Histol Histopathol (2020) 35: 279-287

http://www.hh.um.es

Histology and Histopathology

From Cell Biology to Tissue Engineering

Distribution and microstructure of intrarenal arteries in Bactrian camels (*Camelus bactrianus*)

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Summary. Studies and reports focusing on the Bactrian camels' kidney structure from an anatomical perspective are scanty, therefore, this work aims to systemically investigate the anatomical structure of the kidney and examine the distribution and microstructure of intrarenal arteries. Ten pairs of healthy adult kidneys from male and female Bactrian camels were used in the study. The kidney of Bactrian camel appeared like a broad bean with a smooth surface. Using artery casting, we observed that the renal artery divided into dorsal and ventral branches; the dorsal branch continuously divided into a shorter anterior branch and a longer posterior branch, while the ventral branch directly divided into interlobar arteries. The number of interlobar arteries in the left and right kidneys were slightly different, 14 to 16 in left while 16 in the right kidney. No anastomosis was found between the dorsal and ventral branches or their sub-branches. To further study the microscopic structure, microanatomy and scanning microscope were used. Surprisingly, we observed two other ways afferent arteriole arose apart from the interlobular artery. They were the arcuate artery and conjoint afferent arteriole. Two afferent arterioles supplied one glomerulus and occasionally the absence of glomerulus was also observed, where the arteriole kept extending, and no typical glomerulus formed. Since branching of arteries and urologic function of kidneys are physiologically integrated, these features of Bactrian camel may help to further investigate their adaptations to desert climate.

Key words: Kidney, Distribution, Microstructure, Bactrian camel, Artery

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DOI: 10.14670/HH-18-166

Introduction

The Bactrian camel is one of the most important livestock in northwestern and northern China, which lives mainly in desert and semi-desert climates. Their characteristic tolerance to extreme temperature differences and drought makes them the most adapted mammals to the desert and they are colloquially known as the boat of the desert on the Silk Road (Hasi et al., 2018). The Bactrian camels' adaptation manifests not only in their contour and physique, but also closely relates to their different organ structures and idiosyncratic physiologic function (Wang and Cuisheng, 1998; Wang et al., 2000b; Laursen et al., 2016).

The kidney is the main organ of the urinary system, and it plays an important role in metabolism and ion balance, and its function is inseparable from the blood vessels. Nephron is the structural and functional unit of urine formation, when blood flows through the renal glomerulus of the nephron, due to the pressure inside capillaries and filtration membranes, the renal corpuscle forms original urine by filtration, and it flows through the renal tubule to the collecting tubule, the original urine is reabsorbed and the final urine is formed

(Bissinger, 1995).

The final urine concentration of different animals is quite different. Generally, animals living in humid or water-rich areas produce more and diluted (hypotonic) urine, whereas animals living in drought areas or marine high-salt environments produce less but highly concentrated (hypertonic) urine. These differences are closely related to the structural characteristics of the kidney (Mbassa, 1988; Beuchat, 1996).

There are some reports on Bactrian camel anatomic structures in relation to their adaptations (Wang and Cuisheng, 1998; Wang et al., 2000a,b; Cui et al., 2004), however, these reports rarely emphasize the anatomic structure of the kidney, and the studies about kidney mostly focus on the structures of the renal pelvis only (Sheng and Yi, 2002; Chen and Liu, 2003), with scanty data on the distribution of arteries in kidney of Bactrian camels.

The distribution of renal artery and renal circulation has unique anatomical and functional significance, just as the formation and concentration of urine have a close relationship with the renal circulation. Taking all these into consideration, this present work aimed at studying the structure of the kidney and the arterial system, from the gross and microscopic perspective, to provide some useful basic data for future studies on Bactrian camel's adaptation.

Materials and methods

Animals and tissue collection

Ten healthy Bactrian camels (5-12 years old) were enrolled for the study, 6 males and 4 females, with no apparent diseases. All Bactrian camels used in the study were sacrificed via exsanguination at the local slaughterhouse in Inner Mongolia, China, and 10 pairs of fresh kidneys were collected for use.

All procedures and protocols involving the use of animals were in accordance with the Animal Ethics Procedures and Guidelines of the People's Republic of China, and the study was approved by the Institutional Animal Care and Use Committee (IACUC) (No. GSAUAEC-2015-007) of the College of Veterinary Medicine, Gansu Agricultural University.

Gross anatomy and vessel casting

Five fresh kidneys were used for gross anatomy, 2 from females and 3 from male Bactrian camels. The kidneys were cut from the median sagittal plane in order to observe their structures.

For the purpose of demonstrating the distribution and extent of arteries in the kidney, 6 kidneys were used for vessel casting, 2 from females and 4 from males. Prior to vessel casting, 5% red acrylonitrile butadiene styrene (ABS)-acetone containing 5 g acrylonitrile butadiene styrene resin (ABS), 50 ml acetone, 50 ml butanone and appropriate amounts of Sudan III were prepared for usage. In addition, 10% and 15% red ABS-acetone containing 10g and 15g ABS respectively were also prepared. The compounds were agitated continuously for 3-4 d on a shaker until the ABS dissolved completely. A blunt needle was connected to a rubber tube at one end and the other end of the rubber tube connected to a 20 mL syringe. The whole needle was then inserted into the renal artery, and the 5% red ABS-acetone injected into the renal artery under high pressure, when the artery was filled and no compound could enter any longer, the rubber tube was tightened with a cotton cord to prevent leakage, the set-up was left to stand for 1-2 hours, to enable an even distribution of the solution deep within the arteries and arterioles, after this, 10% and 15% red ABS-acetone were injected in a sequential order, and all other steps following the addition of 5% red ABS-acetone were repeated. After injecting the 15% red ABS-acetone, the set-up was quickly put under running water for at least 24 h so the compound would solidify to make the kidney hard. The 5% red ABS-acetone easily entered the microvasculature, whereas the addition of the 10% and 15% red ABS-acetone compound ensured that the blood vessels were completely full. After hardening, the kidney was corroded in 25% HCL for 5-6 d. The corroded kidney was then put into a basin filled with water, washed with running water, and stereoscopic microscope was used to observe the rinsed kidney to examine if it was clean.

Scanning microscope

Nine kidneys were used for scanning microscopy works, 4 from females and 5 from male Bactrian camels. The protocol used was similar to that of vessel casting, however, just 5% red ABS-acetone was injected. After hardening in running water, the sample was allowed to freeze for 10 h. Different parts of frozen kidney were chosen and cut to 1.0 cm^3 cubes according to standard procedure. After 3-4 days corrosion and cleansing by running water, the samples with dense blood vessels and clear branches were coated with gold by ion-plating machine (Eiko-Ib-3) upon dehydration with alcohol. Samples were then prepared for observation and photography using low vacuum scanning electron microscope (SEM) (TSM-5600LV).

Measurement and statistics

Measurements were taken for afferent arteriole, glomerulus, and efferent arteriole that were observed to have intact structures. Care was taken to avoid any misidentification of the afferent and efferent arterioles, thus the incomplete structures that were destroyed by our operations or covered by other structures were not measured and counted. The measurement of afferent arteriole, glomerulus and efferent arteriole were all performed by SEM.

Results

Anatomic structure of the kidney

The kidney of Bactrian camel showed a broad bean shape with a smooth surface (Fig. 1A). The renal medulla was much thicker than the cortex (Table 1) and the medullary/cortical thickness ratio was about 4:1, the boundary between them was clear. On the median sagittal plane, about 10 conical renal pyramids were visible and while their boundaries were unclear, these pyramids converged to a renal crista which was concave and parallel to the cortex (Fig. 1B). With detailed observation, the medullary ray was obvious at cut surface and extended into the cortex (Fig. 1C). The renal pyramids were separated from each other by branches of renal pelvis at 0.5 cm away from the median sagittal plane, where the pseudopapillae formed, after blunt stripping the renal crista, the funnel-shaped renal pelvis and its fan-shaped branches could be observed, the spaces between these branches were the renal recesses. The renal pelvis narrowed to form the ureter (Fig. 1D). The pattern of the renal recesses is presented in Fig. 1E.

Branches of artery

The renal artery was separated from the abdominal aorta and divided into dorsal and ventral branches at 4-8

Table 1. The thickness of cortex and medulla.

Region	Maximum diameter	Minimum diameter	Average diameter
Cortex	1.6	1.1	1.3
Medulla	4.9	4	4.5

Note: 22 positions of 5 kidneys were selected for measuring the thickness of cortex and medulla. Unit: cm.

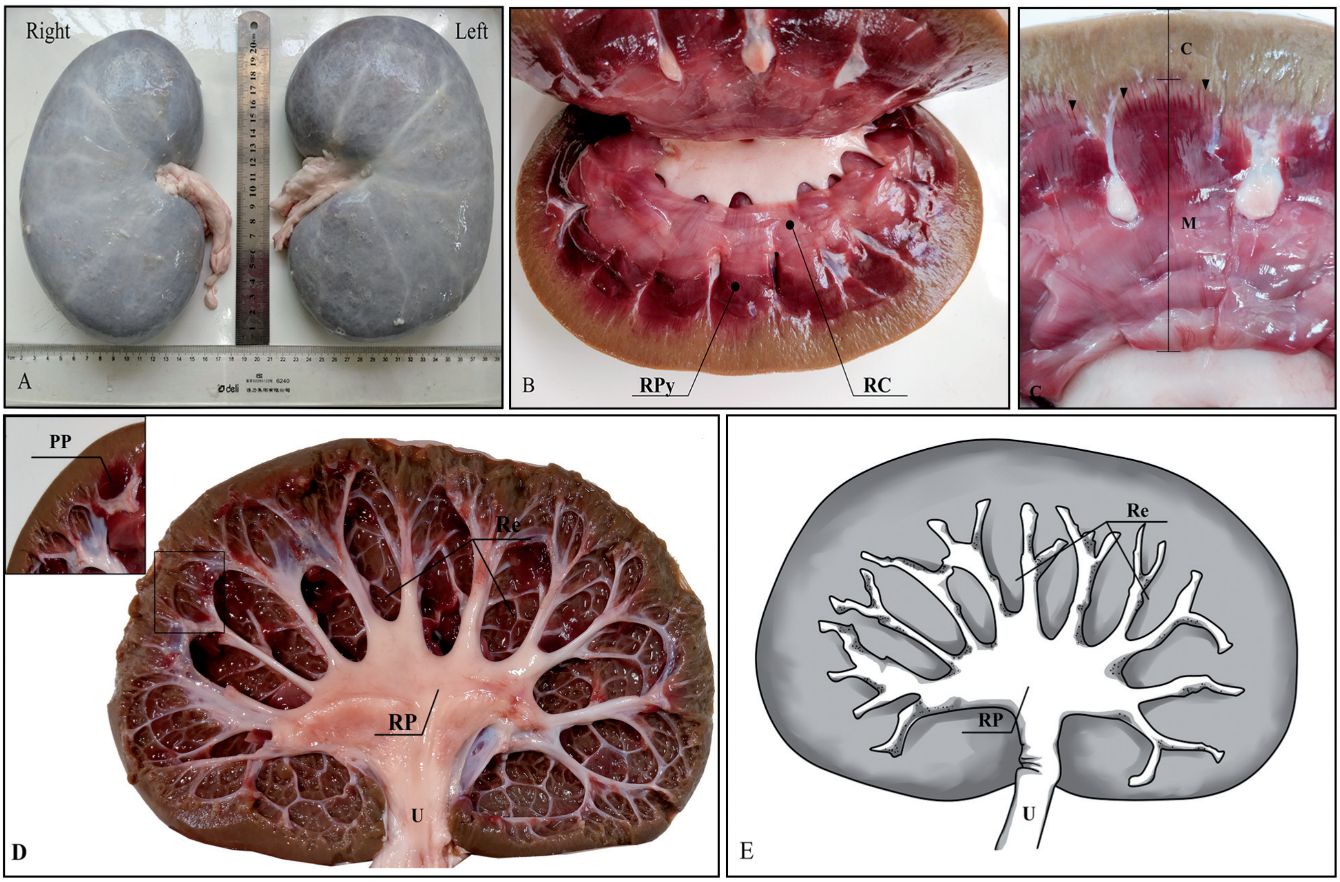

Fig. 1. Anatomy structure of Bactrian camels' kidney. **A.** Appearance the kidney. The kidney has a broad bean shape with smooth surface; the left kidney is a little bigger. **B.** Median sagittal plane of kidney, renal pyramids with unclear boundary and dark red color are visible, these pyramids converge to renal crista and the color is lighter than the pyramid. **C.** Renal medullary ray extends to renal cortex, and the ratio of renal cortex to renal medulla is about 1:4. **D.** The renal pelvis and recesses. 0.5 cm away from the median sagittal plane, the renal pelvis branches to a fan-shaped structure, the spaces between the branches are the renal recesses, at the end of the branch it forms a structure in the shape of a crescent that separates renal pyramids from each other and pseudopapillae form. **E.** Pattern of renal recesses. RPy: Renal pyramids; RC: Renal crista; C: Renal cortex; M: Renal medulla; RP: Renal pelvis; U: Ureter; Re: Recesses; PP: pseudopapillae; Arrowhead: Renal medullary ray.

cm away from the renal hilum; they extended forward to the renal parenchyma and kept branching. (Fig. 2A). No anastomosis was found between the dorsal and ventral branches or their sub-branches (Fig. 2B). The dorsal branch divided into a shorter anterior branch and a longer posterior branch in both left and right kidneys. The anterior and posterior branch appeared to continuously branch to form the interlobar artery, but the number of interlobar arteries was different in the left and right kidneys; there were 6 to 8 in the left while there were 8 in the right kidney (Fig. 2C). Unlike the dorsal branch, the ventral branch directly divided to 8 interlobar arteries in the left and right kidneys with no anterior and posterior branches. As branching continued, the arcuate artery divided, appeared slanted, and not parallel to the surface of the kidney. Interlobular artery shot off from the arcuate artery at an acute angle and appeared smooth and orderly (Fig. 2D), it passed through the renal cortex and shot out the afferent arteriole.

Afferent arteriole

The afferent arteriole extended forward in a spiral manner with lots of narrowing toroidal rings seen on the surface (Fig. 3A). Through measurements and statistics, it was found that the diameter and length of afferent arterioles changed significantly in different zones of the renal cortex. At the superficial zone, the terminal of the interlobular artery extended to the afferent arteriole directly and resulted in an afferent arteriole with the shortest length, having an average diameter of 42.8 μm (range: 36 μm ~71 μm). There was more stenosis compared to the other zones (Fig. 3B). At the deep zone, afferent arteriole separated from interlobular artery at a right angle or an acute angle, with the length being a

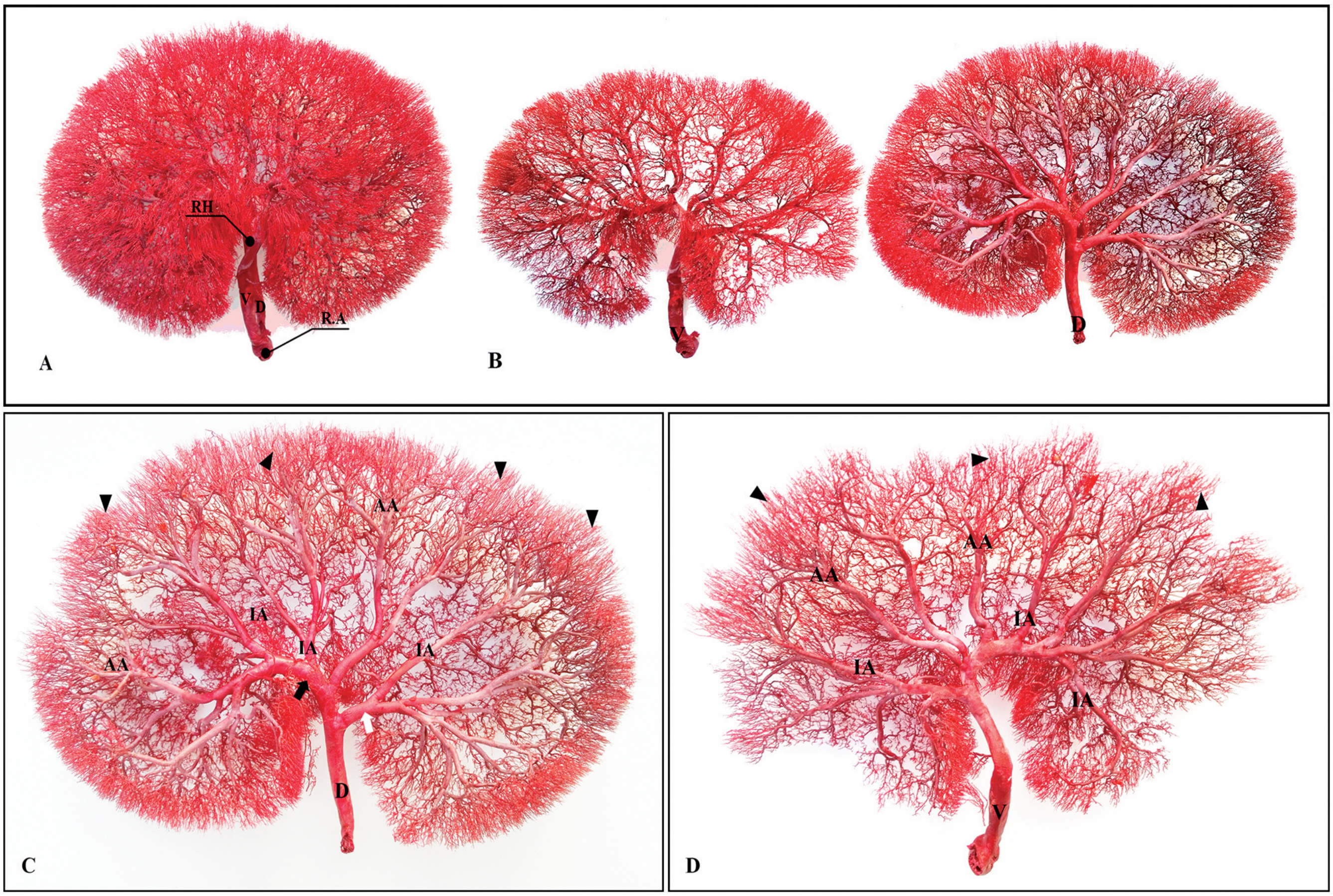

Fig. 2. Artery distribution of Bactrian camels' left kidney through renal artery vasculature casting, illustrating the branching patterns of the renal artery. **A.** The renal artery divides into dorsal branch and ventral branch at a place 4-8cm away from the renal hilum, they branch forward to the renal parenchyma, and the arteries of kidneys are sparsely internal and densely surfaced. **B.** Separate from dorsal branch and ventral branch, the kidney artery can be divided into two unbroken parts. **C.** Branches of dorsal branch, it divided to anterior branch and posterior branch first, and progressively branches to interlobar artery, arcuate artery and interlobular artery. **D.** The ventral branch divides into the interlobar artery directly, and then branches to arcuate artery and interlobular artery. R.A: Renal artery; D: Dorsal branch; V: Ventral branch; RH: Renal hilum; White arrow: Anterior branch; Black arrow: Posterior branch; IA: interlobar artery; AA: Arcuate artery; Arrowhead: Interlobular artery.

little longer than in the superficial zone (Fig. 3C) and the diameter was 46.7 μm (range: 33-65 μm). The afferent arteriole had the longest length and the largest diameter (54.0 μm) at the juxtamedullary zone. The diameter ranged from 37-66 μm (Fig. 3D, Table 2).

In our study, the number of afferent arterioles in glomerulus was not consistent, most of them had just one afferent arteriole, but occasionally two were also observed to supply a glomerulus (Fig. 4A). We also found that apart from the interlobular artery, arcuate artery could also give rise to afferent arteriole (Fig. 4B). An afferent arteriole divided into two branches before supplying the glomerulus, one branch supplied a glomerulus and the other extended forward to supply a different glomerulus (Fig. 4C). This means some of the afferent arterioles arose from other afferent arterioles that connected them, rather than from the interlobular artery or arcuate artery. In other words, we found three ways that afferent arteriole arose in Bactrian camels. In addition to interlobular artery which is well known, afferent arteriole could also arise from the arcuate artery, and a conjoint afferent arteriole.

Table 2. The diameter contrasts of afferent arteriole.

Region	Afferent arteriole		
	Maximum diameter	Minimum diameter	Average diameter
Superficial zone	71	36	42.8
Deep zone	65	33	46.7
Juxtamedullary zone	66	37	54.0

Note: 100 Bactrian camel afferent arterioles were observed, measured and quantitatively analysed. Unit: μm.

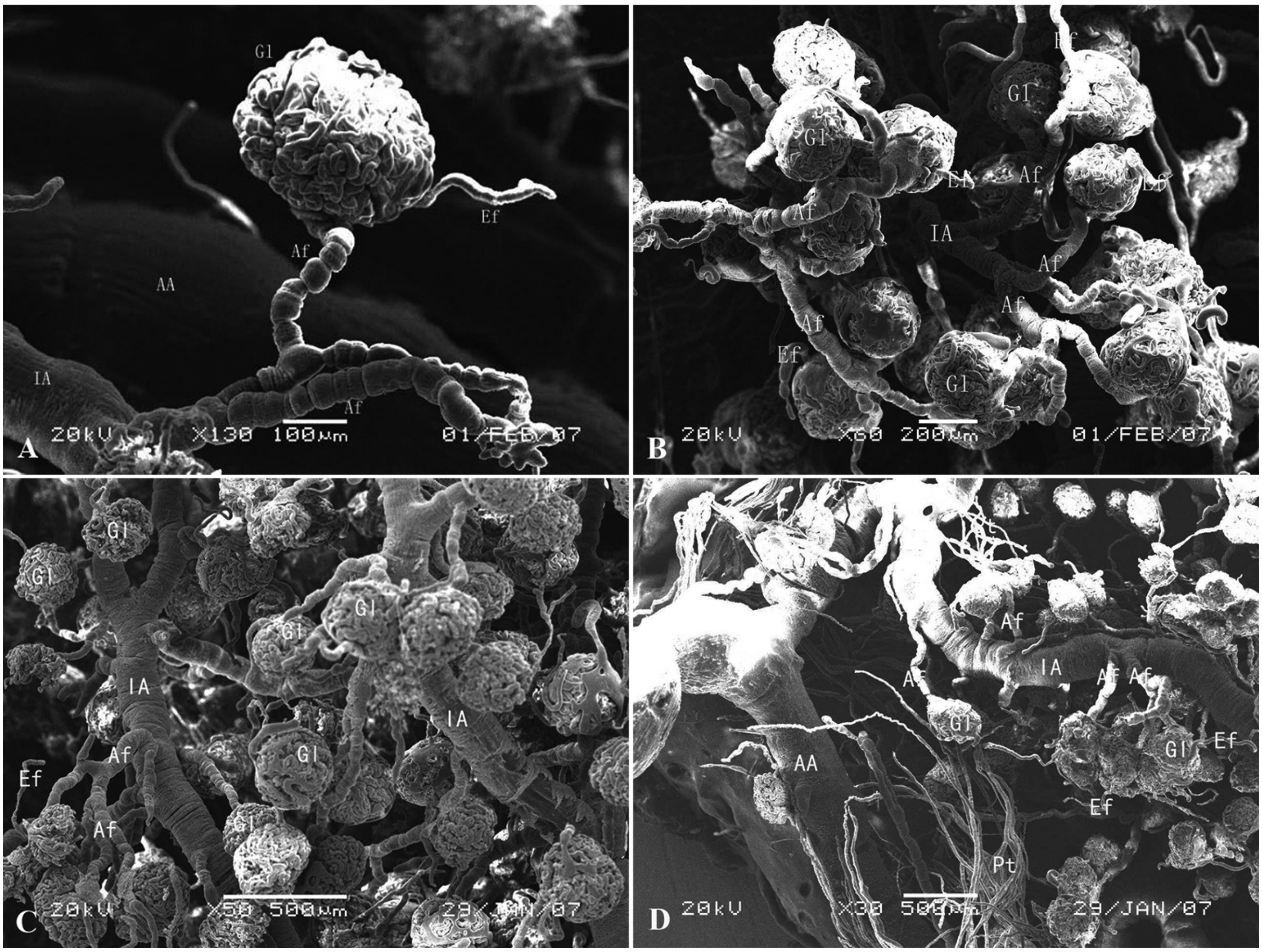

Fig. 3. Characteristic structures of afferent arteriole. **A.** Appearance of afferent arteriole, it shoots forward spirally with lots of narrowing toroidal rings (×130). **B-D**, Diameter and length of afferent arteries in superficial (×60), deep (×50) and juxtamedullary zone (×30). AA: Arcuate artery; IA: Interlobular artery; Af: Afferent arteriole; Gl: Glomerulus; Ef: Efferent arteriole.

Glomerulus

Renal glomerulus of Bactrian camel were mostly round (92%) or oblate (7%), and occasionally irregular in shape (1%), with a shape of "grape-string" on the distal end of interlobular artery. The glomerular capillaries were wrapped loops in renal capsule and their diameters ranged from 5 µm to 25 µm. Studies under microanatomy and scanning microscopy have shown that differences in glomeruli exist in different cortical zones, not only in their shapes and sizes, but also in their densities. The glomerulus at superficial zone had the smallest volume, its average diameter was 273.1 µm (range: 221-369 µm), and with the highest distribution density, most glomeruli in the kidney were concentrated here. It was bigger at deep zone with average diameter of 291.7 µm (range: 252-339 µm), but the number was slightly reduced. The glomerulus with average diameter of 317.7 µm (range: 267-383 µm) had the largest volume at the juxtamedullary zone whereas the number was significantly reduced (Table 3). While comparing and

Table 3. The diameter contrasts of glomerulus.

Region	Maximum diameter	Minimum diameter	Average diameter
Superficial zone	369	221	273.1
Deep zone	339	252	291.7
Juxtamedullary zone	383	267	317.7

Note: 300 Bactrian camel renal glomerulus were observed, measured and quantitatively analysed. Unit: µm.

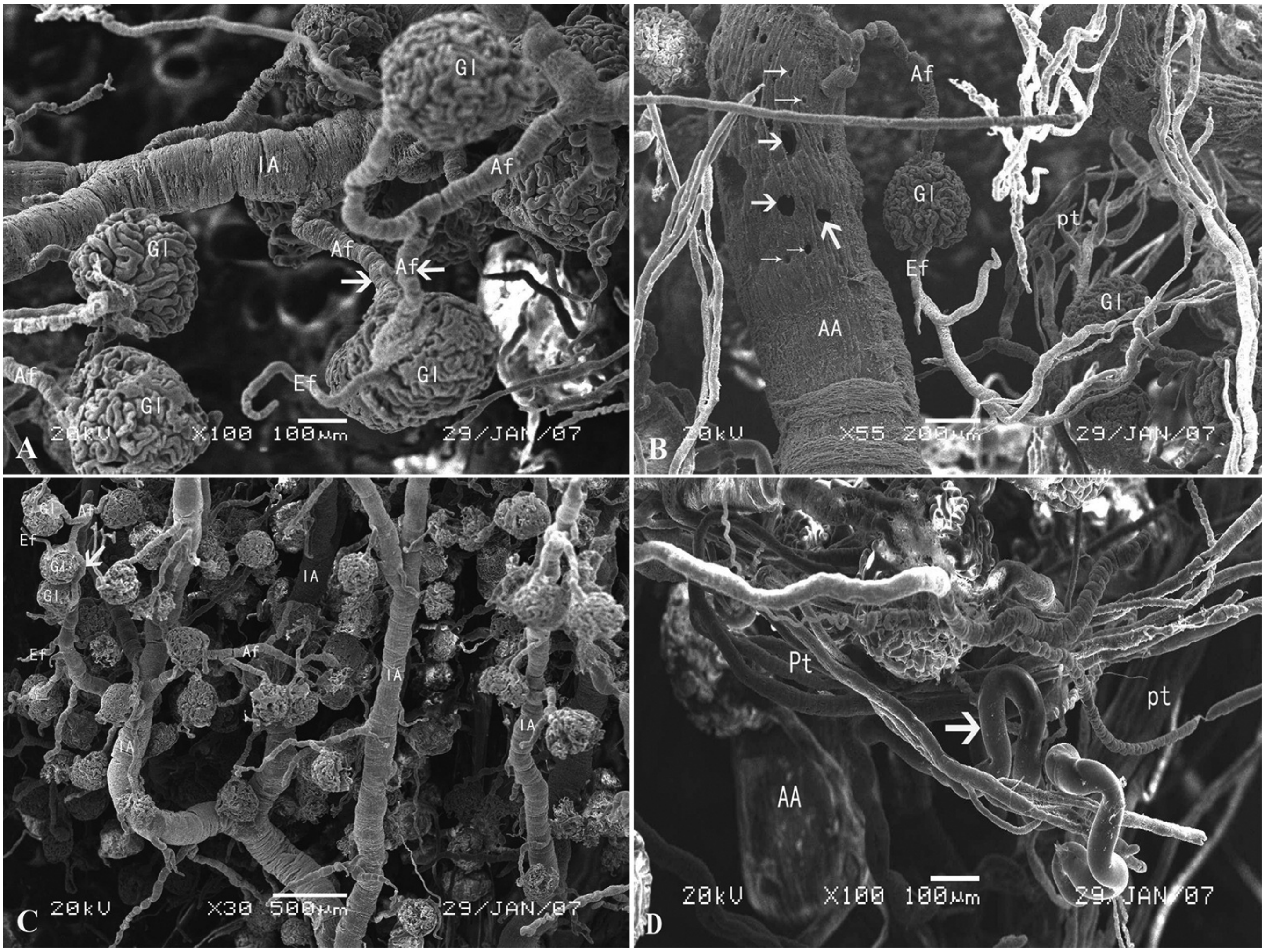

Fig. 4. Character of afferent arteriole and glomerulus. **A.** Two afferent arterioles supplying a glomerulus (arrow) were observed occasionally in Bactrian camels (×100). **B.** The afferent arteriole arises from the arcuate artery, there is some man-made damage (big arrow) in arcuate artery and impression of endothelial cell nucleus (small arrow) (×55). **C.** The afferent arteriole arise from another afferent arteriole (arrow) (×30). **D.** Disappearance of the glomerulus, the arteriole kept extending but no afferent arteriole and typical glomerulus formed (×100). AA: Arcuate artery; IA: Interlobular artery; Af: Afferent arteriole; Gl: Glomerulus; Ef: Efferent arteriole; Pt: Post-glomerular capillary.

counting the glomeruli, we found it disappeared sometimes in the kidney, where the arteriole kept extending but no afferent arteriole and typical glomerulus formed (Fig. 4D).

Efferent arteriole

The appearance of efferent arteriole was similar to the afferent arteriole which extends forward in a spiral manner, but the diameter was smaller compared to the afferent arteriole (Fig. 5A). The diameter also changed regularly in different zones of the renal cortex (Table. 4).

The efferent arteriole at superficial and deep zone supplied branches and formed the postglomerular capillary that surrounded proximal and distal tubules. The efferent arteriole at the juxtamedullary zone was extraordinarily different; the branches extended from the efferent arteriole divided into two types according to its number and length, the first type (fewer and shorter) was distributed in the cortex and formed the postglomerular capillary with branches, whereas the second type (many and longer) extended directly down to the renal medulla, and formed the descending vasa recta (DVR). The DVR kept branching in the renal medulla and constituted the postglomerular capillary which surrounded the renal tubules, after that the postglomerular capillary gathered into ascending vasa recta (AVR) and drained the venous blood of the renal medulla (Fig. 5B).

Table 4. The diameter contrasts of efferent arteriole.

Region	Efferent arteriole		
	Maximum diameter	Minimum diameter	Average diameter
Superficial zone	51	25	33.4
Deep zone	57	27	36.5
Juxtamedullary zone	56	27	42.6

Note: 100 Bactrian camel efferent arterioles were observed, measured and quantitatively analysed. Unit: µm.

Discussion

The kidney is mainly composed of renal tubules, renal artery, and the renal vein, and Stephenson (Stephenson et al., 1976) emphasized that they were integrated functionally. The branching and distribution of renal artery not only determines the blood supply to the kidney, but also affects its function to a large extent. Although all mammalian kidneys are somewhat similar, there are several species-specific differences in terms of organization, microstructure and function (Horacek et al., 1987).

Camels have physiological, anatomical and behavioral adaptation mechanisms to the desert environment (Berlyne et al., 1978; Gebreyohanes and Assen, 2017). The Bactrian camel and the Dromedary are extant Old World camelids, although the qualitative difference between them is subtle, they are considered to be two species (Martini et al., 2018). The anatomical structure of the kidneys in the dromedary and the Bactrian camel are almost the same, both have a renal crista and the same medullary/cortical thickness ratio. The medullary/cortical thickness ratio is the most important factor in relation to the ability to generate concentrate urine, rather than the short and long looped nephrons with similar structure and function (Schmidt-Nielsen and O'Dell, 1961; Abdalla and Abdalla, 1979). The greater the ratio, the higher osmotic pressure

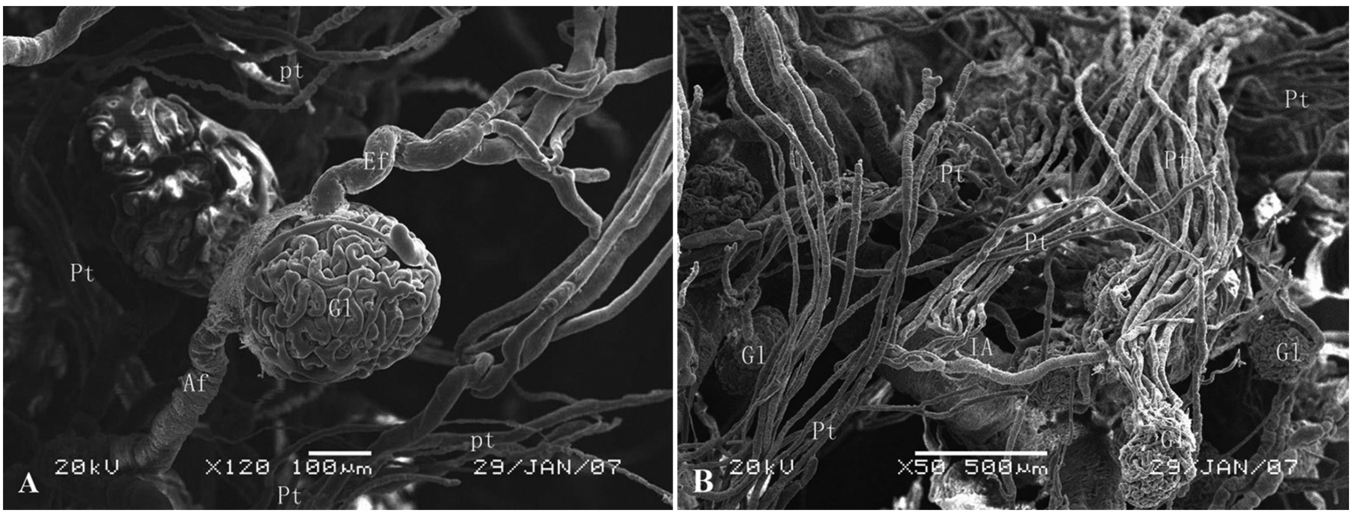

Fig. 5. Characteristic structures of efferent arteriole and post-glomerular capillary. **A,** Appearance of efferent arteriole, it extends forward spirally and has a smooth surface (×120). **B.** Post-glomerular capillary extends from efferent arteriole (×50). Af: Afferent arteriole; Gl: Glomerulus; Ef: Efferent arteriole; Pt: Post-glomerular capillary.

gradient which eventually produces hypertonic urine (Xu et al., 2009). The features that help dromedary to survive in deserts are also key factors for the Bactrian camel to reduce water loss; these features are closely linked to the environmental adaptability of the Bactrian camel.

The anatomical structure and branching of artery in Bactrian camel's kidney are different from humans. In humans, a renal artery always has branches which can be identified as posterior (P), upper (U) and lower (L). It will also have some or all (rarely none) of the following branches: intermediate (I), middle (M) and suprahilar (Fine and Keen, 1966). The anatomical structure of Bactrian camels' kidney is like that of a horse, sheep, dog, or rabbit (Pfeiffer, 1968; Bragulla et al., 2006; Marques-Sampaio et al., 2007). But in the horse, the renal medulla heals completely while in the Bactrian camel, it heals incompletely and has some pseudopapilla. This feature makes it more similar to the kidney structure in sheep, but the number of renal pyramids is different from Bactrian camel's. The renal artery in Bactrian camel divides into dorsal and ventral branches, and it is the same as in sheep (Michalczyk et al., 1985; Buys-Goncalves et al., 2016), and rabbit (Shalgum et al., 2012).

The numbers of interlobar arteries in the left and right kidneys are different in different Bactrian camels (14 to 16), and our result is also different from previous reports (12 or 13) (Sheng and Yi, 2000). Numerous reports have indicated the common variation of arteries in kidneys (Khamanarong et al., 2004; Pereira-Sampaio et al., 2004; Marques-Sampaio et al., 2007; Daescu et al., 2012), we also speculate that this anatomical variation in arteries may exist in multiple species, and the variation in Bactrian camel is mainly reflected in interlobar artery. The distinction in numbers is not a pathological phenomenon or experimental error, but due to individual differences. Anastomosis and distribution of the renal artery are key points during clinical local resection of the kidney. The multiple variations of arteries in different species further remind us about the need for preoperative angiography.

In our research on microstructure, the afferent arteriole, efferent arteriole, glomerulus, and post-glomerular capillary, all have linear changes in different zones in the kidney, and it's the same as in other mammals. Our interest rests on the sometimes disappearance of the glomerulus, where the arterioles keep extending but no typical glomerulus is formed. A distinct feature of the renal circulation is the double capillary bed in the glomerulus and around the tubules, which is a key component in the filtration, secretion, and reabsorption of minerals and removal of unwanted substances from the filtrate toward the formation of urine (Chade, 2013). As Burger and Cross (1982) pointed out, glomerulus loss is rare, and Casellas and Mimran (1981) added that its frequency of occurrence had nothing to do with age, although the reasons for the existence of this phenomenon have not been clearly established. The disappearance or appearance of this structure in Bactrian camel needs to be explored and researched further. Attention should also be paid to the two other ways (via arcuate artery and conjoint afferent arteriole) from which the afferent arteriole arise apart from the interlobular artery. This means the glomerulus can sometimes connect to each other directly through afferent arteriole. Since this character has not been observed in other species, further studies need to be conducted to ascertain whether this phenomenon in Bactrian camel relates to function or not.

In conclusion, although some results in our data need to be explored further, we have conducted a more systematic and in-depth study on the general structure of intrarenal arteries in Bactrian camels. It gives us a clearer and more detailed understanding of the Bactrian camels' environmentally adaptive nature. These results will not only provide an understanding of the kidney structure of Bactrian camels and insights into the diversity of species, but are also helpful for further investigations into adaptations to desert climate.

Acknowledgements. This research was funded by the National Science Foundation of China with Grant No. 31772691.

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Accepted September 19, 2019