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RESEARCH ARTICLE

Computer-aided three dimensional morphometric measurements of cervical vertebrae variations compared with manual measurements in throughbred horses

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Safkan atlarda boyun omurları varyasyonlarının bilgisayar destekli üç boyutlu morfometrik ölçümlerinin manuel ölçümlerle karşılaştırılması

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Öz

Abstract

Amaç: Çalışmada, bilgisayarlı tomografi (BT) görüntüleri ile her bir servikal omurun üç boyutlu (3B) modellerini oluşturmak; manuel ve dijital ölçüm yöntemleri ile her servikal vertebranın morfometrik parametrelerini belirlemek ve iki yöntem arasındaki doğruluğu karşılaştırmak amaçlanmıştır.

Gereç ve Yöntem: Morfometrik analizler için dört erkek safkan atın son beş boyun omurları kullanıldı. Tüm omurlar 256 multidetektörlü BT cihazı ile tarandı. 3D Slicer yazılımı ile 3B rekonstrüksiyon modeller elde edildi. Yirmi üç morfometrik parametre, 3B modeller ve dijital kumpas kullanılarak manuel olarak hesaplandı.

Bulgular: Ölçüm yöntemleri*servikal omur etkileşim terimi, omur yüksekliği, processus articularis cranialis'in dış ve iç genişliği, fossa vertebralis'in genişliği, foramen transversalis'in yüksekliği, incisura vertebralis'lerin yüksekliği ve pedikül genişliği için istatistiksel olarak anlamlı bulundu (p<0,001). Omur gövdesinin uzunluğu her iki ölçüm yönteminde de üçüncü servikal omurdan yedinci servikal omura azalıyordu, ancak ölçüm yöntemi*servikal omur etkileşim terimi arasında fark bulunmadı (p=0,685). Bu çalışma ile, servikal omurların kendi aralarında karşılaştırması yapılarak ayrıntılı morfometrik veriler sağlandı. 3B yaklaşımlarla toplanan dijital veriler, anatomik varyasyonları analız etmek için faydalı bilgiler verecektir.

Öneri: Bu çalışmada elde edilen morfometrik verilerin sadece anatomik araştırmalara katkıda bulunmayacağı, aynı zamanda atların bu bölgesi üzerinde cerrahi ve ortopedik araştırmalar veya klinik müdahaleler için veri tabanı sağlayacağı düşünülmektedir.

Anahtar kelimeler: Anatomik varyasyon, at, bilgisayarlı tomografi, 3B anatomi, 3B ölçüm **Aim:** The aim of this study was to create three-dimensional (3D) models of each cervical vertebra with computed tomography (CT) images; to determine the morphometric parameters of each cervical vertebra with manual and digital measurement methods; to compare the accuracy between the two methods.

Materials and Methods: The last five cervical vertebrae of four male thoroughbred horses were used for morphometric analyses. All vertebrae were scanned with 256-multidetector CT device. 3D reconstructed models were acquired with 3D slicer software. Twenty-three morphometric parameters were calculated on 3D models and manually by using a digital caliper.

Results: Measurement methods*Cervical vertebra interaction term was found statistically significant for height of vertebra, external and internal width of the cranial articular process, width of the vertebral fossa, height of transverse foramen, height of cranial and caudal vertebral notch, and width of the pedicle (p<0.001). The length of the vertebral body was decreasing from third to seventh cervical vertebra in both measurement methods but was not different between measurement methods*cervical vertebra interaction term (p=0.685). This study provided detailed comprehensive morphometric data to compare cervical vertebrae among each other. The digital information gathered with 3D approaches will give useful information for analysing anatomical variations.

Conclusion: These morphometric data cannot only contribute to anatomic investigations but also provide database for surgical and orthopaedic researches or clinical interventions on this region of equine species.

Keywords: Anatomical variation, computed tomography, horse, 3D anatomy, 3D measurement

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Introduction

Due to the long, massive musculature and huge robust head and neck bones, equine neck is considerably stronger when compared to related species (Henson 2018). Common diseases of the neck include congenital cervical vertebral malformations with secondary spinal cord impingement, acquired stenosis secondary to osteoarthritis, as well as traumatic injuries from falling (Rivera et al 2017). Furthermore, the anatomy of this region has great importance for diagnosis and treatment due to various malformations or inconsistencies such as cervical vertebral stenotic myelopathy (Varol et al 2006, Claridge et al 2010).

In common with all mammals there are seven cervical vertebrae. Third to seventh vertebra consists of a body, an arch, and various processes with related structures (Liebich and König 2004, Seo et al 2014). There are some distinctive features used in the identification and differentiation of cervical vertebrae (Santinelli et al 2016). The formal difference of the first two vertebrae, the slight variations among the third to fifth cervical vertebrae and the presence of the ventral crest, the absence of a transversal foramen in the seventh cervical vertebra can be listed among them (Liebich and König 2004, Derouen et al 2016, Santinelli et al 2016, Henson 2018).

Morphometric measurements taken from anatomic structures can be quite effective for various clinical applications and provide a reference for surgeons. The results and parameters of several morphometric studies on the cervical region have become efficient references today and still being used by the researchers focused on vertebral column problems, spinal deformities, and cervical vertebral malformations (Gupta et al 2013, Yu et al 2014). Manual measurement methods were frequently used in morphometric studies in the past. This was a sine qua non for the researchers on that field. However, computer-aided measurement methods have gained priority in the last decade with the development of modern imaging techniques (Yu et al 2014).

In parallel to that development, the usage of advanced imaging methods such as Computed Tomography (CT) and Magnetic Resonance Imaging (MRI) has been increased for the evaluation of the neck region in equine clinics (Zafra et al 2012, Jones 2016, Veraa 2016). Besides, with the improvements in CT technology, imaging and software enhanced Three-Dimensional (3D) modeling of the desired region became a convenient method for different fields. The advantages of this method are practical visualization of osseous structures from different aspects and detailed digital measurement and investigation of anatomical formations (Zafra et al 2012, Seo et al 2014, Özkadif et al 2017). The use of the 3D reconstruction method made it easier to understand some disorders and to support a clinical evaluation in the neck region of equine species (Zafra et al 2012, Özkadif et al 2017). In addition to those mentioned above, these technological approaches provide different opportunities for the effective education of anatomy. And also pave the way for creating various anatomical models and inorganic training specimens (Cai et al 2019, Low et al 2019).

This study aimed to perform a convenient morphometric measurement of cervical vertebrae on organic specimens and 3D reconstructed digital images as well. It was hypothesized that digital morphometric measurements of 3D reconstructed models would be used instead of manual measurements for the osteological analyses and therefore the potential superiority of imaging technologies would be revealed. The objectives of this study were: 1) to create 3D models of the cervical vertebra with CT images; 2) to determine the morphometric parameters of each cervical vertebra with manual and digital measurement methods; 3) to compare the accuracy between two methods.

Material and Methods

Third to seventh cervical vertebrae (C3, C4, C5, C6 and C7) of four male thoroughbred horses (11 to 14 years old) from the collection of Anatomy Department in the Faculty of Veterinary Medicine, Ankara University were used for morphometric analyses and 3D reconstruction modelling. Vertebrae were scanned with a 256-multidetector computed tomography device (Siemens Somatom Definition Flash, Germany). The slice thickness was 0.75 mm on the transverse plane. The scanning parameters were recorded as follows; 120 kV, 600 mAs, window level 200 Hounsfield unit (HU), and window width 50 HU. Two-dimensional (2D) images were obtained in DICOM format. Then the segmentation stage of these images was performed. 3D reconstructed images of the vertebrae were acquired with 3D slicer software (3D Slicer, GitHub, San Francisco). The MeshMixer software (Autodesk Inc., version 3.5, San Francisco) was used to calculate the morphometric measurements on the 3D reconstructed models, and the values were given in (Table 1). After the 3D measurement process, the same measurements were performed manually by using a digital calliper (Mitutoyo Corporation, CD-15D, Japan) on the corresponding organic specimens. Each measurement was made by three examiners and the average value was taken into consideration. The measurement parameters were determined in accordance with the relevant literature (Von den Driesch, 1976, Sheng et al 2010). All procedures and configurations were described schematically in Figure 1 from the specimen imaging to the statistical analyses.

Before the statistical analyses, data were examined with the Shapiro-Wilk test for normality and Levene test for homogeneity of variances as parametric test assumptions. Descriptive statistics for each variable were calculated and presented as "Mean ± Standard Error of Mean". Data were subjected to two-way mixed ANOVA (analysis of variance) using the



General Linear Model procedure. In the model, "Measurement methods" and "Cervical vertebra" were analysed as main effects and "Measurement methods*Cervical vertebra" were also analysed as interaction effects. The measurement methods included in "computer-aided three dimensional morphometric measurements" and "manual measurement". The cervical vertebra also includes the C3, C4, C5, C6 and C7 vertebra. Post hoc testing was only carried out for significant interactions and was performed using a simple effect analysis. A probability value of less than 0.05 was considered significant unless otherwise noted. SPSS 14.01 was used for statistical analysis.

Results

Anatomical structures and measurements were easily determined on 3D reconstructed models. All values and statistical analyses of measurements obtained from all vertebrae were given in Figure 2, Figure 3 and Table 2. The measurement methods*cervical vertebra interaction term was found statistically significant for Vh, CAPew, CAPiw, VFow, TFh, CVNh, CauVNh, and Pw which means there was a change in the simple main effects of the measurement methods over the cervical vertebra (p<0.001).

There was a statistically significant difference among all cervical vertebra for VBl in both measurement methods (p<0.001). And VBl was distinctively decreasing from C3 to C7. There was a statistically significant difference among all cervical vertebra for VAl in both measurement methods (p<0.001). The VAl value was increased from C3 to C4 and decreased from C4 to C7. However, there was no statistically significant difference between measurement methods (p=0.356).

There was a statistically significant difference among all cervical vertebra for VAw in both measurement methods (p<0.001). The VAw value was increased from C3 to C7 in each. The difference between the measurement methods was not significant (p=0.511). The summary of body and arch parameters were indicated in Table 2.

There was a statistically significant difference among all cervical vertebra for CauAPew, CauAPiw, CAStw, and CASlw in both measurement methods (p<0.001) but the diffe-

Table 1. T	he various measurement de	finitions and symbols on the vertebral parts
Vertebral part	Symbol	Definition
Body	VBl	Length of the vertebral body
	Vh	Height of the vertebral body
Arch	VAw	Width of the vertebral arch
	VAl	Length of the vertebral arch
Foramen	VFcauw	Caudal width of the vertebral foramen
	VFcauh	Caudal height of the vertebral foramen
	VFow	Width of the vertebral fossa
	VFoh	Height of the vertebral fossa
	TFh	Height of the transverse foramen
Process	CAPew	External width of the cranial articular process
	CAPiw	Internal width of the cranial articular process
	CauAPew	External width of the caudal articular process
	CauAPiw	Internal width of the caudal articular process
	CAStw	Lateromedial width of the cranial articular surface
	CASlw	Craniocaudal width of the cranial articular surface
	CauAStw	Lateromedial width of the caudal articular surface
	CauASlw	Craniocaudal width of the caudal articular surface
	SPw	Width of the spinous process
	SPh	Height of the spinous process
Pedicle	Pw	Width of the pedicle
	LPw	Width of the lower part of the pedicle
Notch	CVNh	Height of the cranial vertebral notch
	CauVNh	Height of the caudal vertebral notch

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Figure 1. An overview of the procedure workflow from the specimen imaging to the statistical analyses



Figure 2. 3D reconstructed illustrations prepared from digital CT images of the fourth cervical vertebra. Dorsal view (A). cranial view (B). left lateral view (C), caudal view (D) of the 3D model images were given. The calculated measurements were explained in Table 1. (A-B) was shown the measurements of the vertebral arch, spinous, and pedicle. (A-B-C) demonstrated the measurements of the vertebral body and process. (B-D) were shown the measurements of the vertebral notch and foramen. Abbreviations in Table 1.



Figure 3. Manual measurement of the length of the vertebral body from the original specimen (A) and digital measurement of the third cervical vertebra from 3D reconstructed images (B)

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502.6.140.330.2.2014931.3740.4932.6440.4431.9830.6439.6330.49TFh3024.9140.6421.266.06431.6740.6431.6740.6430.1440.6460.001TFh3024.9140.6413.55540.6431.6740.6431.6740.6430.1440.6460.001TFh3012.9340.39 cf15.55540.6431.6740.6431.6740.6430.1440.6460.001TFh3013.02±0.39 cf15.55540.6431.6740.6431.6742.639 af<0.001	VFcauw	Σĉ	27.1±0.5	28.99±0.49	29.73±0.49	31.12±0.49	37.15±0.49	<0.001	<0.001	0.661
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$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	VFcauh	3D	24.91 ± 0.64	23.20 ± 0.64	15.55 ± 0.64	31.67 ± 0.64	36.14 ± 0.64	<0.001	<0.001	0.463
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	тер	Μ	12.32±0.39 d	14.91±0.39 cf	16.69±0.39 b.f	20±0.39 af	ı	100.02	-0000	-0.001
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	11711	3D	13.02±0.39 c	13.53±0.39 cg	15.1±0.39 bg	17.42±0.39 a.g				
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	CVNh	X	12.93±0.36 bg	11.38 ± 0.36 cg	11.6±0.36 bc	10.72±0.36 c	16.29±0.36 a,f	0.098	<0.001	0.002
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$		3D	14.05±0.36 ab.f	12.72±0.36 bc.f	12.42±0.36 °	11±0.36 d	14.66±0.36 a.g			
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	CauVNh	M C	14.3±0.44 ^b 13 q3+0 44 c	12.54±0.44 c 12 75+0 44 cd	11.12±0.44 ° 11 31+0 44 d	14.9±0.44 bg 17 27+0 44 bf	ZZ:9±0.44 a.r 21 2+0 44 a.g	0.608	<0.001	0.002
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	1	Υ	5.04 ± 0.2 by	5.77 ± 0.2 ab.g	5.1 ± 0.2 bg	6.48±0.2 a.g				
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3D 20510.35 202740.35 21.040.35 21.040.35 21.040.35 19.3540.35	LPw	Σ	20.83±0.35	21.37±0.35	22.33±0.35	21.66±0.35	18.96 ± 0.35	0.088	<0.001	0.274
h = h = h, $h = h$, $h = h$		3D	20.51±0.35	20.27±0.35	21.6±0.35	21.51±0.35	<u>~ 19.35±0.35</u>	- m - r]-		-



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Figure 4. The graphs indicating the variations in the length of the vertebral body (VBI), length of the vertebral arch (VAI), and width of the spinous process (SPw) values of the manual (M) and 3D measurements (mm) in different cervical vertebrae (C3, C4, C5, C6, and C7)

rence between measurement methods was not significant (p>0.05). There was a statistically significant difference among all cervical vertebra for CauASlw and CauAStw in both measurement methods (p<0.001). While these values were increasing from C3 to C5, an orderly decrease from C5 to C7 in each method was observed. The difference between measurement methods was not significant for CauAStw (p=0.495) but significant for CauASlw (p=0.007). There was a statistically significant difference among all cervical vertebra for SPw in both measurement methods (p<0.001).

The SPw values were decreasing from C3 to C7 in each. The difference between measurement methods was not significant (p=0.946). The summary of the process, spinous and pedicle parameters are stated in Table 2.

There was a statistically significant difference among all cervical vertebra for VFcauw in both measurement methods (p<0.001) and VFcauw values were increasing from C3 to C7 in each method. There was a statistically significant difference among all cervical vertebra for VFcauh in both measurement methods (p<0.001). VFcauh values were decreasing from C3 to C4 but increasing from C4 to C7 vertebra in the measurement methods. The difference between measurement methods was significant for VFcauw and VFcauh (p<0.001). There was a statistically significant difference among all cervical vertebra for VFoh in both measurement methods (p<0.001). When the vertebrae examined orderly, the VFoh was decreasing from C3 to C5 and increasing from C5 to C7 in each method. However, there was no statistically significant difference between measurement methods (p=0.426). The summary of foramen and notch parameters are indicated in Table 2.

Discussion

It can be clearly stated that using 3D based techniques are considerably increasing not only in researches but also in various training programs in recent years. This approach provides a new and dynamic perspective for all fields of the veterinary profession (Estai and Bunt 2016). In addition to clinical use, CT images assisted with 3D modelling have widely been used to increase the quality of medical education. These improved digital techniques and associated 3D models are supporting the clinical diagnosis of various disorders such as cervical vertebral malformations, degenerative changes in the joints or cervical stenosis in domestic animals (Zafra et al 2012, Janes et al 2014). In a previous study, it was reported that the majority of osteoarthritis is in the joints between C3, C4, C5, C6, and C7 of the articular processes in horses. It was emphasized that CT provides good quality images for diagnosing cervical pathologies (Lindgren et al 2021). In another study, it was indicated that distinctive features of C3 and C5 could not be visualized with radiography technique in horses (Gee et al 2020). In this study, it was shown that the 3D reconstruction of the cervical vertebrae can be easily distinguished by CT images and the difference of statistical data of the bones were estimated through the reconstructions (Table 2). Compared to conventional radiography, CT images allow detailed examination in multiple planes. These images have higher sensitivity on bones in veterinary clinics, especially when supported with improved digital techniques like 3D modelling (Veraa et al 2016, Gough et al 2019). Studies related to anatomical measurements on morphometric variations are relatively few in horses (Zafra et al 2012). However, anatomical knowledge (e.g. morphometry, relationship, etc.) is crucial for surgical techniques, pathological alterations, and anatomical variations (Gupta et al 2013, May-Davis and Walker 2015). It was thought that the use of the 3D images and data in this study will contribute to the methodology of anatomy education.

In addition to educational purposes, newly developed methods and simulations are applied to animal experiments. The anatomical differences among the species are very important for choosing the convenient animal model for these approaches. Large animal models are generally preferred for studies on the human spine (Sheng et al 2010). The data stated in our study can be taken into consideration in newly developed experimental models.

The length of bodies of the cervical vertebrae were decreased from C3 to C7 (Nickel et al 1986, Liebich and König 2004). VBl was also lower for C7 compared to C6 in horses (Derouen et al 2016). A significant difference was observed between C1, C2, C3, C4, C5, and C7 in terms of vertebral body length in chinchilla in a previous study (Özkadif et al 2017). In another study conducted in rabbits, no statistically significant difference was determined between C3, C4, C5, C6, and C7 in terms of vertebral body length (Amiri et al 2020). In our study, a gradual decrease in VBI from C3 to C7 was observed in both manual and 3D methods. There was a statistically significant difference among length of the vertebral body in both methods.

There are some features that cannot be measured with standard manual callipers such as the spinous process and therefore digital measurements of the 3D reconstructed models provides more flexibility. Authors can confidently state that using improved digital imaging techniques and 3D reconstruction for morphometric measurements instead of manual methods provide great comfort. All measurements can be easily calculated on 3D images regardless of their location in the vertebra (Figure 3).

One of the previous studies reported that C6 and C7 have no lamellar connection with the nuchal ligament in order to provide high mobility (May-Davis 2014). SPw had the highest value on C3 (Table 1) and the value steadily descended from C3 to C7 in our study (p<0.001). It was considered that the cause of this reduction is due to the connection of the nuchal ligament lamellae and the wider spinous process can create a stronger connection. The spinous process was determined to be well-defined with straight cranial and caudal margins for C7. This was also noted by a previous study (Santinelli et al 2016).

The cervical parts of the vertebral column are known to be commonly used in surgical interventions such as pedicle screw fixation. The width of the pedicles is a useful parameter for osteological approaches (Gupta et al 2013, Yu et al 2014). In our study, pedicle measurements were examined in two different aspects (Pw and LPw). A gradual increase in Pw from C3 to C4 and C5 to C6 and a gradual decrease from C4 to C5 was noted in both methods (Table 2). The difference among C3, C4, and C5 values was not statistically significant in the manual method. C3 - C4 and C5 - C6 were not also statistically significant in the 3D method. However, there was a statistically significant difference between the two methods for all cervical vertebra (p<0.001).

Stenosis and limb nerve problems can be forecasted by morphometric measurements of the vertebral and intervertebral foramen dimensions (Varol et al 2006, Santinelli et al 2016). In our study, we also examined these dimensions, and a gradual increase in VFcauw value from C3 to C7 was noted in both methods, similar to the previous studies (Janes et al 2014). Also, a gradual increase in VFcauh value was observed from C3 to C7 in both methods. The values showed similarity to the control group of the previous study (Janes et al 2014). Although the measurement methods*cervical vertebra interaction term was not found statistically significant for VFcauh and VFcauw (Table 2), there was a statistically significant difference between measurement methods and cervical vertebrae (p<0.001). As already mentioned, the height of the cranial and caudal vertebral notch is the parameter that needs to be evaluated for spinal stenosis (Varol et al 2006). Intervertebral foramen height was very low in horses suffering from cervical stenotic myelopathy (Janes et al 2014). In our study, the measurement methods*cervical vertebra interaction term was found statistically significant for CVNh and CauVNh (Table 2) and the values were quite similar to horses in the healthy group of the previous study (Janes et al 2014). The major limitation of this study was the number of specimens. Therefore, it will be the recommendation of the authors that researchers focused on anatomical variations on horses should include a considerably higher number of objects for their future studies in order to understand the impact of these parameters.

Conclusion

In conclusion, despite the limitation mentioned above, these preliminary findings will play an important role in examining morphological variations among cervical vertebrae of horses for further anatomical, surgical or pathological researches. 3D reconstructed models prepared with reliable modern imaging techniques and improved software also can have a significant role not only for estimating morphometric measurements but also for the efficient education in veterinary clinics. It is predicted that these detailed morphometric measurements will provide basic data for researchers who will work on equine vertebral morphometry. In addition, it is thought that 3D images and measurement data acquired due to high technology imaging system superiorities, regardless of the reason for the approach, will provide important contributions to the researcher compared to manually estimated

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measurements.

Conflict of Interest

The authors did not report any conflict of interest or financial support.

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During this study, any pharmaceutical company which has a direct connection with the research subject, a company that provides and / or manufactures medical instruments, equipment and materials or any commercial company may have a negative impact on the decision to be made during the evaluation process of the study or no moral support.

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Author Contributions

Motivation / Concept: Caner Bakıcı Design: Caner Bakıcı, Barış Batur Control/Supervision: Okan Ekim, Çağdaş Oto Data Collection and / or Processing: Caner Bakıcı, Barış Batur, Remzi Orkun Akgün Analysis and / or Interpretation: Ufuk Kaya Literature Review: Caner Bakıcı, Barış Batur Writing the Article: Caner Bakıcı, Barış Batur, Okan Ekim Critical Review: Çağdaş Oto, Reşide Merih Hazıroğlu

Ethical Approval

The present study was approved by the Ankara University Local Ethics Committee for Animal Experiments (Decision Number: 2019-3-20).

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