diameters of the afferent arteriole, glomerular capillary and efferent arteriole were greater in the early diabetic animals than in the control groups.

An elevated GFR (glomerular filtration rate) is a well established feature in early diabetes in humans [13] as well as in rats [19].The mechanism of this elevated GFR is activated by an enhanced RPF (renal plasma flow), an increased transglomerular hydrostatic pressure gradient and an increased glomerular ultrafiltration coefficient [14]. The GFR determinants are closely related to the hemodynamics of the glomeruli.

Although we cannot directly determine the functional significance of the GFR determinants, it is reasonable to assume that an increase in the diameter of glomerular vessels may affect the kidney function in early diabetes. In support of this concept, Poiseuille's Law [20] states that when the diameter of a vessel increases by 10 to 20 %, blood flow in the vessel increases by 20 to 40%. Interestingly, this 46 to 200% blood flow increase in the kidney has been reported in humans with early diabetes compared with non-diabetic control groups [15]. The increased renal blood flow may be the factor behind the enhanced RPF and increased glomerular ultrafiltration coefficient ultimately affecting the GFR. It is also well known that parallel reductions in afferent and efferent resistances induce a blood flow increase in early diabetes. The present study demonstrates an increase in diameters of the afferent and efferent arterioles in diabetic rats. This increase in diameter of arterioles may allow increased blood flow into the glomerulus, resulting in an increased RPF.

The present study has clearly shown enlargement of the capillary lumen, which represents an expansion of the surface area available for filtration. Since the ultrafiltration coefficient is determined by the fluid permeability of the glomerular capillary and the area of its surface available for filtration [13], the enhanced ultrafiltration characteristic of early diabetes may well be caused by the enlargement of glomerular capillaries. Furthermore, an increase in the caliber of the afferent arterioles has been shown in the present study. Dilation of the afferent arteriole increases the glomerular pressure, with a corresponding increase in glomerular filtration rate [13].

It was reported that the streptozotocin-diabetic mouse model is characterized by both growth hormone (GH) hypersecretion and glomerular growth in early experimental diabetes in mice [21]. Although segmental thickening of the basement membrane and proliferation of cells has been observed in diabetic KK mice from two to four months old [12,16], we could find in the present study that these glomerular changes occur in four month old diabetic rats. However, our findings regarding the dilation of capillaries in the glomeruli of diabetic rats are consistent with the findings of other authors [12]. These glomerular changes in rats increase in severity with age and are similar to those observed in human diabetes. The typical nodular glomerular sclerosis seen in humans does not develop in rats but severe sclerosis of arterioles develops in older diabetic mice more severely than in humans [22]. The striking glomerular vascular changes we observed in young diabetic rats beara certain similarity to those described in early-onset human diabetes. The enlargement of glomerular fine vessels may be considered a common feature. In the late and/or severe diabetic, an increase of mesangium and thickening of basement membranes encapsulating glomerular capillaries was found in the present study and was consistent with other findings [11,22,23]. These mesangial and basement membrane lesions cause a distortion and narrowing of glomerular capillaries that leads to a decreased GFR [13]. The fine vascular configuration of the glomerulus in later stages appears to be quite different from that seen at earlier stages of diabetes.

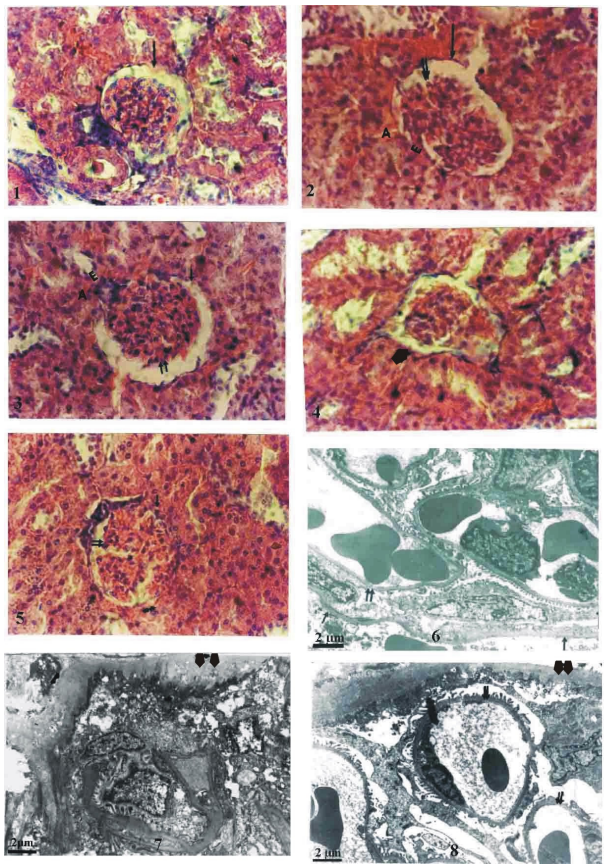

Figure (1): Photomicrograph of glomerulus (arrow) of control non-diabetic rat. H&E stain. X 250.

Figure (2): Photomicrograph of glomerulus of ten days old hyperglycemic rat showing enlargement of the glomerulus (arrow) and dilated capillaries (double arrows), afferent (A) and efferent (E) arterioles H&E stain. X 250.

Figure (3): Photomicrograph of glomerulus of two months old hyperglycemic rat showing more enlargement of the glomerulus (arrow) and more dilated capillaries (double arrows), afferent (A) and efferent (E) arterioles H&E stain. X 250.

Figure (4): Photomicrograph of glomerulus of four months old hyperglycemic rat showing glomerular basement membrane thickening (arrowhead) beside glomerular enlargement and vessels dilation. H&E stain. X 250.

Figure (5): Photomicrograph of glomerulus after two months of insulin treatment of rat with two months diabetes showing recovery of glomerular enlargement (arrow), vessels dilation (double arrow) and structural alteration of basement membrane (arrow) nearly to the normal. H&E stain. X 250.

Figure (6): Transmission electron micrograph of glomerulus of two months old hyperglycemic rat showing enlargement of glomerulus (arrow) and dilatation of glomerular capillaries (double arrow). X 4000.

Figure (7): Transmission electron micrograph of glomerulus of four months old hyperglycemic rat showing thickening of basement membrane (arrowheads), beside to the enlarged glomerulus. X 4000.

Figure (8): Transmission electron micrograph of glomerulus of two months insulin treatment for rat having diabetes for two months duration showing recovery of glomerular enlargement, vessels dilatation (double arrows) and decreased alteration of basement membrane thickening. X 4000.

Conclusion

Results from our study indicated that in early stages of diabetes, there was a moderate enlargement of glomeruli and dilatation of glomerular vessels. In late stages of diabetes, the glomerular walls were thickened. Insulin treatment has a good role for reversing these changes but further studies may be required to observe long-term vascular changes and the exact pathogenesis of diabetic nephropathy.

References

1- Jacob van den Born, Brenda Pisa, Marinka AH Bakker, Johanna WA M Celie, Carin Straatman, Steven Thomas, Gian C Viberti, Lena Kjellen, and Jo HM Berden. No change in glomerular heparin sulfate structure in early human and experimental diabetic nephropathy. Journal of Biological Chemistry 2006; 281: 29606–29613.

2- Tapia Ceballos L. Metabolic syndrome in childhood. An Pediatr (Barc) 2007; 66(2):159-66.

3- Curcio A, Ceriello A. Decreased cultured endothelial cell proliferation in high glucose medium is reversed by antioxidants: New insights on the pathophysiologic mechanisms of diabetic vascular complications. In vitro cell Dev BIOL 2003; 28A: 787-790.

4- Kamoi K, Ishibashi M and Yamaji T. Endothelin - 1 and big endothelin - 1 in NIDDM patients with and without microangiopathy. Diabetes Res Clin Pract 1994; 24:125-129.

5- Sank A, VVel D and Reid J. Human endothelial cells are defective in diabetic vascular disease. J Surg Res 1994; 57:647-653.

6- Katsuo K, Masao H, Takayuki M and Tsuneo K. Acetylcholine-induced vasodilation in the perfused kidney of the streptozotocin-induced diabetic rat: role of prostacyclin J. Smooth Muscle Res 2006; 42 (5): 159–170.

7- Prospective Diabetes Study Group UK (A). Tight blood pressure control and risk of macrovascular and microvascular complications in type 2 diabetes: UKPDS 1998; 38. Br Med. 2006 J 317:703-713.

8- Prospective Diabetics Study Group UK (B). Intensive blood - glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 38). Lancet 1998; 352:837-853.

9- Mitruka, B.M., Rawnsley, HM and vadehra DV Models for study of human disease. Jon Willy & Sons. New York. 1976.

10- Shibata M, Yasuda B And Kobayashi K (1973). Diabetes 16-5:412-416.

11- Treser G, Oppermann W, Ehrenreich T, Lange K, Levine R And Camerini-Davalos R (1978). Proc. Soci. Exp. Biol. Med 1978; 129:820-823.

12- Wehner H, Hohn D, Faix-Schade UH and Walzer P. Lab. Invest. 2001; 26: 331- 340. cited by Hiroyshi, et al. (1997)

13- Christiansen J.S., Gammelgaard J., Frandsen M. And Parving H. Diabetologia 1981; 20:451-456.

14- Mogensen CE. And Andersed M. Diabetes 2002; 22-9:706-712.

15- Oka Y. Diabetes, (Akasaka, A, ed.), Medical review, Tokyo (in Japanese). 1987; PP, 164-169. Cited by Hiroyshi, et al. 1997.

16- Wehner, H., Hohn, D., Faix-Schade, U., Huber, H, Walzer, P. Adv. Exp. Med. Bio. 2003; 246: 147-156. Cited by Hiroyshi, et al.,

17-Van den Born J, Van Kraats AA, Bakker MA, Assmann KJM, Dijkman HB, Van der Laak JA, and Berden JHM. Diabetologia 1995; 38, 1169–1175.

18- Adam H, Caihak G. Grosses zo¬ologisches parktikum tell. Arbeits¬methoden der makroskopischen und mikroskopischen anatomic Mit 283 Ab¬bildungen Gustav. Fischer Verlag Stutt¬gart. 1964.

19- Hostetter TH, Troy JL. And Brenner BM. Elevated GFR is a well established feature in early diabetic rats. Kidney International 1981; 19:410-415.

20- Ruch TG and Patton HD. Physiology and Biophysics. BW Saunders, Philadelphia. P; 1965; 527.

21- Gronbaek H, Nielsen B, Schrijvers B and Vogel I. Inhibitory effects of octreotide on renal and glomerular growth in early experimental diabetes in mice. J. Endocrinol. 2002; 172: 637-43.

22- Doucet M, London I, Gomez A and Bendayan M. Glomerular basement membrane selective permeability in short-term streptozotocin-induced diabetic rats. Int. J. Exp. Diabetes-Res 2000;1:19-30.

23- Isogai S, Mogami K, Shiina N and Yoshino C. Initial ultrastructural changes in pore size and anionic sites of the glomerular basement membrane in streptozotocin-induced diabetic rats and their prevention by insulin treatment. Nephron 1999; 83: 53-8.