American-Eurasian J. Agric. & Environ. Sci., 7 (5): 505-511, 2010
ISSN 1818-6769

Morphological and Histological Study of Kidney in Juvenile Great Sturgeon (*Huso huso)* and Persian Sturgeon *(Acipenser persicus*)

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Abstract: Sturgeons are ancient and commercial fishes which posses' primitive characteristics and due to their great inflections are able to live in both freshwater and marine environments. So their kidney has great ability for adaptation process. In order to find the structure and distribution of kidney nephrons in juvenile *Huso huso* and *Acipensr persicus*, sampling of head, body and caudal parts of kidney in fishes were carried out. Histological samples were dehydrated by routine methods and embedded in paraffin wax. They were sectioned by microtome and stained with H & E and PAS stain. The results revealed that the kidney of young *Huso huso* and *Acipenser persicus* consists of two lobes that are slender and long and is divided into three parts: a head, body and caudal part. Also, the kidney of sturgeons was consisted of: Glomeruls, Bowman's capsule, Proximal (I & II), Distal and Collecting tubule. The renal corpuscles of sturgeons consist of a glomerulus and a glomerular (Bowman's) capsule. The proximal tubules consisted of cuboidal cells with well- developed brush border. Two proximal tubule segments distinguished by the different heights of the cells and the brush border. The distal tubules have cuboidal epithelium without brush border. The collecting tubules lined with high cuboidal epithelium. Their nuclei are mainly spherical that are located in the central part of the cells. The kidney of *H. huso* and *A. persicus* was Y shape and distribution of different cells was not hemogene in all sections of kidney. The head of the kidney composed exclusively of hematopoietic tissue and islets of interrenal tissue. But, in the body and caudal part the amount of hematopoeitic tissue and interrenal glands were decreased and substituted by renal tubules and glomeruli. So, there are not any difference in kidney structure and nephrons morphology between *A. persicus* and *H. huso*.

Key words: Nephron · Urine unite · Renal Corpuscle · Sturgeon · Acipenser · Morphology · Histology

INTRODUCTION

The kidney of the vertebrate is the main organ which balances the body fluid homeostasis [1]. The kidney of fish receives majority of postbranchial blood and renal lesions may be expected to be good indicators of environmental stress [2]. Sturgeons have large inflection and have the ability to live in both freshwater and marine environments. So their kidney has great ability for adaptation process [1]. Because they are migratory fishes with preliminary property which has determined their wide adaptation to varying environmental conditions [3, 4].

The sturgeons are ancient and commercial fishes that had the freshwater origin [4] which exposed to decline and their morphology and function of kidney have been altered during the evolution in many years. The kidney of such fishes usually is a fused organ lying in a retroperitoneal location just ventral to the spinal column. All of the kidneys are paired and located in abdominal cavity. The kidney of all vertebrates is made up of nephrons which are the functional, structural and morphological unit [5].

The head of kidney containes endocrine elements, the chromaffin cells and interrenal tissue which are

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located around the blood vessels. The posterior kidney contains the nephrons with variable quantities of hemopoietic and lymphoid tissue in the interstitium. In vertebrates, three kind of kidney are found: the pronephros, mesonephros and metanephros [5, 6].

Doubtless more further understanding of the lower vertebrates kidney will lead to detailed improvement in the relationship between structure and function of mammals nephrons [7].

The other histological studies on the kidney of sturgeons consist of: *Acipenser persicus* [8], juvenile *Huso huso* [9], juvenile *Acipenser naccarii* [10] and sturgeons of Caspian Sea basin [11]. Beluga (*Huso huso)* is a brackish-water species with breeding pattern in fresh water [12]. So, increased understanding of the kidney structure, Morphology and histology pattern in this commercial species will be of great importance in some progressive information about its adaptation and environmental condition effects.

This study aimed at the Morpho-histological examination of kidney based on histology, features and distribution of cells in *Huso huso* and *Acipenser persicus*.

MATERIALS AND METHODS

Fishes: 6 specimens of young farmed beluga *Huso huso* (1 and 2 year old, average length 51.14 ± 0.14 cm and weight 550.23 ± 15.65 g) and *A. persicus* (1 and 2 year old, average length 40 ±1.2 cm and weight 470 ± 13.00 g) that reared in freshwater with: pH 6.81, total hardness (as $CaCo_3$): 406 mg L^{-1} and with water temperature 10°C, after transferred from fiber glass tank to laboratory (in International Sturgeon Research Institute) were studied. The part of water of each tank was changed every day. Fishes were removed from each tank for biometry and then anesthetized with *Caryophillium aromaticus* powder and then killed for sampling.

Sampling: Samples from the head, body and caudal part of kidney were removed. They were fixed in Bouin's liquid for light microscopy. Then they were dehydrated through ethanol series, cleaned with xylol and embedded in paraffin [13]. Then the sections were prepared (5 – 7 μm) and stained with H&E stain [14] and PAS [15]. After that the slides were studied by light microscopy & photographs were prepared.

RESULTS

Kidney Structure: The kidney of young *Huso huso* and *Acipenser persicus* consists of two lobes that are slender and long. So, the kidney is divided into three parts: a head, body and caudal part. The head of the kidney composed exclusively of hemato-poietic tissue and islets of interrenal tissue devoid of renal tubules and glomerulus. This structure changes in the body, where the hematopoietic tissue is gradually decreased, but the numbers of tubules and glomeruli are increased. In the caudal part of the kidney dispersion of hematopoietic tissue is completely reduced and substituted by numerous glomeruli and convoluted tubules. The hematopoietic tissue filles among the nephrons. Each nephron consists of glomerulus that is enclosed by Bowman's capsule, the proximal, distal and collecting cells (Fig. 1).

Renal Corpuscle: The renal corpuscles of *Huso huso* and *Acipenser persicus* consist of a glomerulus and a glomerular (Bowman's) capsule. The glomerulus is a globular network of densely packed anastomosing capillaries that is invaginates Bowman's capsule. The relatively wide diameter afferent arteriole enters Bowman's capsule at the vascular pole of the renal corpuscle and then forms the network of glomerular capillaries. The efferent arteriole drains the glomerulus and leaves the capsule at the vascular pole which is usually situated opposite the entrance to the renal tubule, the urinary pole. Bowman's capsule consists of a single layer of flattened cells resting on a basement membrane; it forms the distended, blind end of the renal tubule. Bowman's capsule has two layers: visceral and parietal layers.

The internal or visceral layer of the glomerular capsule surrounds the glomerular capillaries with modified epithelial cells, called podocyte. At the vascular pole of the renal corpuscle, the epithelium of the visceral layer reflects to form the simple squamous parietal layer of the glomerular capsule. The space between the visceral layer and the parietal layer of the renal corpuscle is called the capsular (urinary) space. There are numerous nuclei in the glomerulus that are capillary endothelial cells, mesangial cells and podocytes.In 1 year *Huso huso* kidney, two to four glomeruli were observed within each cluster. But, in 2 year *Huso huso* the number of glomeruli was increased in each cluster about three to seven (Fig. 2 & 5).

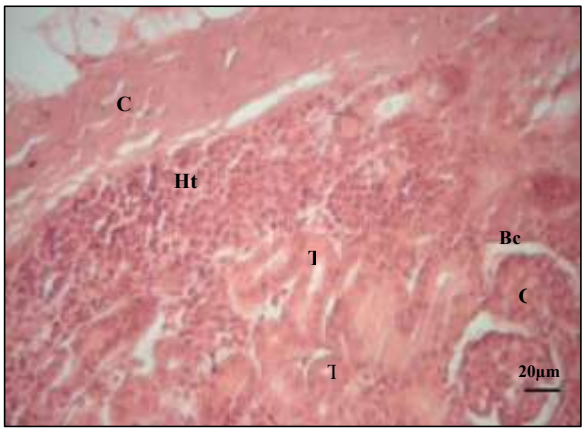

Fig. 1: The kidney of 2 year old *H. huso*, Kidney Capsule (C), hematopoietic tissue (Ht), tubules (T), Glomerulus (G) and Bowman's capsule (Bc); (H&E, 20X)

Fig. 2: The kidney of 1 year old H. huso, Glomerulus (G), Bowman's capsule (Bc), Tubule (T), in glomerular clusters; (PAS, 20X)

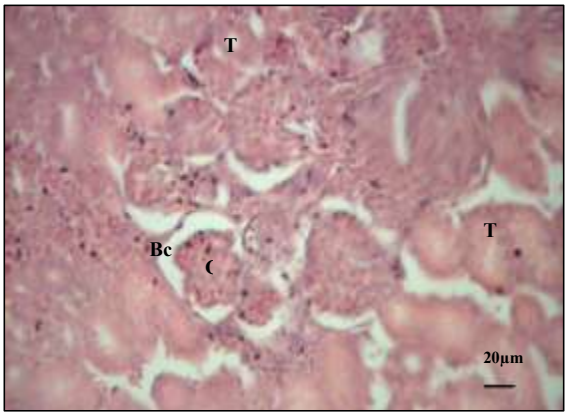

Fig. 3: The kidney of 2 year old *H. huso*, Glomerulus (G), Bowman's capsule (Bc), Tubule (T), in glomerular clusters; (H&E, 20X)

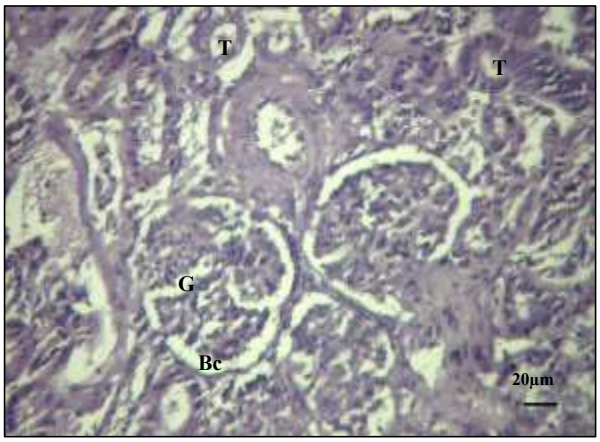

Fig. 4: The kidney of 1 year old *A. persicus*, Glomerulus (G), Bowman's capsule (Bc), Tubule (T), in glomerular clusters; (PAS, 20X)

Fig. 5: The kidney of 2 year old *A. persicus,*Glomerulus (G), Bowman's capsule (Bc), Tubule (T), in glomerular clusters; (H&E, 20X).

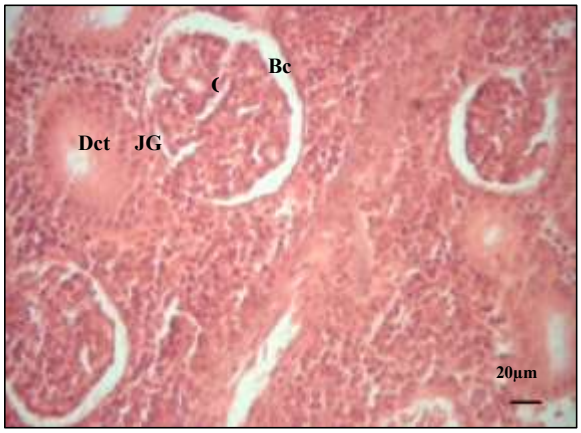

Fig. 6: The kidney of 2 year old *H. huso*, Glomerulus (G), Bowman's capsule (Bc), Distal tubule (Dct) and Juxta Glomerular apparatus; (H&E, 20X).

Fig. 7: The kidney of 2 year old *H. huso*, Proximal tubule I (Pct I), Proximal tubule II (Pct II), Lumen (L) and Brush border (Bb); (H&E, 20X).

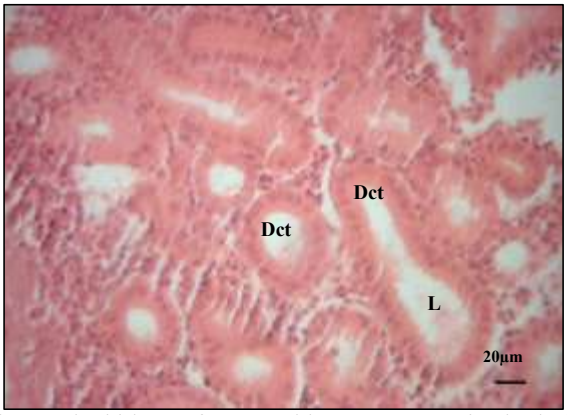

Fig. 8: The kidney of 2 year old *A. persicus*, Distal tubule (Dct), Lumen (L); (H&E, 20X).

In 1 year *Acipenser persicus* the kidney consists of dispersed glomeruli, mostly. But in 2 year *Acipenser persicus* glomeruli were grouped as small clusters (Fig. 3, 4 & 6).

Juxta Glomerular Apparatus: The juxtaglomerular apparatus (JGA) in *Huso huso* and *Acipenser persicus* is a specialization of the glumerular afferent arteriol and the distal convulated tubule of the corresponding nephron. The apparatus consists of two components, the juxtaglomerular cells and the macula densa. Juxta glomerular cells are specialized smooth muscle cells of the wall of the afferent arteriole before it enters the glomerulus. The macula densa is an area of closely packed, specialized cells lining the distal convulated tubules adjacent to the vascular pole. These cells are taller and have larger, prominent nuclei than the distal tubule cells. The macula densa and the juxtaglomerular cells are separated by a thin basement membrane (Fig. 6).

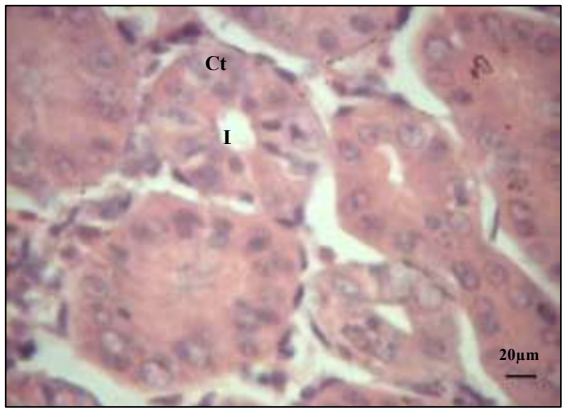

Fig. 9: The kidney of 1 year old *A. persicus*, Collecting tubules (Ct), Lumen (L); (H&E, 20X)

Proximal Convoluted Tubule: The proximal convulated tubule in *Huso huso* and *Acipenser persicus* is the longest, widest and most developed segment of the nephron. This tubule is lined by eosinophilic-granular simple cuboidal cells with a well-developed brush border. In these cells, the nuclei are mainly spherical and situated in the lower part of the cells. There were two proximal tubule segments: the first proximal tubule has a well developed brush border with cuboidal cell. But the second proximal tubule has distended lumen and the brush border was lower and sparser than the first type with the high columnar cells and central nucleous (Fig. 7).

Distal Convoluted Tubule: Distal convulated tubules in *Huso h uso* and *Acipenser persicus* are short and not encountered as frequently in sections as proximal tubules. Their luminal diameters are greater than in proximal tubules. More cells line them and the brush border is not seen. They have cuboidal epithelium and their cytoplasm stains less acidophilic than proximal tubules. The elliptical or spherical nuclei are more centrally to Para basally located. Some of the tubules look oval but others had the spherical feature (Fig. 8).

Collecting Convoluted Tubule: The collecting tubules in *Huso huso* and *Acipenser persicus* are quite large, with walls formed of high cuboidal epithelium. Their nuclei are mainly spherical that are located in the central part of the cells. They are light-staining cells with distinct lateral border. Collecting tubules are larger than proximal and distal tubules (Fig. 12 & 13).

DISCUSSION

The morphology of kidney in fishes varies [16]. Based on the result of this study, the kidney of both species (*H. Huso*, *A. persicus*) is paired and slender which is elongated from the anterior to caudal part along the spinal cord that resembles with Silver sea bream (*Sparus sarba*) [17] and Killfish (*Fundulus heteroclitus*) [18]. But the kidney of trout is a long couple with massive body [19]. Indeed, the mesonephric kidney in amphibian is a long and slender organ located on either side of the aorta [5]. The kidney of *H. Huso* and *A. persicus* is Y shaped like that of the Perciformes consistsing of a Y- shaped organ [20]. Like the teleostean [21] and Osteichthyans [22] the kidney of *H. huso* and *A. persicus* is divided into three parts: the head, body and caudal part. However, as a result of Milano *et al*. [20], the kidney of Cyprinodontiformes has a homogene structure and there are no any distinction between the head and caudal part. Therefore, the anterior and posterior part may not be distinguished. These sections are composed of renal and hematopoietic tissues [22]. The head of the kidney in Salmoniformes, such as *Salmo trutta fario* and *Coregonus lavaretus,* consists of lymphoid, endocrine and hematopoietic tissue with isolated malpighian bodies and nephrons [23]. As a result the head of kidney in *H. huso* and *A. persicus* mainly consists of hematopoietic tissue and interrenal glands without any malpighian body or nephron tubules.

The kidney is composed of anterior, which consists of hematopoietic tissue without any renal tubules and glomeruli and the posterior, which containes of hematopoietic tissue and excretory parts like in Perciformes and Cyprinodontiformes [20]. Fange [24] showed that the head of kidney in sturgeons is a universal hemopoietic organ, which is in agreement with the results of the present study. Also, Krayushkina *et al*. [9] and [25] belived that the most part of caudal tissue of kidney containes the highest number of nephrons and the caudal kidney is composed of nephrons surrounded by hematopoietic tissue dispersed throughout the organ. The anterior of kidney in *A. persicus* such as teleosteans consists of lymphoid and hematopoietic tissue [26]. Indeed, Charmi *et al*. [27], reported that in the head of the kidney of *Huso huso* there are not any nephron cells and only hemopoietic tissue and interrenal cells are distributed in this part. Moreover, the results showed that the hemopoietic tissue covers the head of kidney and it is similar to Hoar and Randall`s report [7] which shows that the variable amounts of hemopoietic tissue are distributed among the tubules and vascular spaces in the body of the kidney in teleosts.

The kidney consists of nephrons, which are functional and structural unite [5]. Also, an overall zonation would appeare in sturgeon kidney [1].

Proximal: In young *H.huso* and *A.persicus,* the proximal tubules have a tall epithelium with the brush border on the apical surface with the basal nuclei [9]. In *A. naccarii,* these cells are composed of squamous and slightly rounded cell [1]. The proximal tubules of *H. huso* and *A. persicus* like *A. naccarii* [10], *A. brevirostrum* and *A. oxyrhynchus* [28], Euryhaline teleostean [29], Elasmobranches [30] have two types, namely the first and the second proximal tubule. The first proximal tubules were also reported in lamprey by Hoar & Randall [7]. But, the *Polyodon spathula* had just one type proximal tubule and the second proximal tubule was not observed [23]. The proximal tubular epithelium of columnar cells has a prominent brush border [31]. As described by Cataldi *et al*. [10], two proximal tubule segments were identified by different heights of cells with different sparse of brush border.

Distal: The distal tubules have lower epithelium than proximal tubules and the nuclei are not basal and spherical [9]. As a result of this study, like Elasmobranches [7] distal tubules in sturgeons have cuboidal epithelium without brush border. In general, the proximal and distal tubules characteristics in sturgeons are similar to those of other teleosteans [21, 32].

Collecting Tubules: The collecting tubules in sturgeons, due to of their regular feature and columnar epithelium [7, 18, 17] differ from other nephron cells. Similar to our results, the collecting tubules in Silver sea bream (*Sparus sarba*) have a circular lumen without any curvature or folding [17]. While, in Perciformes the collecting tubules have high dispersion in the caudal part of kidney [20]. In line with Cataldi *et al*. [33] issue, report that these cells in tilapia are recognized by the high columnar cells and the connective layer around them. In addition, they noted that in tilapia, the collecting tubule has basally located nuclei. While, in sturgeons, the cells are high cuboidal with centrally located nuclei.

Renal Corpuscles: The glomerulus is a tuft of capillary which occupies the Bowman`s spaces [10, 34]. Cataldi *et al*. [10], stated that the glomerulus in *A. naccarii* has multilobed glomerulus. Glumeruli were grouped as clusters in *A. persicus* and *H. huso* kidney. So, the clusters in 2-year-old fishes were greater and more numerous than 1-year-old ones. Indeed, clusters of glumeruli were larger and more numerous than in *A. persicus.* Harder [35] described that in the kidney of adult sturgeons, the glomeruli were grouped as 5 to 6 clusters. The development of diameter, number and size of the glumeruli clusters is strongly age-dependent. As described by Won & Woo [17], the glomeruli were grouped as small clusters in the peripheral regions of the kidney in Silver sea bream (*Sparus sarba*). Unlike the previous-mentioned findings, many teleostean kidneys consist of dispersed glomeruli [36]. The renal corpuscles are globular and small, relatively larger than proximal tubules. The present findings also show that the size of renal corpuscle is not related to its location. Hematopoietic tissue surrounds the renal corpuscles [1]. The structure of the sturgeon`s renal corpuscles differ from mammals and other fishes [37]. The function of different layers of parietal layer in sturgeon renal corpuscle is still unknown [1]. The presence of mesangial cells in glomerulus in other sturgeons was reported by Gambaryan [11]. The mesangial cells are obstacles for free molecules and ions through the filtration process [1]. In addition, Hickman [29] described that mesangial cells are vital for quick adaptation to different environments. As described by Mobjerg *et al*. [5] renal corpuscles in amphibian have two structures; Glomerulus and Bowman's capsule. The Bowman's capsule consists of a parietal layer and a visceral layer which surrounds the glomerular capillaries. So, this structure is similar to glomerulus of *A. naccarii* [1], amphibians [5].

Considering all the above mentioned facts, Gambarian come to the conclusion that the special characteristic of sturgeon nephrons is their heterogeneity, which cause to their adaptation to different salinities.

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