Vertebral heart score and cardiothoracic ratio in Wistar rats

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ABSTRACT

The purpose of this study was to determine and compare the Vertebral Heart Size (VHS) and Cardio-Thoracic Rate (CTR) in normal Wistar Rats. The size of the organs in the chest cavity and the size of the heart can be measured by taking thorax radiographs. Thorax radiographs of 85 male Wistar rats were taken under anesthesia, and VHS and CTR were calculated in the digital environment. The mean VHS was 7.22 mm in the right lateral position and 7.34 mm in the left lateral position. In the radiography taken in the ventrodorsal position (P = 0.3530), the mean CTR was calculated as 0.89 mm. Also, no significant correlation was measured between VHS and CTR. In conclusion, our study results can be reference values, as no previous study has been found for Wistar rats.

Introduction

Radiographic examination of heart tissue is one of the routines in veterinary medicine (22). Chest radiography has an important place in the diagnosis of heart diseases, especially in terms of changes in the size of the heart, edema, and the shape of pulmonary vessels (27). Radiographic evaluations are equivalent to other cardiac diagnostic methods (3). Besides, vertebral heart size (VHS) measurement, one of these methods, has advantages such as monitoring the heart with continuous images, and the method being accessible and applicable (13). In this method, which Buchanan and Bucheler defined as VHS in 1995 the long and short axis lengths of the heart are summed and compared with the thoracic vertebrae (7). Studies have been published about the wider range of VHS reference values since the study of Buchanan and Bucheler (4, 6, 15). One of the most important aspects of these methods is to mark the method of measuring heart size by comparing the length of the fourth thoracic vertebra to the heart size on chest radiography, as described by Ljubica (18). These methods give the same results as echocardiographic and electrocardiographic measurements. There is no race variation and there is no difference between right and left lateral recumbency while radiography is taken (23, 32). However, since normal heart size and shape are different in each race, these differences should be taken into account when considering VHS (2). The breed recumbency, gender, and body weight affect VHS (6, 36). Therefore, reported reference values do not apply to all breeds, and breed-specific values must be established (14). Although the VHS measurement system has been described in cats, dogs (19), sheep (31), monkeys (28), ferrets, and rabbits (12, 24, 35), little information is available on rodents (10, 35). Literature on the VHS values of Wistar rats is lacking.

Thorax radiographs are widely used as a non-invasive method in Veterinary Medicine to investigate the chest cavity (25). In addition to VHS measurements, it is also important to determine the cardiothoracic ratio.
(CTR), which is widely used in human medicine. The main goal of CTR is to relate systolic dysfunction with the left ventricle (30). Besides, this method plays an important role in the diagnosis of heart diseases by revealing the difference between normal heart rate and enlarged heart size (5). To our knowledge, no studies are stating VHS and CTR values or comparing these measurement methods for rats. Therefore, the purpose of this study was to determine the VHS and CTR values for use in cardiac studies using Wistar rats as experimental animals, and to correlate the VHS and CTR values measured on radiographs taken in the right and left lateral position.

**Materials and Methods**

**Animals:** This study was conducted on healthy, male, 10 months old Wistar rats (n=85), and free from cardiothoracic diseases upon the approval by Atatürk University Animal Experiments Local Ethics Committee (HADYEK) (2018/226). After clinical examinations of the rats including respiratory system (respiratory rate and type), cardiovascular system (heart rate, oxygen saturation), and body temperature, no heart disease was confirmed by monitoring via monitor (Cardell, 9404, Sharn Veterinary Inc., FL, USA), and right and left lateral thorax radiographs (Meditronics 3L 103, Japan) of all rats were obtained under xylazine-ketamine anesthesia (10 mg/kg IP xylazine HCl, 75 mg/kg IP ketamine HCl).

**Measuring the Vertebral Heart Size:** The VHS was measured according to the protocol established by Ljubica (18). The long heart axis (LA) was measured from the ventral border of the main stem bronchi (carina cranioventral border) to the apex of the heart (the farthest point in the ventral contour of the cardiac radiographic image) on the radiographs. The short axis (SA) was measured at the widest cardiac image point on a line perpendicular to the long axis at the level of the clavicle vena cava. Both measurements (long and short axes) were compared with the distance from the cranial edge of the 4th thoracic vertebra (T4) to the cranial edge of the 5th thoracic vertebra (Figure 1). The VHS was calculated according to the formula given below:

\[
\text{VHS} = \left( \frac{\text{LA}}{\text{T4}} \right) + \left( \frac{\text{SA}}{\text{T4}} \right)
\]

**Measuring the Cardio-Thoracic Ratio:** The CTR was calculated as described by Schillaci et al (29). In the dorso-ventral position, the distance between the thoracic walls and the width of the heart was measured at the T8 level. The thoracic diameter was measured as the longest thoracic distance (MTD) at the T8 level, and the longest distance (ML and MR) from the line passing through the middle of the heart to the right and left sides between the heart width organ boundaries. (Figure 2). The CTR was calculated according to the formula given below:

\[
\text{CTR} = \left( \frac{\text{MR} + \text{ML}}{\text{MTD}} \right)
\]

**Statistical analysis:** The data were subjected to the Student t-test and regression (PROC. REG) and correlation (PROC. CORR) analyses after testing for normality (Version 9.1; SAS Institute Inc., Cary, NC, USA). Statistical significance was declared at \( P < 0.05 \).
Results

Thorax radiographs were performed on all rats. The VHS measurements were made on thorax radiographs of the rats taken at right (R-VHS) and left lateral (L-VHS). The mean R-VHS and L-VHS was not different (P = 0.3530) and was 7.22±0.78 mm and 7.34±0.78 mm, respectively (Figure 3). The mean CTR was 0.89 mm. There was no correlation between VHS and CTR (Figure 4).

Discussion and Conclusion

This study determined and described the VHS and CTR in health Wistar Rats (18). It is important to determine the VHS for the diagnosis of heart diseases in veterinary medicine (21). The VHS is an effective method for objectively determining heart size on chest radiographs, and especially for detecting cardiomegaly caused by eccentric hypertrophy (27). In addition to method by Ljubica (18) suggesting calculation of the ratio of the long and short axis lengths of the heart to the width of the 4th thoracic vertebra, Buchanan and Bucheler (7) suggests transposing the long and short axes of the heart into the vertebral column. This method was preferred because it is a measurement that can be calculated automatically in the x-ray machine used in our study. The closest study of our study, has been newly published by Dias et al (9). Dias et al. measured the right lateral, ventrodorsal and dorsoventral VHS in Sprague-Dawley Rats (both male and female) and reported a mean of 7.70. In the present study the VHS was 7.22-7.34 mm and the CTR was 0.89 for Wistar rats with no evidence of cardiac disease.

Abdolvahed Moarabi et al. (21) reported that the techniques used for VHS measurement are easy and the measurements taken are relatively independent of the patient (e.g., gender, right, or left lateral). However, there are studies in the literature suggesting that the VHS measurements vary depending on the side of lying down. For example, Onuma M et al. (24) reported that the VHS values were significantly different in rabbits whose thorax radiographs were taken during right and left lateral. Also, Bavegems et al. (2) suggested that the heart silhouette would always be larger on the left lateral radiographs, as the heart is located on the left side of the chest cavity. Kraetschmer et al. (16) reported that the heart was significantly larger on the right lateral radiography in beagle dogs. In our study, we considered having chest radiographs in both the right lateral and left lateral positions, as different opinions were found in the literature reviews. There was no difference between the VHS measurements taken in both positions. De Moura et al. (8) and Marin et al. (20) reported the same results in right and left lateral radiographs on ferrets and greyhounds. Many studies have been conducted on VHS measurements, but since there is no study on rats, we cannot compare our results with other studies. Lamb et al. (17) reported different VHS values in male and female dogs. Since the use of generic references determined for other species and breeds may cause misdiagnosis (33), detailed studies on rats should be performed and breed-specific reference ranges should be determined.
The CTR is a method in which heart size is determined by measuring the heart and thorax cavity on radiographs (34). The main purpose of CTR measurement in humans is to compare the abnormalities of the left ventricle and systolic dysfunction (29). Studies reporting many types of CTR measurements have previously been published (1, 5, 26). However, as in the VHS, there is no study in which CTR measurements were made in Wistar rats in the literature review. The probability of getting the myocardial disease is higher with a high CTR value (30). Likewise, it is suggested that the CTR may be useful in the early diagnosis of heart diseases by detecting latent variations in the heart silhouette (26). In our study, the mean CTR was 0.89. These measurements should be evaluated by working together with different factors as in the VHS.

In disagreement with the current study, Oana Bîrsan et al. (5) found a significant correlation between VHS and CTR in healthy cats. They also reported that the CTR measurement can be used to evaluate VHS in cats. When heart width is examined within the scope of previously determined reference values, it appears as an important parameter in the diagnosis of heart diseases. The best measurement of this parameter can be made on thoracic radiographs (11). Therefore, as a result of our study, we think that previously undetermined VHS and CTR values should be evaluated as the first findings in rats. Simultaneous determination of the CTR and VHS may have merit for cardiology patients. The limitations of this study include not taking gender and body weight into consideration and using only healthy animals.

In conclusion, species, and breed-specific reference values should be determined to provide an accurate reference for clinical practitioners, to avoid misdiagnosis of heart diseases, and to be used in experimental cardiology studies.

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Conflict of Interest
The authors declared that there is no conflict of interest.

Author Contributions
ED, SO, AH and ZO conceived and planned the experiments. ED and SO carried out the experiments. ED, SO and ZO planned and carried out the simulations. ED, SO, AH and ZO contributed to sample preparation. AH, and ZO contributed to the interpretation of the results. ED took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

Data Availability Statement
The data supporting this study’s findings are available from the corresponding author upon reasonable request.

Ethical Statement
This study was approved by the Atatürk University Animal Experiments Local Ethics Committee (2018/226).

Animal Welfare
The authors confirm that they have adhered to ARRIVE Guidelines to protect animals used for scientific purposes.

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