Original Article

Morphometric and Normal 2D CT Anatomic Study of the Vertebral Column of the European Pond Turtle (*Emys orbicularis*)

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ABSTRACT

Background: European pond turtle is one of the two species of freshwater turtles in Iran. Regarding clinical examinations and diagnostic imaging techniques, it is necessary to have complete anatomical information on this turtle.

Objectives: This study provided complete morphometric and normal two-dimensional computerized tomographic scanning information of the vertebrae of European pond turtles.

Methods: Ten European pond turtles were used in this study. Computerized tomography (CT) scans were taken from each anesthetized turtle. Then, morphometric parameters were measured in the CT scans of the vertebral column.

Results: Atlas was the shortest of the cervical vertebrae, and the eighth cervical vertebra was shorter than the previous vertebrae. The articular surface of the caudal articular processes of the eighth cervical vertebra was bent, and these surfaces were almost vertical. Transverse process width had remained constant in the cervical vertebrae. The transverse process was not observed in the dorsal vertebrae. The first dorsal vertebra had a different shape than others.

Conclusion: The particular shape of the last two cervical vertebrae, especially the arched shape of the eight vertebrae. The seventh and eighth cervical vertebrae have the largest transverse distance between caudal articular processes that seem necessary for cervical motion. The limited space of the caudal cervical vertebrae inside the shell chamber can be the reason for the reduction in the length of these vertebrae. The absence of a spinous process in the seventh and eighth cervical vertebrae of the neck may be related to their specific position in the neck retraction.

Keywords: 2D CT Anatomy, European pond turtles, Morphometry, Vertebral column

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1. Introduction

The skeletal system is a very important organ of the body, and the position of other systems is usually defined in relation to this system. Also, the components of this system are used as a topographic guide in diagnostic imaging methods, so the direct study of bones is necessary (Sisson and Grossman, 1975).

The European pond turtle is one of the two species of freshwater turtles in Iran. No diagnostic imaging studies have been performed on the skeletal system of this species. However, similar studies have been done on other species. So far, various studies have been performed on different organs of the body in different species of turtles in the world, including joint radiological and anatomical works of the following researchers.

Valente et al. (2006) examined radiographs of the neck and trunk of the red sea turtle (Caretta caretta). They provided helpful indicators for identifying internal organs, including the bronchi, coracoid bone, and acetabulum. In 2007, they studied the radiographical anatomy of the limbs of C. caretta and described their normal radiographic profiles. The researchers used three-dimensional (3D) computerized tomography (CT) scans to describe these profiles. Valente et al. (2007a) analyzed a CT scan of the vertebrae and coelomic cavity of Caretta caretta. They noted some essential points, such as the position of the various organs of the coelomic cavity compared to the carpus and vertebrae. One of the essential points of their study was that the trachea is bifurcated more cranially than in other turtles in this species, which has been attributed to the inability of this species to contract its neck. Young et al. (2019) did a comparative limb bone scaling study in turtles. Ampaw et al. (2019) did a compressive study of the deformation and failure of trabecular structures in a turtle shell. Schachner et al. (2017) studied the pulmonary anatomy of a common snapping turtle. Ricciardi et al. (2019) did a multidetector computed tomographic study of the lungs in the loggerhead sea turtle.

Lyson & Joyce (2012) studied the relationship between the scapula and the rib cage topologically. They found that the shoulder girdle was located inside the shell and in front of the rib cage. Sheil (2003) examined the morphology of bones during the embryonic period in the Apolone spinifera and compared it with another tortoise species. Adult tortoise bones have also been studied in detail in this study. Sanchez-Villagra et al. (2007) studied bone morphogenesis during the embryonic period of the Pelodiscus sinensis, a Chinese soft-shelled tortoise, focusing on the pattern and ossification sites. They found differences between this species and Apolone spinifera at different bone formation times. Joyce & Bell (2004) reviewed a comparison of the bone morphology of the Testudines order, which includes wetland and terrestrial species. Their initial results suggest that ontogenic changes in skeletal structure may be one of the main reasons for differences within species of this order.

Sheil and Greenbaum (2005) re-examined the development of different bones in the body of Chelydra serpentine and noted differences between species based on previous studies of other species. Sanchez-Villagra et al. (2007) studied the carpal and tarsal bones in 25 species of adult side-necked turtles and found greater diversity in manus and pes morphology. Davari et al. (2020) studied the anatomical features of the lungs in the Caspian pond turtle by CT scan. Zehtabvar et al. (2014) studied the anatomical features of the coelomic cavity in the European pond turtle by CT scan and radiography. In 2015, these researchers also studied the anatomical features of the non-respiratory organs of the European pond turtle coelomic cavity.

Regarding clinical examinations and diagnostic imaging techniques, it is necessary to have complete anatomical information of the examined animal and to consider these features in various studies. Also, to interpret the injuries to the spine and the shell and better understand the relative position of the internal organs of the body, normal radiographs and CT scans are suitable tools to achieve the above goals. This study analyzed the anatomical and two-dimensional (2D) CT scans of the vertebral column and compared the results with other available sources. By doing this study and similar studies, the first and necessary steps can be taken to identify, preserve, and maintain this biological reserve better. In addition, using morphometric measurements interpret the spine structure in the European pond turtle better.

2. Materials and Methods

Study samples

Our study samples were 10 male adult European pond turtles (*Emys orbicularis*) with Mean±SD weight of 450±45.22g. The specimens were kept in reptile-suitable conditions for one week to get used to the environment. During this period, whole fish carcasses (Black Sea sprat, Clupeonella cultriventris) were used to feed the turtles.

The identification keys provided in the references were used to select the turtles and separate the males from the females. In this species, the iris in males is reddish and orange, while it is almost yellow in females. The number of yellow spots on the head and neck of males is smaller and fewer than in females. In addition, males have sunken plaster compared to females (Alinezhad et al. 2019).

Computed tomography (CT) scanning

First, the turtles were transferred to the Radiology Department of the Small Animal Hospital of the Faculty of Veterinary Medicine, University of Tehran, Tehran City, Iran. Then, they were anesthetized by intramuscular injection of ketamine (25 mg/kg) and diazepam (1 mg/kg) (Carpenter and Marion, 2018). The Siemens Somatom Spirit 2 CT-Scan machine was used to prepare images.

Technical parameters for this imaging protocol were as follows: rotation time, ls; slice thickness, 1mm; reconstruction interval, 0.5-1mm; pitch, 1; x-ray tube potential, 120kV; and x-ray tube current, 130mA.

Appropriate window width (WW) and window level (WL) were selected to take each section's graph mentioned in each section's CT scan results. Bone windows were used to check the images. The turtles were not euthanized after the study, and studies were performed on CT scans. The turtles studied in this article are still alive and well at the time of writing this article.

Morphometric study

After analyzing the CT scan and identifying the different sections, the parameters were measured in the CT scan images of the vertebral column. The measured parameters are described in Table 1. The results of the measurements are shown in Tables 2-4. Morphometric mensuration from digital CT images was performed with Syngo MMWP VE40A software.

Statistical analysis

Statistical analyses were done by SPSS software v. 24.0. The descriptive statistics are mean and standard deviation. Parameters were compared by running paired sample t test. P values less than 0.05 was statistically considered significant.

3. Results

2D CT scan

This species had eight cervical vertebrae, 10 dorsal vertebrae, 2 sacral vertebrae, and 25 caudal vertebrae. The cervical vertebrae were highly mobile, and there were no cervical ribs. The dorsal vertebrae were immobile and fused. The neural spines were fused and integrated from the back with a carapace neural plate. The sacrum had two vertebrae. The tail also had 25 highly mobile caudal vertebrae (Figure 1).

The cervical region had 8 vertebrae; each had a specific shape. The first and eighth vertebrae were significantly wider compared to their length.

Atlas (the first cervical vertebra) had two neural arches, a centrum, and an intercentrum. The cranial part of the vertebra included a cranial articular cavity to articulate with the occipital condyle. The ventral part of the centrum had a crest. The centrum had a foramen on either side (lateral vertebral foramen) (Figures 1 and 2).

Axis (the second cervical vertebra) was more elongated than the atlas. The width was significant in the region of caudal articular processes. Transverse processes were located on both sides of the cranial surface of the centrum (Figures 1 and 2).

The third, fourth, and fifth cervical vertebrae were very similar. The intervertebral foramen was formed between the vertebrae. This foramen was also formed between the second and third vertebrae. The transverse process was located in the cranial part of the vertebrae (Figures 1 and 2).

The sixth cervical vertebra was similar in appearance to the preceding vertebrae. The caudal articular processes were arched (Figures 1 and 2).

The general shape of the seventh vertebra was similar to the preceding vertebrae but is wider. No spinous process was observed in this vertebra. Caudal articular processes were arched (Figures 1 and 2).

The eighth cervical vertebra had a unique shape. The length of the vertebra was shorter than that of the preceding vertebrae. The articular surface of the caudal articular processes was sharply indented and bent, and the surface was almost vertical. Caudal articular processes were larger than cranial articular processes and had more

Table 1. Morphometric parameters of the vertebral column of the European pond turtle (*Emys orbicularis*)

Abbreviations	Parameters	Descriptions
VBH	Vertebral body height	Distance from the base of the vertebrae to the vertebral canal in the transverse view, the maximum distance was measured. In the atlas, the height of the ventral arch was measured before the vertebral foramen.
VBL	Vertebral body length	The length of the vertebral body in the midsagittal view, the maximum distance, was measured.
TPW	Transverse process width	Distance between the end of the right and left transverse processes in the transverse view was measured just in the cervical, sacral and caudal parts. Regarding the measurement of this parameter in the atlas, the transverse distance between the right and left wings was measured.
TDCA	Transverse distance between caudal articular processes	Distance between the right and left caudal articular processes in the transverse view was measured; in the case of dorsal vertebrae, the width of the posterior part of the body was measured as this parameter.

Table 2. Measured parameters of the cervical vertebrae (C1-C8) of the European pond turtle (*Emys orbicularis*) (mm)*

Parameters	Mean±SD							
	C1	C2	C3	C4	C5	C6	C7	C8
VBH	4.73±0.18^{a}	3.27±0.05^{b}	3.32±0.08^{b}	3.06±0.14^{b}	2.75±0.18^{c}	1.96±0.22^{d}	1.80±0.17^{d}	2.67±0.05^{e}
VBL	2.86±0.11^{a}	7.40±0.10^{b}	11.12±0.15^{c}	12.46±0.15^{d}	10.17±0.22e	9.76±0.07^{f}	8.45±0.11^{g}	5.51±0.17^{h}
TPW	8.13±0.09^{a}	7.95±0.09^{a}	8.14±0.18^{a}	8.25±0.08^{a}	8.21±0.08^{a}	7.99±0.13^{a}	7.96±0.08^{a}	8.19±0.18^{a}
TDCA	-	7.87±0.07^{a}	7.56±0.22^{a}	6.71±0.11^{b}	6.56±0.15^{b}	6.34±0.10^{b}	7.85±0.23^{c}	7.88±0.10^{c}

$^{a\text{-}h}$: Different letters in each cell represent a significant difference between parameters of the different vertebrae ($P<0.05$). The statistical analysis of the parameters recorded in different columns has been done, and a comparison between rows has not been made.

VBH: vertebral body height; VBL: vertebral body length; TPW: transverse process width; TDCA: the transverse distance between caudal articular processes.

arches than other vertebrae. No spinous process was observed in this vertebra (Figures 1 and 2).

The ribs, dorsal vertebrae, and dermal bones formed a single bone called the carapace. The count of dorsal vertebrae was 10, to which a pair of ribs (costal heads) was attached (Figures 1 and 2).

The first dorsal vertebra had a different shape than the rest of the vertebrae, and its shape was significantly altered to articulate with the eighth cervical vertebra. A large articular surface was seen between the eighth cervical vertebra and the first dorsal vertebra. From the cranial part of the first dorsal vertebra's centrum, two delicate bone rods were elongated toward the second rib and finally attached to the cranial rim of the second rib head (Figure 1). The rest of the dorsal vertebrae were similar in appearance. It should be noted that the transverse process was not observed in the dorsal vertebrae.

Table 3. Measured parameters of the dorsal vertebrae (D1-D10) of the European pond turtle (*Emys orbicularis*) (mm)*

Parameters	Mean±SD									
	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10
VBH	1.34±0.12^{a}	1.06±0.11^{a}	0.91±0.05^{a}	1.31±0.12^{a}	1.33±0.14^{a}	1.47±0.09^{a}	1.25±0.18^{a}	1.49±0.33^{a}	1.98±0.08^{b}	2.14±0.25^{b}
VBL	5.53±0.18^{a}	10.08±0.19^{b}	10.03±0.09^{b}	10.01±0.22^{b}	10.04±0.15^{b}	10.01±0.22^{b}	9.84±0.13^{b}	4.55±0.24^{c}	4.40±0.12^{c}	4.63±0.06^{c}
TDCA	7.61±0.08^{a}	7.77±0.05^{a}	7.84±0.15^{a}	7.61±0.18^{a}	7.69±0.19^{a}	7.85±0.31^{a}	7.77±0.22^{a}	7.76±0.05^{a}	7.88±0.19^{a}	7.75±0.19^{a}

$^{a\text{-}c}$: Different letters in each cell represent a significant difference between parameters of the different vertebrae ($P<0.05$). The statistical analysis of the parameters recorded in different columns has been done, and a comparison between rows has not been made.

VBH: vertebral body height; VBL: vertebral body length; TDCA: the transverse distance between caudal articular processes.

Table 4. Measured parameters of the sacral (S1, S2) and caudal vertebrae (Ca1-Ca3) of the European pond turtle (*Emys orbicularis*) (mm)*

Parameters	Mean±SD				
	S1	S2	Ca1	Ca2	Ca3
VBH	2.12±0.22[a]	2.18±0.34[a]	2.03±0.07[a]	2.12±0.05[a]	2.09±0.25[a]
VBL	3.66±0.32[a]	3.60±0.22[a]	3.50±0.24[a]	2.73±0.27[b]	2.77±0.06[b]
TPW	19.51±0.06[a]	13.02±0.15[b]	5.67±0.31[c]	5.48±0.33[c]	5.35±0.13[c]
TDCA	4.71±0.19[a]	4.53±0.09[a]	3.08±0.07[b]	3.12±0.12[b]	3.02±0.21[b]

[a-c]: Different letters in each column represent a significant difference between parameters of the different vertebrae ($P<0.05$).

The statistical analysis of the parameters recorded in different columns has been done, and a comparison between rows has not been made.

VBH: vertebral body height; VBL: vertebral body length; TPW: transverse process width; TDCA: the transverse distance between caudal articular processes.

The costal heads were connected proximally to the vertebrae and distally to the dermal bones. These plates were the costal dermal bones. The first and second costal heads were attached to the first costal dermal bone, and the ninth and tenth costal heads were attached to the eighth costal dermal bone (Figure 1). Due to the fusion of the dorsal vertebrae, there were no intervertebral foramina between them; however, very small lateral vertebral foramina were observed.

The sacrum consisted of two vertebrae. These vertebrae were not bonded to the carapace. Transverse processes in the first sacral vertebra had an articular surface for the ilium (Figures 1 and 2).

The tail had 25 caudal vertebrae. Transverse processes became smaller in the caudal vertebrae. The length of the vertebrae towards the caudal was gradually reduced (Figure 2).

Morphometric study

Results of morphometric studies of different parts of the vertebral column have shown in Tables 2-4. As shown in Table 2, in the cervical vertebrae, the size difference between C1 and C2 was statistically significant concerning vertebral body height (VBH), which became shorter ($P<0.05$). The difference in VBH size from C2 to C4 was not significant, and the VBH remained constant ($P>0.05$). The difference in VBH size from C4 to C6 was significant, and the VBH became shorter ($P<0.05$). The difference in VBH size between C6 and C7 was not significant, and the VBH remained constant ($P>0.05$). The difference in VBH size between C7 and C8 was significant, and VBH became longer ($P<0.05$).

In the cervical part of the vertebral column, the difference in vertebral body length (VBL) size from C1 to C8 was significant, and the VBL became longer from C1 to C4 and shorter from C4 to C8 ($P<0.05$). Atlas was the shortest of the cervical vertebrae.

Regarding the transverse process width (TPW), the size difference from C1 to C8 was not significant, and the TPW remained constant ($P>0.05$). Concerning the transverse distance between caudal articular (TDCA) processes, the size difference between C2 and C3 was not statistically significant, and the TDCA remained constant ($P>0.05$). The difference in TDCA size between C3 and C4 was statistically significant, and the TDCA became shorter ($P<0.05$). The difference in TDCA size from C4 to C6 was not significant, and the TDCA processes remained constant ($P>0.05$). The difference in TDCA size between C6 and C7 was significant, and the TDCA processes became longer ($P<0.05$). The difference in TDCA size between C7 and C8 was not significant, and the TDCA processes remained constant (Table 2) ($P>0.05$). The seventh and eighth vertebrae have the largest TDCA processes.

As presented in Table 3, in the dorsal vertebrae, the size difference from D1 to D8 was not statistically significant concerning VBH, and it remained constant ($P>0.05$). The size difference between D8 and D9 was statistically significant, and the vertebral body height became longer ($P<0.05$). There was no significant difference between the VBH size of D9 and D10, and vertebral body height remained constant ($P>0.05$). It should be noted that the difference in VBH size of C8 and D1 was statistically significant, and the VBH became shorter ($P<0.05$).

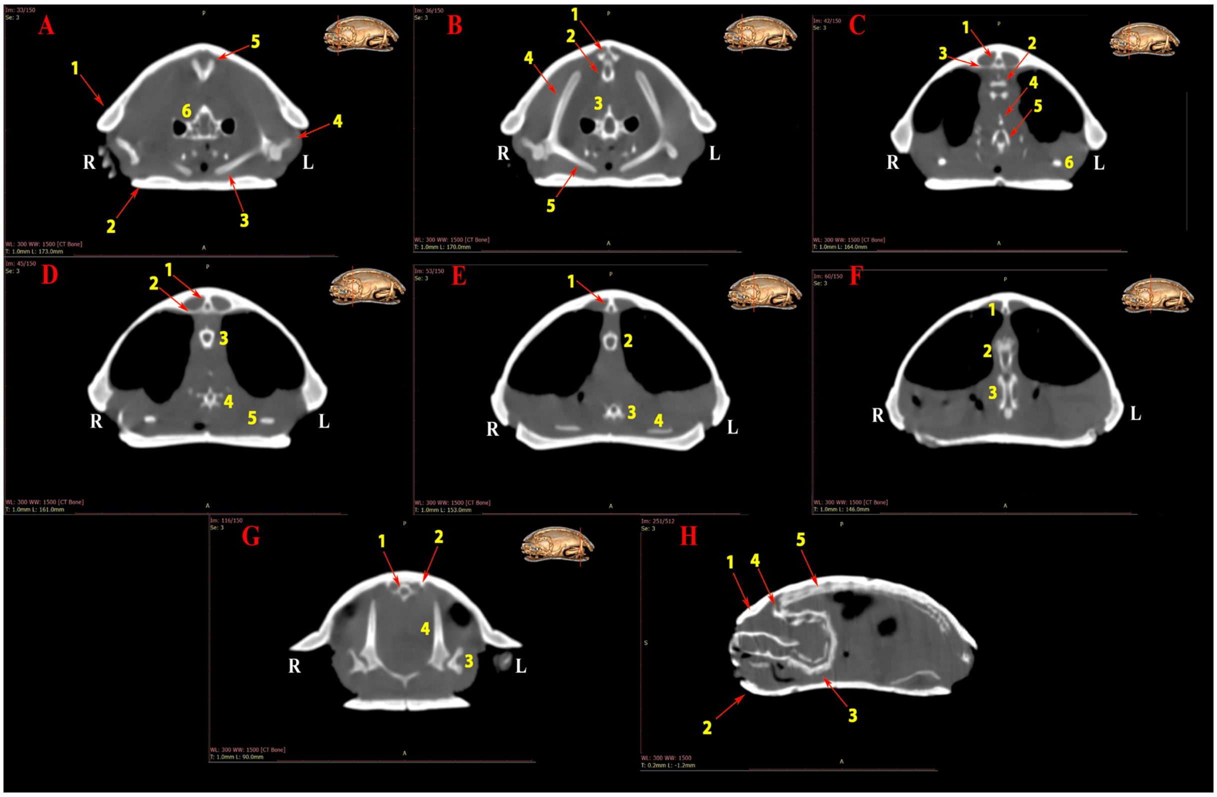

Figure 1. A-G: Transverse computerized tomography (CT) images of the European pond turtle (bone window)
Different parts of the image labeled. H: Sagittal CT image (Bone Window)

A: 1. Carapace, 2. Plastron, 3. Acromion, 4. Humerus, 5. The eighth cervical vertebrae, 6. Skull;

B: 1. the first dorsal vertebrae, 2. The eighth cervical vertebrae, 3. Skull, 4. Scapula, 5. Acromion;

C: The 1st dorsal vertebrae, 2. The seventh cervical vertebrae, 3. The head of the first rib, 4. The supraoccipital process, 5. Atlas, 6. Coracoid;

D: 1. The 2nd dorsal vertebrae, 2. Head of the second rib, 3. The seventh cervical vertebrae, 4. Axis, 5. coracoid, E: 1. The third dorsal vertebrae, 2. The sixth cervical vertebrae, 3. The third cervical vertebrae, 4. Coracoid;

F: 1. The 4th dorsal vertebrae, 2. The fifth cervical vertebrae, 3. The fourth cervical vertebrae;

G: 1. The 10th dorsal vertebrae, 2. Head of the ninth rib, 3. Femur, 4. Ilium;

H: 1. Carapace, 2. Plastron, 3. Cervical part of the vertebral column, 4. the eighth cervical vertebra, 5. the dorsal part of the vertebral column

Concerning dorsal vertebral VBL, as seen in Table 3, the size difference between D1 and D2 was statistically significant, and the VBL became longer ($P<0.05$). The difference in VBL size from D2 to D7 was not significant, and the VBL remained constant ($P>0.05$). The difference in VBL size between D7 and D8 was statistically significant, and the VBL became shorter ($P<0.05$). VBL size difference from D8 to D10 was not significant, and VBL remained constant ($P>0.05$). It should be noted that the difference in VBL size between D1 and C8 was not statistically significant, and the VBL remained constant ($P>0.05$).

As shown in Table 3, in the dorsal vertebrae, the size difference from D1 to D10 was not statistically significant for TDCA, and the TDCA processes remained constant ($P>0.05$). It should be noted that the difference in TDCA size between D1 and C8 was not statistically significant, and TDCA processes remained constant ($P>0.05$).

As presented in Table 4, in the sacral and caudal vertebrae, the size difference from S1 to Ca3 was not statistically significant regarding VBH, and it remained constant ($P>0.05$). It should be noted that the difference in VBH size between S1 and D10 was not statistically significant, and the VBH remained constant ($P>0.05$).

As shown in Table 4, in the sacral and caudal vertebrae, the size difference from S1 to Ca1 was not statistically significant concerning VBL, and the VBL had remained constant ($P>0.05$). It should be noted that the difference in VBL size of D10 and S1 vertebrae was statistically significant, and the VBH became shorter ($P<0.05$). The difference in VBL size between Ca2

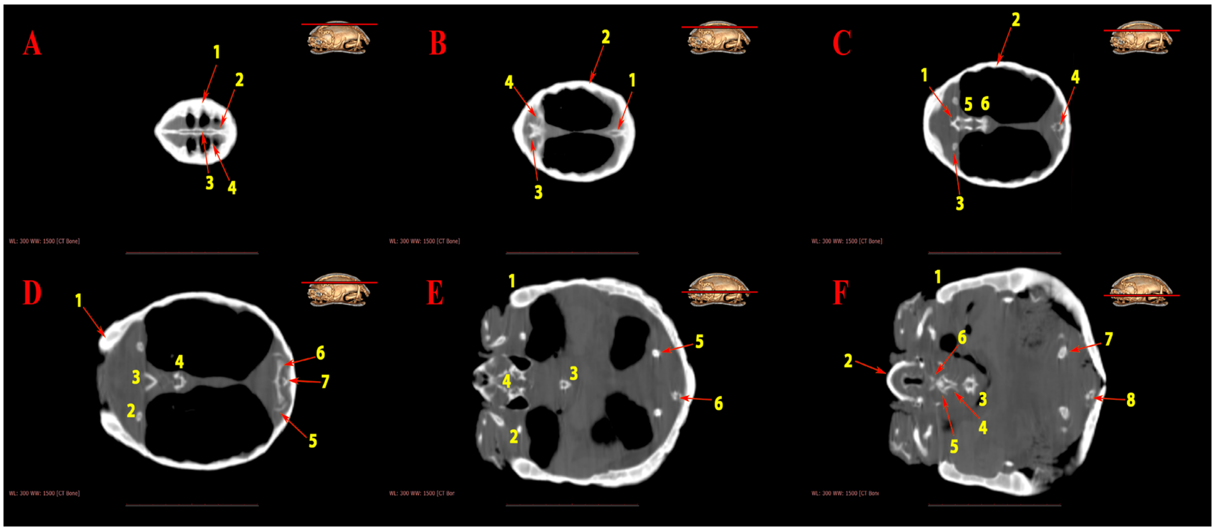

Figure 2. A-F: Dorsal CT image of the European pond turtle (Bone Window)

Different parts of the image labeled:

A: 1. Carapace, 2. Dorsal part of the vertebral column, 3. Vertebral canal, 4. Head of the rib;

B: 1. Dorsal part of the vertebral column, 2. Carapace, 3. The 1st dorsal vertebra, 4. Head of the 1st rib;

C: 1. The 8th cervical vertebra, 2. Carapace, 3. Scapula, 4. Dorsal part of the vertebral column, 5. The 7th cervical vertebra, 6. The 6th cervical vertebra;

D: 1. Carapace, 2. Scapula, 3. The seventh Cervical vertebra, 4. The 5th cervical vertebra, 5. Ilium, 6. The first sacral vertebra, 7. The second Sacral vertebra;

E: 1. Carapace, 2. Scapula, 3. The 4th cervical vertebra, 4. Skull, 5. Ilium, 6. Caudal vertebrae.

F: 1. Carapace, 2. Mandible, 3. The 3rd cervical vertebra, 4. Axis, 5. Atlas, 6. Skull, 7. Ilium, 8. Caudal vertebrae

and Ca1 was statistically significant, and the length became shorter (P<0.05). The difference in size from Ca2 to Ca3 was not statistically significant, and the VBL remained constant (P>0.05).

As seen in Table 4, in the sacral and caudal vertebrae for TPW, the size difference between the S1 and S2 vertebrae was statistically significant, and the TPW became shorter (P<0.05). The difference in TPW size between Ca1 and S2 was statistically significant, and the TPW became shorter (P<0.05). The difference in TPW size between Ca1 to Ca3 vertebrae was not statistically significant, and the TPW remained constant (P>0.05).

As shown in Table 4, in sacral and caudal vertebrae, the difference between S1 and S2 vertebrae was not statistically significant regarding TDCA, and it remained constant (P>0.05). It should be noted that the difference in TDCA size between S1 and D10 was statistically significant, and the TDCA became shorter (P<0.05). The difference in TDCA size between Ca1 and s2 was significant, and the TDCA became shorter (P<0.05). The difference in TDCA size between Ca1 and Ca3 was not significant, and the TDCA remained constant (P>0.05).

4. Discussion

Few studies have investigated turtles' bone anatomy and radiological appearance (Valente et al., 2006; Valente et al., 2007). In some of these studies, the settings of the radiology device and the required voltage for preparing radiographs of the desired quality in sea turtles have been considered. According to the findings of these studies, it is better to increase the kilo voltage in the anterior one-third of the carapace length and decrease it in the posterior one-third. It has also been suggested that it is best to use mammography films for more details in reptiles (Valente et al., 2006). In the present study, considering the used CT scan technique, the exact location of the bone structure was detectable, and the problems seen due to bone overlap on radiography were resolved.

In the present study, a large articular surface was seen between the eighth cervical vertebra and the first dorsal vertebra. In other words, since the head moves closer to or farther away from the body by moving these two vertebrae, the range of motion between the two vertebrae was wide, the joint surface was semicircular, and the contact surface between them was increased. To increase the range of motion between these two vertebrae, an arch has been created on the neck to allow the head to move as much as possible toward the shell. The cervical vertebrae of European ponds are very similar to those of Apolone spinifera, and both have eight highly mobile vertebrae (Sheil, 2003). However, in sea turtles, the first seven vertebrae of the neck are mobile, and the eighth cervical vertebra is fused to the carapace. Since sea turtles cannot pull their heads toward the shell, they do not have a caudal arch of the neck. Their vertebrae's length is approximately equal, while the European pond turtle's caudal cervical vertebrae's length is shorter than the cervical cranial vertebrae. The particular shape of the last two cervical vertebrae, especially the arched shape of the eight vertebra, is noticeable. The seventh and eighth vertebrae have the largest transverse distance between caudal articular processes and seem necessary in cervical motion. The absence of a spinous process in the seventh and eighth cervical vertebrae of the neck is related to their specific position in the neck retraction.

The limited space of the caudal cervical vertebrae inside the shell chamber can be the reason for the reduction in the length of these vertebrae (Zehtabvar et al., 2022). Valente et al. analyzed radiographs of the neck and trunk of the Caretta caretta (Valente et al., 2007b). They developed a series of landmarks to identify internal organs, such as the bronchi, sternum, and acetabulum. They mentioned that by viewing radiographic images, it is possible to determine a relationship between lateral and medial landmarks and to address the location of the coelomic cavity organs compared to the dermal plates of the carapace and the spine (Valente et al., 2006). In another study in 2007, Valente et al. analyzed the CT scan of the spine and the coelomic cavity of the Loggerhead Sea turtle (Caretta caretta) (Valente et al., 2007b). The researchers used anatomical slices to interpret the CT scan images better. They also determined the position of various organs of the coelomic cavity in relation to the carapace and spine, which facilitated the interpretation of other diagnostic techniques, such as radiography and sonography, and could ease biopsy and surgery (Valente et al., 2007a). These researchers also noticed that the intervertebral and lateral vertebral foramen in the Loggerhead Sea turtle is similar to the European pond turtles.

Since the carapace is attached to the spine, carapace trauma can lead to spinal cord injury and neurological symptoms. Radiography is the best way to diagnose this fracture type in turtles. Radiographs taken by Valente et al. in 2006 from the trunk of a Caretta caretta showed significant overlap, especially in the cranial part of the carapace, in such a way that nuchal bones, entoplastron, and vertebrae in this part were indistinguishable (Valente et al., 2006). As mentioned in the European pond turtle (*Emys orbicularis*) dorsal vertebrae, the structure of the transverse process was not observed. As mentioned in the references, the transverse process has located in the thoracic region and attached to the intertransverse ligaments and muscles related to this region (König, et al. 2007). It seems that the lack of the transverse process in the dorsal vertebrae of the current study turtle is related to the deformation of the ribs and the absence of the muscles and ligaments mentioned earlier in the coelomic cavity. In addition, it should be noted that the transverse process was observed in the cervical, sacral, and caudal vertebrae, and subsequently, the function of this structure was required in these parts. Transverse processes created the widest width in the first sacral vertebra.

In 2015, Werneburg et al. (2015) studied the development of vertebrae shape and neck retraction in modern turtles with a geometric and morphometric approach. Modern turtles have neck retraction ability, one group is side-necked (pleurodiran), and the other is hidden-necked (cryptodiran). The researchers stated that the anatomical changes that led to the vertebral shapes of modern turtles had not been well understood yet. It has been mentioned that there is no correlation between the construction of formed articulations in the cervical center and neck mobility. Excessive mobility between the vertebrae, together with a change in the shape of the vertebrae, has led to a more advanced ability to contract the neck (Werneburg et al. 2015). European pond turtle is a type of hidden-necked (cryptodiran). Our study examined the spine's structure so that several essential structural features and compatibility of the articular vertebrae were observed for the neck retraction process. It has been noted that in turtles that cannot retract the neck, the cervical vertebrae are compact and short. Generally, it has been pointed out that the morphometric method is one of the best methods to study the structural differences of cervical vertebrae in turtles (Werneburg et al. 2015). In our study, the morphometric method was used to analyze vertebrae changes in different areas.

It has been reported that the cervical vertebrae of cryptodiran are compressed dorsoventrally, which was almost observed in our study (Werneburg et al. 2015). He reported that the articular surface of the caudal articular process of the eighth cervical vertebra is almost vertical, which was also observed in our study. Concerning muscle adaptation involved in the movement, broad cervical vertebrae are more suitable for cryptodiran, whereas long cervical vertebrae are in pleurodires (Werneburg, 2011).

Turtles have a group of HOX genes that influence the arrangement of axial skeletal components. Modifying some of these genes leads to sudden changes in development (Asadi Ahranjani et al. 2016). The shape and position of the bones in the turtle's body can also be evaluated developmentally. It has been said that this position is similar to that of reptiles, amphibians, and early mammals. Lyson & Joyce (2012) examined the relationship between the scapula and the rib cage topologically and found that the scapula in turtles is placed vertically inside the shell and in front of the ribs. This position is also seen in early Amniotes, such as amphibians, laying mammals, and reptiles of the Lepidosaur group. They concluded that turtles' developmental studies should be compared with laying mammals and reptiles of the Lepidosaurus group, which are more similar to turtles, instead of mice and chickens (Lyson & Joyce, 2012).

The necks of cryptodira, by contrast, are characterized by cervical joints that become increasingly more mobile towards the posterior. This observation may explain the orderly anterior-posterior shape patterning their vertebrae form in morphospace (Jones et al., 2012).

Regarding neck retraction, the main part of this movement is done with the retrahens capitis collique muscle, which is done ventrally to the cranium and ventrolaterally in all turtles (Jones et al., 2012). Other muscles that function to revert the retracted neck are attached to the dorsal surface of the vertebrae in cryptodira, so the wide cervical vertebrae are a feature of this group of turtles. In the European pond turtle, we observed that the last two cervical vertebrae are not wider than the others, but the distance between the caudal articular process is greater than the earlier vertebrae.

According to the present study results, diagnostic imaging techniques such as CT scans are beneficial for studying the skeleton system. This technique facilitates determining the correct direction and position of the bones. In this study, the position of different parts of the European pond turtle spine in 2D CT scans was determined, which can be used to diagnose various issues. The course of resizing different parts of the spine was also examined.

Due to the close relationship between the bones and the muscles and the effect of their tension on the shape and the formation of various processes on the bones, it is recommended to analyze the muscles of this species. It is also suggested to compare the skeleton of this species with other freshwater-dependent species and identify their differences.

Ethical Considerations

Compliance with ethical guidelines

This study was extracted from a doctoral dissertation and all experimental procedures were approved by the Faculty of Veterinary Medicine, University of Tehran Local Ethics Committee (30704/6/5).

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Authors' contributions

All authors equally contributed to preparing this article.

Conflict of interest

The authors declared no conflict of interest.

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مقاله پژوهشی

مطالعه مورفومتریک و سی تی آناتومی دو بعدی ستون مهره‌ها در لاک‌پشت برکه‌ای اروپایی (Emys Orbicularis)

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چکیده

زمینه مطالعه: لاک‌پشت برکه‌ای اروپایی یکی از دو گونه لاک‌پشت آب شیرین ایران است. برای انجام معاینات بالینی و روش‌های تصویربرداری تشخیصی، داشتن اطلاعات کامل کالبدشناسی حیوان مورد معاینه ضروری است.

هدف: این مطالعه با هدف تامین اطلاعات کامل مورفومتریک و تصاویر سی تی اسکن دو بعدی طبیعی از مهره‌ها در لاک‌پشت برکه‌ای اروپایی انجام شد.

روش کار: مهره اطلس در بین مهره‌های گردنی کمترین طول را داشت و طول مهره هشتم گردن از مهره‌های قبلی خود کمتر بود. سطح مفصلی زایده مفصلی خلفی مهره هشتم گردن خمش شده و به صورت عمودی قرارگرفته بود. زایده‌های عرضی در مهره‌های پشتی مشاهده نشد. فاصله بین زواید عرضی در مهره‌های گردنی ثابت بود.

نتایج: شکل خاص دو مهره آخر گردنی، خصوصا قوس‌دار بودن مهره هشتم. مهره هفتم و هشتم گردن دارای بیشترین فاصله عرضی زوائد مفصلی خلفی هستند که به نظر می‌رسد برای حرکت گردن این حالت لازم است.

نتیجه‌گیری نهایی: دلیل کوتاه شدن طول دو مهره آخر گردن، فضای کم موجود در لاک برای آنها می‌تواند باشد. به نظر می‌رسد عدم وجود زائده خاری در مهره هفتم و هشتم گردن به دلیل موقعیت خاص آنها در جمع شدن گردن باشد.

کلیدواژه‌ها: ستون مهره، سی تی آناتومی، لاک‌پشت برکه‌ای اروپایی، مورفومتری

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