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Comparative histochemical study of the kidney of six mammalian species

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ABSTRACT

Submited: 2018-09-06 Accepted : 2019-05-20 Comparative anatomy describes the similarities and differences in the anatomy of different species. Comparative anatomical studies do not only provide evidence for evolution but also indicate that various organisms originate from a common ancestor. They assist scientists in classifying these organisms based on similar characteristics of their anatomical structure. The aim of this study was to compare histochemical of the kidney of six mammalian species. The animals were grouped into three based on their dietary patterns i.e herbivores (Capra aegagrus and Bos taurus), carnivores (Canis lupus and Felis catus) and omnivores (Rattus norvegicus and Sus scrofa). Tissues were obtained from each of the studied species and preserved in 10% formal saline for one week until analysis. The tissues were then subsequently processed and stained using general and specific histochemical techniques. It was observed from this study all the mammalian kidneys consisted of similar histological features which explained that these studied mammals undergo the same evolutionary trend, adaptational changes and phylogenic relationship that exist between the kidneys of the studied species. Despite their differences in dietary patterns, the studied species have similar histological features with little observed variation. However, they have little differences in histochemical staining pattern for glycogen and glycoprotein but in reticulin they all stain intensely. This study has established a strong relationship in the histology of the kidney in six mammalian species.

ABSTRAK

Perbandingan anatomi menjelaskan kesamaan dan perbedaan pada anatomi dari spesies yang berbeda. Penelitian perbandingan anatomi tidak hanya menyediakan bukti evolusi tetapi juga menunjukkan bahwa variasi organisme berasal dari pendahulunya. Hal ini dapat membantu peneliti dalam mengklasifikasikan organisme ini berdasarkan karakteristik struktur anatomi yang serupa. Penelitian ini bertujuan untuk membandingkan gambaran histokiam ginjal dari enam spesies mamalia. Hewan dibedakan menjadi tiga kelompok berdasarkan pola diet masing-masing hewan yaitu herbivora (Capra aegagrus dan Bos taurus), karnivora (Canis lupus dan Felis catus), dan omnivora (Rattus norvegicus dan Sus scrofa). Jaringan diambil dari setiap spesies yang dipelajari dan disimpan dalam 10% formal salin selama 1 minggu hingga dilakukan analisis. Jaringan kemudian diproses dan diwarnai menggunakan teknik histokimia umum dan spesifik. Dalam penelitian ini didapatkan bahwa ginjal mamalia memiliki kemiripan gambaran histologi yang menjelaskan bahwa mamalia mengalami tren evolusi yang sama, perubahan adaptasi dan hubungan kekerabatan yang ada pada ginjal spesies yang diteliti. Terlepas dari perbedaan dalam pola makanan, spesies yang diteliti memiliki gambaran histologi yang sama dengan sedikit variasi yang teramati. Namun, mereka memiliki sedikit perbedaan dalam pola pewarnaan histokimia untuk glikogen dan glikoprotein tetapi pada reticulin hasil pewarnaan menunjukkan hasil yang intens. Penelitian ini telah menunjukkan hubungan yang kuat pada gambaran histologi ginjal enam spesies mamalia yang diteliti.

Key words: kidney mammals histochemical comparative herbivores

INTRODUCTION

Comparative anatomy describes the relationship in the anatomy of different species.¹ These anatomical studies do not only provide evidence for evolution but also indicate that various organisms originated from a common ancestor. These studies assist scientists in classifying these organisms based on their similar characteristics of their anatomical structure.²⁻⁴ Based on mode of dietary patterns organisms can be classified into herbivores, carnivores and omnivores. Herbivores animals that get its energy from eating plants, and only plants. Carnivores are organism that get its energy and nutrient requirement animal tissues whether from predation scavenging. through or whereas omnivores feed on both plant and animal.⁵

In mammalia the kidneys were bean shaped organs located retroperitoneally at the level of T12-L3 in herbivores and omnivores, and at L1-L3 in carnivores.⁶ Morphology of the kidneys differs amongst the mammalian species based on their modes of diet. They are firmly encapsulated by a fibrous capsule of dense irregular connective tissue and with a shock –absorbing layer of adipose tissue.⁵ The aim of this study was to compare a detailed histological association between the kidneys in six mammalian species.

MATERIALS AND METHODS

Animals

Approval of the protocol of this study was obtained from ethical committee of the Department of Human Anatomy and Cell Biology, Delta State University Abraka with reference number DELSU/ CHS/ANA/68/85. This was a comparative observational study of the kidneys in six mammalian species. The animals were grouped into three based on their dietary patterns i.e. herbivores (*Capra aegagrus* and *Bos taurus*), carnivores (*Canis lupus* and *Felis catus*) and omnivores (*Rattus norvegicus* and *Sus scrofa*). The animals were purchased from the abattoir which includes: Bos taurus, Capra aegagrus, Canis lupus and Sus scrofa while Rattus norvegicus and Felis catus were purchased from the market.

Histological examination

Animal were euthanized by cervical dislocation, a renal tissue was obtained from each of the studied animals and preserved in 10% formal saline for one week before analysis. The tissues were subsequently processed and stained using general and specific histochemical techniques standard. Micrographs obtained were observed under a digital microscope at 100 x and 400 x.

Analysis

Histological preparations of kidney tissue of six animals were microscopically examination and compared.

RESULTS

Haematoxylin and eosin staining.

Micrograph A shows a section of the kidneys of Bos taurus (cow). There is a cortex and medulla and within the cortex are several renal corpuscles composed of Bowman's capsule and glomeruli. The glomeruli are variable in size and demarcated into 7-10 lobules. The glomeruli form the core of the corpuscle and are composed of capillaries which are present within the fibromyxoid stroma. Podocytes are present in the parietal aspects of the Bowman's capsule juxtaglomerular while apparatus, composed of distal convoluted tubules, afferent, efferent arterioles and lacis cells occur in the polar part of the glomerulus. Varying forms of tubules ranging from proximal convoluted tubule, loop of Henle, collecting tubules and collecting ducts are present and altogether they form the nephron. The proximal part of the nephron, the proximal convoluted tubule, consists of cuboidal cells with brush borders. The loops of Henle are lined by squamous epithelial cell which transit to low cuboidal epithelial pale cells constituting the distal convoluted tubule. The collecting ducts are lined by low columnar epithelial cells. These ducts, also known as duct's of Bellini, open into the lumen of the ureter.

Micrograph B shows the renal apparatus of *Capra aegagrus* (goat kidney) composed of a cortex and medulla. Medullay rays are distinct at the inner medulla with multiple renal corpuscles composed of Bowman's capsule's and glomeruli of variable sizes composed of 2-3 lobules also seen. The glomeruli are attached to the Bowman's capsule with renal tubules disposed between the renal corpuscles which are separated by a loose connective tissue stroma. While the distinct lower duct portions are composed of ducts lined by cuboidal to low columnar cells, the proximal component of the renal tubules are lined by low columnar to cuboidal occasionally and squamous cells. Squamous cells are however difficult to define lining the Bowman's capsule.

In micrograph C the kidneys of Canis *lupus* (dog) are illustrated. The kidneys are observed to possess a cortex with several renal. The cortico-medullay ratio is 1-3 with the glomeruli confined to the cortex within the capsule. The proximal tubules are lined by cuboidal epithelial cells with brush border (PCT) while the distal convoluted tubules (DCT) are lined by low cuboidal epithelial cells. The glomeruli are composed of 3-7 lobules, and podocytes are present in the visceral aspect of the Bowman's capsule. Varying forms of tubules ranging from PCT, LH, CT and CD, normally constituting the nephron, are seen. The LH are lined by squamous epithelium which consists of pale low cuboidal epithelial of pale cells known as DCT, each of the tubules or duct are separated by thin fibro connective tissue.

Micrograph D illustrates the kidneys of the cat species studied (*Felis catus* cat). Within the cortex are multiple renal corpuscles with Bowman's capsule, space, and glomerulus, there are prominent medullay rays seen. The

cortical medulla ratio is 1-3. There were several tubules present which are lined by cuboidal epithelial cells with brush border (PCT), while the DCT are lined by low cuboidal epithelium. The glomeruli are composed of 3-7 lobules with podocytes present in the visceral aspect of the Bowman's capsule. Varying forms of tubules arranging from PCT, LH, CT, and CD constitute the nephron. While the LH are lined by squamous epithelial cells which consists of low cuboidal epithelia of pale cells known as DCT, each of the tubules or duct are separated by thin fibro connective tissue.

Micrograph E is the illustration of the kidneys of *Rattus norvegicus* (rat). It is composed of a cortex and medulla. The juxtaglomerular apparatus' are composed of lacis cell, afferent, efferent arterioles with DCT. The renal corpuscle with several Bowman's capsule, space and glomeruli of variable sizes and shapes consisting of several lobules are present. Podocyte are present in the visceral layer of the capsule with varying forms of tubules ranging from PCT, LH, CD and CT, present from cortex to the medulla. The tubules are lined by cuboidal epithelia with brush borders in the (PCT) and low cuboidal epithelia constituting DCT. CD is lined by low columnar epithelial cells. The tubules are separated from each other by thin fibro-connective tissue stroma.

Micrograph F shown Sus *scrofa* (porcine) cortex, juxtaglomerular apparatus were composed of lacis cell, afferent, efferent arterioles with DCT, presence of renal corpuscle with several Bowman's capsule, space and glomerulus were variable in size and shape consisting of several lobules, podocyte were present in the visceral layer of the capsule, varying forms of tubules arranging from PCT, LH, CD and CT were present. The tubules are lined by cuboidal epithelium with brush border (PCT) and low cuboidal epithelia constituting DCT.



FIGURE 1. Micrograph A (cow), B (goat), C (dog), D (cat), E (rat), and F (porcine) kidneys stained with H&E at x100& x400 magnifications. (G) glomerulus, (RT) renal tubule (DCT) distal convoluted tubule (M) medulla (MR) medulla ray (V) vessel (BS) bowmans space (BC) bowman capsule

Glycogen (Per-iodic acid -schiff)

Micrograph G, kidneys of *Bos taurus* (cow) glomerulus. The glomeruli are enclosed by the Bowman's capsule. Podocytes are presents in the parietal aspects of the Bowman's capsule. There is intense staining of the proximal aspects of the nephron and less so far stroma cell. The basement membranes reveal an intensely dark purple colour with occasional rail-roading. The nuclei of the epithelial cells are bland to vesicular in appearance.

Micrograph H shows Capra *aegagrus* (goat) cortex with several Bowman's capsules, renal tubules and medulla. There is intense staining at the glomerulus. The basement membrane and epithelium of the PCT stain dark purple pale unlike the DCT and CD which are pale in color. Some blood vessels also stain deeply.

Micrograph I shows Canis *lupus* (dog) cortex, prominent medulla ray seen. The proximal tubules are lined by cuboidal epithelia cells with brush borders (PCT) while the DCT are lined by low cuboidal epithelium. Podocytes line the visceral aspect of the Bowman's capsule. The tubules or ducts are separated by thin fibro connective tissue.

Micrograph J shows the kidneys of *Felis catus* (cat). The cortex, capsule and medulla with medullary rays are seen at the inner medulla, multiple renal corpuscles composed of Bowman's capsule, space, glomeruli, which are variable in sizes and composed of 2-3 lobules. The glomeruli are attached to the Bowman's capsule. The capsules are composed of dense connective tissue. The renal tubules within the renal corpuscle are abundant. These tubules are separated by a loose connective tissue stroma while the distinct lower portion composed of duct lined by columnar to low cuboidal, proximal component of the renal tubules are lined by low columnar to cuboidal and occasionally squamous cell which are difficult to define in the Bowman's space. Several tubules are lined by cuboidal epithelium with brush borders (PCT) while the DCT are lined by deeply eosinophilic staining low cuboidal epithelial cells.

Micrograph K shows Rattus norvegicus (rat) cortex with several Bowman's capsule, space and glomeruli of variable sizes and shapes consisting of several lobules. The tubules are lined by cuboidal epithelia with brush border (PCT) and low cuboidal epithelia. Intense staining of the entire basement membrane with the epithelia cell stroma staining dark purple is evident. The PCT stain more at the luminal part unlike the DCT.

Micrograph L shows a section of the kidneys of Sus scrofa (porcine). Distributed within the cortex are juxtaglomerular apparatus' composed of lacis cells, afferent, efferent arterioles and DCT. The renal corpuscle, composed of several Bowman's capsules, space and glomeruli are variable in size and shape consisting of several lobules. Podocytes are present in the visceral layer of the capsule. The tubules are lined by cuboidal epithelial cells with brush border (PCT) and low cuboidal epithelia constituting DCT. Collecting ducts are lined by low columnar epithelia cell. Cortex stains intensely especially the epithelia cell while the basement membrane revealed an intensely dark purple pale with occasional rail-roading also seen.



FIGURE 2. Micrograph G (cow), H (goat), I (dog), J (cat), K (rat), and L (pig) kidneys stained with PAS at x100 and x400 (G) glomerulus, (RT) renal tubule (LH) loop of Henle

Glycogen (Per-iodic Acid –Schiff -Diastase)

Micrograph M shows a section of the kidneys cow (Bos taurus). Varying forms of tubules, ranging from proximal convoluted, loop of Henle, collecting tubules and collecting ducts constitute the nephron. The tubular aspects of the nephron, made up of proximal convoluted tubule, consist of cuboidal cell with brush borders. These cells are distinctively eosinophilic with cast present in the lumen of some of the structure. The loops of Henle are lined by squamous epithelial cell which transited to low cuboidal epithelial of pale cells constituting the distal convoluted tubule. The basement membrane nuclei of the epithelial cells stain less intensely with clear evidence of rail-roading.

Micrograph Ν shows Capra (goat) kidnevs aegagrus showing several Bowman's capsules and renal tubules within the cortex. Tubules are distributed abundantly in the cortex and are separated by a loose connective tissue stroma. Lower portions are composed of ducts lined by columnar to low cuboidal. Proximal component of the renal tubules are lined by low columnar to cuboidal cells. Squamous cell is difficult to define in the Bowman's space. There is intense staining of the glomerulus. The basement membrane and epithelia cell of the PCT stain more intensely dark purple than that of previously described mammals.

Micrograph O shows the kidneys of *Canis lupus* (dog). There is intense staining of the basement membrane all

through the nephron with prominent staining of the luminal material in the lumen of the PCT.

Micrograph P shows *Felis catus* (cat) There is intense staining of the basement membrane all through the nephron with prominent staining of the luminal material in the lumen of the PCT.

Micrograph Q shows *Rattus norvegicus* (rat) glomerulus with Bowman's capsule, space, capsule and medulla with presence of medulla rays seen at the inner medulla. Multiple renal corpuscles composed of Bowman's capsule, space, glomeruli, variable in size and composed of 2-3 lobules, are also seen.

Micrograph R shown Sus scrofa (porcine) cortex, capsule and medulla with presence of medulla rays seen at the inner medulla. Multiple renal corpuscle composed of Bowman's capsule, space, glomeruli, which were variable in sized composed of 2-3 lobules. The glomeruli were attached to the Bowman's capsule. The capsule was composed of dense connective tissue. The renal tubules disposed within the renal corpuscle were abundantly. These tubules were separated by a loose connective tissue stroma while the distinct lower portion composed of duct lined by columnar to low cuboidal. Proximal component of the renal tubules was lined by low columnar to cuboidal and occasionally squamous cell were difficult to defined in the bowman's space renal tubules with glomerulus and Bowman's space. The cytoplasm of the cell stained more intensely.



FIGURE 3. Micrograph M (cow), N (goat), O (dog), P (cat), Q (rat), and R (pig) kidneys stained with PAS-D at x 100 and x 400 (G) glomerulus, (RT) renal tubule (BM) basement membrane

Gomoris Method for Identification of Reticulin

Micrograph S shows *Bos taurus* (cow) cortex, capsule and medulla with presence of medulla rays seen at the inner medulla. Multiple renal corpuscle composed of Bowman's capsule, space, glomeruli, which were variable in sized composed of 2-3 lobules. The renal tubules are disposed within the renal corpuscle abundantly. The tubules are separated by a loose connective tissue stroma while the distinct lower portion composed of duct lined by columnar to low cuboidal. Proximal component of the renal tubules are lined by low columnar to cuboidal epithelial cells. The cytoplasm of the epithelia cells also stained intensely with reticulin.

Micrograph T shows *Capra aegagrus* (goat) kidneys stained intensely at the glomerulus, collecting ducts, andtubular epithelia unlike the epithelial of the proximal tubules. The basement membrane is also distinctively stained by reticulin.

Micrograph U had shows *Canis lupus* (dog) glomerulus and renal tubules. It

shows intense staining in the basement membrane at the apical part of the cortex. The cytoplasm of the epithelia cells also stains intensely.

Micrograph V shows *Felis catus* (cat) cortex. There is intense staining in the entire basement membrane of the renal structure. The cytoplasm of the epithelia cells and glomerulus stain intensely.

Micrograph W shows *Rattus norvegicus* (rat) cortex. There is deep staining of the basement membrane of the renal parenchyma.

Micrograph X show Sus scrofa (porcine) renal tissue composed of a cortex and medulla. Juxtaglomerular apparatus is composed of lacis cell, afferent, efferent arterioles with DCT. Several renal corpuscle composed of Bowman's capsule, space and glomerulus with variable sizes and shapes consisting of several lobules are clearly evident. Podocyte are present in the visceral layer of the capsule. Varying forms of tubules arranging from PCT, LH, CD and CT are present. The tubules are lined by cuboidal epithelium with brush border (PCT) and low cuboidal epithelia constituting DCT, CD lined by low columnar epithelia cells.





FIGURE 4. Micrograph S (cow), T (goat), U (dog), V (cat), W (rat), and X (pig) kidneys stained with reticulin at x 100 and x 400 (G) glomerulus, (RT) renal tubule (BM) basement membrane (EC) epithelia cell lining.

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Features	Herbivores		Carnivores		Omnivores	
	Cow	Goat	Dog	Cat	Rat	Porcine
Glomerulus	+	+	+	+	+	+
РСТ	+	+	+	+	+	+
DCT	+	+	+	+	+	+
LH	+	+	+	+	+	+
Podocytes	α	А	α	β	А	α
BC	+	+	+	+	+	+
Glycogen distribution	+	+	+	+	+	+
Glycoprotien	+	+	+	+	+	+
Reticulin	+	+	+	+	+	+

+=Present, β =visceral, α =parietal layer, PCT= proximal convoluted tubule, LH=loop of Henle, DCT= distal convoluted tubule, BC=bowman's capsules.

TABLE 1 presents the summary histo-architecture between and histochemical staining pattern of the kidney in six mammals. Histoarchitecture of kidney in six mammalian species show the glomerulus which are variable in shape but composed of different size of lobules. The proximal convoluted tubule are lined by cuboidal cells with brush border or microvilli except the goat which were lined by low columnar to cuboidal cell while the distal convoluted tubules are all lined by low cubiodal cells. The loop of Henle consists of squamous epithelia cell and the podocytes varies. The podocytes are present in in the parietal layer of cow, goat, dog rat and pig. Glycogen distribution showed intensely staining at the basement membrane in herbivores, at the cytoplasm in carnivores and proximal convoluted tubule in omnivores respectively. Glycoprotein intensely stained at the cytoplasm of pig, rat and cat.

DISCUSSION

From this study, it was observed that the mammalian kidneys consisted of similar histological features. It explained evolutionary trend of adaptation changes and its phylogenic organization that exists among the kidney in six mammals. In cow however, the kidneys revealed structural modification indicating preferential filtration in the nephron.⁷ The tubular aspects of the nephron consist of proximal convoluted tubule lined by cuboidal epithelial with brush border which facilitate re-absorption in the tubule. This finding agreed with Mbassa⁸ who showed that the proximal convoluted tubule was lined by cuboidal epithelial with brush border and high renal blood flow.

Intense stain response to PAS, and PAS-D in herbivores (cow) indicates large amount of glycogen, glycolipid and glycoprotein present in the basement membrane of the renal structure. This is likely due to ingested carbohydrate that is stored in the fore-stomach and fermented by microbes.⁹

Similarly goat kidney showed a similar pattern with little differences on the proximal components of the renal tubules which were lined by low columnar to cuboidal epithelium. The squamous cells were difficult to define in the bowman's space occasionally. In goat kidney, re-absorption is very slow compared to cow kidney. Mbassa⁸ argued that the cow proximal convoluted tubules were lined by cuboidal cells with micro villi which helped to increase the rate of re-absorption. Although this species belongs to the same group of herbivores, the re-absorption rate is slightly different.

In the contrary, the proximal convoluted tubules of the dog kidney lined by cuboidal epithelia with brush border while the distal convoluted tubules were lined by low cuboidal epithelia without brush border. Although the dog's renal corpuscle consisted of several Bowman's capsule, loop Henle and glomerulus. These findings were similar to that of Ali et al, ¹⁰ who observed large amount of renal corpuscle in juxtamedullary and mid-cortical region that are characterized by long loop of Henle of which they regarded as the structural properties of dog to enable voiding of concentrated urine. The proximal convoluted tubules have wide luminae lined by cuboidal cells containing brush border and spherical nuclei while the distal convoluted tubule possess low cuboidal cells without brush border. Glycogen, glycoprotein and protein are well distributed in the basement membrane of the renal cell. The cat kidneys showed a similar pattern in containing cells.

Rat kidneys revealed presence of glycogen, glycoprotein, neutral mucins and protein in the luminal part of the convoluted tubules. proximal This is likely because the consumption of food with large amounts of these macromolecules (sugars, fat and protein) in proximal convoluted tubules unlike the herbivores and carnivores. This finding can be explained by the advancement of omnivores although

they share a common ancestral origin. This finding was in line with studies by Salam and Agha¹¹ who observed large amount of carbohydrate in the cytoplasm of herbivores.

Protein were found in the epithelia cells lining of the renal tubule, and lack of carbohydrate in the nuclei of the kidney cells. The Bowman's capsule and glomeruli were variable in size, the juxtaglomerular apparatus was composed of lacis cell, afferent and efferent arterioles (vessels) with distal convoluted tubule. The result obtained in this study conformed to that of Al-Samawy¹² who reported that the kidney of albino rat consists of two regions, the outer cortex and the inner medulla. The presence of nephron composed of renal corpuscle, and renal tubules. Hassaneen¹³ found similar result that the renal corpuscle was spherical in shape and structure which formed glomerulus enclosed bv Bowman's capsule. Basuony¹⁴ and William¹⁵ claimed that the distal convoluted tubule was fewer in number, recognized by pale cuboidal epithelia cell, smooth internal surface and lack brush border. The presence of thin fibroconnective tissue capsule enclosing the entire capsule. Contrary with Junqueira¹⁶ who establish that proximal convoluted tubule was lined with columnar epithelia, but in agreement with other features the proximal tubule were more narrow than the distal convoluted tubule, the distal convoluted tubules were lined with low cuboidal types, round in shape and large nuclei possess no brush border. The distal tubules tend to be shorter than the proximal tubules and were fewer in number in the cortex region.

Hassaneen¹³ revealed a similar result in pig that proximal convoluted tubules lumen was wide, lined by cuboidal epithelial cells resting on a basement membrane; the cells were characterized by ground eosinophilic cytoplasm, large centrally nuclei and the apical surface covered by a brush border called microvilli, which explained that these species underwent a similar evolutionary trend based on adaptation.¹⁷

CONCLUSION

Species with difference dietary have similar histological patterns features with little observed variation which explained that these studied mammalia shared common ancestral origin. However, they have few differences in histochemical staining pattern for glycogen and glycoprotein, but in reticulin they all stained intensely. This study established a strong relationship in the kidney histology of six mammalian species and provided histological details associated with the kidneys of the six mammalian species extensively for the first time. It also highlighted the glycogen, glycoprotein, neutral mucins and reticulin in kidneys of the studied mammals and associated the histological features of the kidney with the pattern of diet in three categories of mammals.

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