Diabetic Nephropathy in KK and KK-A' Mice

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Abstract: KK mice and KK-A' mice were examined for age related changes in blood and urinary biophysiological parameters. Blood hemoglobin A\textsubscript{1c} levels were significantly higher in KK-A' and KK mice as compared to non-diabetic ddY mice. In both diabetic mice, especially KK-A' mice, plasma insulin levels markedly increased at 2 to 4 months of age, and the urinary glucose and microalbumin levels and albumin-to-creatinine ratios increased dependent on age. Plasma thrombomodulin levels significantly increased at 2 to 4 months of age in both KK and KK-A' mice. Mild enlargement of mesangial matrix and segmental proliferative glomerular nephritis were revealed in KK and KK-A' mice, respectively, at 4 months of age. KK-A' mice with insulin resistance and high urine mAlb level might be useful as models for the early stage of diabetic nephropathy.

Key words: diabetic nephropathy, KK mice, KK-A' mice

Diabetic nephropathy is a major complication of diabetes mellitus with high morbidity and mortality [4], and high plasma glucose concentration predicts fatal cardiovascular disorders and diabetic nephropathy in Pima Indians with type II diabetes, non-insulin-diabetes mellitus (NIDDM) [14]. The predictive diagnosis is essential for decreasing premature morbidity and mortality, and blood hemoglobin A\textsubscript{1c} (HbA\textsubscript{1c}) level is very useful in the diagnosis of diabetic mellitus, since it correlates with the plasma glucose level, and plasma thrombomodulin (TM) levels which increase with damage to the endothelial cells [3, 16]. The urine microalbumin (mAlb) level is indicative of early and mild states of kidney injury [7]. These markers are important for diabetes mellitus with nephropathy. Animal models for diabetic nephropathy are required for analyzing the usefulness of HbA\textsubscript{1c}, mAlb and TM in the diagnosis of diabetic nephropathy. Experimental models of type II diabetes are divided into naturally occurring models and chemically induced ones. The KK mouse is an inbred mouse strain established from Japanese native mice [11], and Fujita et al. [2] reported that the KK-A' mice produced by the transfer of the yellow obese gene (A') into KK mice were obese-diabetic showing hyperglycemia, hypertriglycerideremia and hyperinsulinemia. However, the plasma TM and urine mAlb levels in these diabetic mice remain unknown. The present study aimed to observe the age related changes in blood and urinary biophysiological parameters as well as the renal pathology of KK and KK-A' mice and compare them with those in non-diabetic ddY mice.

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Specific pathogen-free 6-week-old male KK and KK-A<sup>v</sup> mice were purchased from CLEA, Inc., Tokyo, and male ddY mice 6 weeks of age were bought from Japan SLC, Inc., Shizuoka. Each animal was housed in an individual cage (16 × 12 × 12 cm) and given ad libitum pellets (MR standard; Nihon Nosan Ind. Ltd., Kanagawa) and tap water. The animals were maintained on a 12-hr light-dark cycle at a room temperature of 21 ± 1°C. This study design was approved by the Showa University Animal Care and Use Committee and followed the Showa University Guideline for Animal Experimentation. At 2, 3 and 4 months of age, blood was collected between 10:00 a.m. to 12:00 p.m. and urine samples were collected from 9:00 a.m. to 4:00 p.m. Blood specimens were taken from the inferior vena cava using a plastic syringe and a silicon-coated needle under pentobarbital anesthesia. Plasma and urinary glucose levels were determined using commercially available diagnostic kits (Wako Pure Chemical Industries, Ltd., Osaka). Plasma TM and HbA<sub>1c</sub> were determined using an enzyme-linked immunosorbent assay (ELISA) (TM test F, International Reagents Co., Kobe) and HbA<sub>1c</sub> immunoassay (DCA2000 system, Bayer Diagnostics, Elkhart, U.S.A) [8], respectively. Plasma insulin levels were determined by ELISA (Shibayagi, Wako Pure Chemical Industries, Ltd., Osaka). Urine was centrifuged at 1000 r.p.m. for 5 min, and the supernatant was examined for the mAlb level and albumin-to-creatinine (A/C) ratio using an immunoassay (DCA-2000 system). The mean values ± S.E.M. from 10 to 12 mice per group were analyzed using Student’s t-test, and statistical significance was noted at the p<0.01 and p<0.05 levels in comparison with ddY or KK mice. For histopathology, kidneys from 6 mice per each strain at 2, 3 and 4 months of age were fixed in 10% buffered formalin, and tissues were embedded in paraffin and stained with hematoxylin and eosin (HE) and periodic acid Schiff (PAS) stain.

As shown in Fig. 1, the body weight of ddY, KK and KK-A<sup>v</sup> mice increased with the duration of feeding and food (g/day), and water intake (ml/day) of the KK-A<sup>v</sup> mice was significantly greater than age-matched ddY and KK mice. As shown in Fig. 2, plasma glucose and HbA<sub>1c</sub> levels in KK and KK-A<sup>v</sup> mice were significantly higher than age-matched ddY mice. The glucose level was markedly increased in KK-A<sup>v</sup> mice from 3 to 4 months of age, and the HbA<sub>1c</sub> level was also significantly higher in KK and KK-A<sup>v</sup> mice than in ddY mice. As presented in Fig. 3, urinary glucose tended to increase in KK mice at 3 to 4 months of age, when KK-A<sup>v</sup> mice showed a marked increase. The urinary mAlb level and A/C ratio also increased depending on age in both KK and KK-A<sup>v</sup> more remarkably than in ddY mice. Using multiple regression analysis, a significant correlation was observed between urinary mAlb levels and urinary A/C ratios (y=4.368 x –25.646, r=0.9403). In ddY, KK and KK-A<sup>v</sup> mice, both urinary mAlb and A/C ratios were significantly correlated with plasma HbA<sub>1c</sub> levels, as shown in Fig. 4. Although the body weight gain was similar among ddY, KK and KK-A<sup>v</sup> mouse,
plasma insulin level was markedly lower in the ddY mice than in the KK and KK-A' mice, as shown in Fig. 5. The TM levels of the KK and KK-A' mice were 2 to 3 times higher than those of the ddY mice.

Minimal changes were observed in the glomerular mesangium in the 3-month-old KK mice, and enlargement of mesangial matrix was more apparent at 4 months of age (Fig. 6-A, B). KK-A' mice showed apparent segmental proliferative glomerular nephritis at 4 months of age (Fig. 6-C, D).

The KK mouse is considered a polygenic model for human diabetes mellitus type II [15], and the KK-A' mouse has been used for studies on a novel oral anti-diabetic agent [6, 19]. In the present study, age related changes in physiological and biochemical parameters were observed in the blood and urine from KK and KK-A' mice, and both mice were shown to be useful models for diabetic nephropathy.

Blood HbA1c correlating with plasma glucose concentration has been shown to be an important marker of diabetic mellitus [9], and the present study revealed that blood HbA1c levels were significantly correlated
with urine mAlb levels and/or A/C ratio, as well as with pathological changes in the kidney in KK and KK-\(A\)' mice at 3 to 4 months of age. Microalbuminuria is also an important marker for vascular disease, and is used to detect the early phase of renal diseases. NIDDM patients with microalbuminuria are highly predisposed to cardiovascular disease [13], and persistent microalbuminuria is believed to be a major predictor of diabetic nephropathy in cases with insulin-dependent diabetes mellitus (IDDM) [1].

Patients with thrombocytopenic purpura and disseminated intravascular coagulation, show a significant elevation in plasma TM [18]. Nakano et al. [8] reported increased levels of serum TM in streptozotocin induced diabetic rats reflecting endothelial injury. In the present study, since the plasma TM level increased in KK and KK-\(A\)' mice at 2 to 4 months of age more remarkably than in ddY mice, both diabetic mice were also suggested to suffer from endothelial injury. Volkman and Wehner [17] reported that in KK mice
the dilatation of small intra-renal arteries and arterioles might be the result of progressive impairment of vaso-constriction causing glomerular hyperfiltration in diabetes. Reddi et al. [12] also reported mesangial matrix accumulation in KK mice. Scanning electron microscopy showed vascular changes and enlarged glomeruli without capillary proliferation or distortion in diabetic 4-month-old KK-A' mice [10].

In the present study, KK and KK-A' mice showed mild or moderate enlargement of the glomerular mesangial regions, and KK-A' mice showed more significantly increased levels of plasma insulin and urinary mAlb indicating it is a better model for early diabetic nephropathy than KK mice.

References


