

Morphometric Evaluation of Chinchillas (*Chinchilla lanigera*) Femur with Different Modelling Techniques

Sema ÖZKADİF¹  Abdullah VARLIK² İbrahim KALAYCI² Emrullah EKEN³

¹ Cukurova University, Faculty of Ceyhan Veterinary Medicine, Department of Anatomy, TR-01930 Adana - TURKEY

² Necmettin Erbakan University, Faculty of Architecture and Engineering, Department of Geodesy and Photogrammetry, TR-42090 Konya - TURKEY

³ Selcuk University, Faculty of Veterinary Medicine, Department of Anatomy, TR-42003 Konya - TURKEY

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Abstract

Together with technological developments, different methods in anatomic and morphometric studies have been started to be used. In fact, in this study, more than one method was used and the reliability of these methods was presented. This study was performed in the aim of getting 3D model with three dimensional (3D) reconstruction and photogrammetric methods obtained from multidetector computerized tomography (MDCT) images of femur of chinchilla by mimics program, comparing the data of both methods and presenting whether there was a difference between genders as well as left and right sides. For this purpose, 6 chinchillas of both sexes were used. First of all, MDCT images of animals were taken under general anaesthesia and 3D reconstruction was obtained after transferring the data to personal computer as DICOM format. After taking MDCT images, the femurs of the animals killed by the conventional methods were cleaned of muscle and fats by boiling and their 3D models were formed by using images via photogrammetric methods. The measurements were taken from the models of two methods and volume and surface area of femur were found significant at a level of $P < 0.05$ as a result of statistical processes. According to this, a statistically difference was not found in morphometric measurement values except volume and surface area. Moreover, while a statistical difference was not found between right and left femur of both sexes, a difference was determined between sexes and it was observed that the measurement values of females were higher than males. As a result, since 3D models obtained by both methods had almost same values, it was considered that these methods could be used in anatomic and morphometric studies.

Keywords: *Chinchilla, Femur, Three dimensional reconstruction, Photogrammetry, Morphometry*

Chinchilla (*Chinchilla lanigera*) Femur'unun Farklı Modelleme Teknikleri ile Morfometrik Değerlendirilmesi

Özet

Teknolojik gelişmelerle birlikte anatomik ve morfometrik çalışmalarda farklı yöntemler kullanılmaya başlanmıştır. Hatta bir çalışmada birden fazla yöntemle çalışılarak, bu yöntemlerin güvenilirliği ortaya konulmaya çalışılmaktadır. Sunulan bu çalışma şinşilla'nın femur'u üzerinde multidedektör bilgisayarlı tomografi (MDBT) görüntülerinden mimics programı ile elde edilen üç boyutlu (3B) rekonstrüksiyon ve fotogrametrik yöntemlerle elde edilen 3B model üzerinden gerçekleştirilerek, her iki yöntem verilerini karşılaştırmak ve cinsiyetler ile sağ ve sol taraf arasında fark olup olmadığını ortaya koymak amacıyla gerçekleştirilmiştir. Bu amaçla her iki cinsiyetten 6'şar şinşilla kullanılmıştır. Önce hayvanların genel anestezi altındayken MDBT görüntüleri çekilerek, veriler DICOM formatında kişisel bilgisayara aktarılarak daha sonra 3B rekonstrüksiyonu yapılmıştır. MDBT görüntüleri alındıktan sonra usulüne göre hayvanların ölümü gerçekleştirilerek, maserasyon yöntemi ile femur elde edilmiştir. Kas ve yağlardan kaynatılarak temizlenen femur'un fotoğrafları çekilerek, görüntülerden fotogrametrik yöntemlerle 3B modeli oluşturulmuştur. Her iki yöntemle oluşturulan modeller üzerinden ölçümler alınmış ve istatistiki işlemler yapılarak, femurun hacmi ve yüzey alanı $P < 0.05$ düzeyinde anlamlı bulunmuştur. Buna göre hacim ve yüzey alanı dışında morfometrik ölçüm değerlerinde istatistiki bir fark görülmemiştir. Ayrıca her iki cinsiyetin de sağ ve sol femur'u arasında istatistiki farklılık yokken, cinsiyetler arasında farklılık tespit edilmiştir ve dişilerde ölçüm değerleri erkeklerden fazla olduğu görülmüştür. Sonuç olarak her iki yöntemle oluşturulan 3B modeller hemen hemen aynı değerlere sahip olmasından dolayı, anatomik ve morfometrik çalışmalarda bu yöntemlerin kullanılabilceği düşünülmektedir.

Anahtar sözcükler: *Şinşilla, Femur, Üç boyutlu rekonstrüksiyon, Fotogrametri, Morfometri*



İletişim (Correspondence)



+90 322 6133507



semaerten80@gmail.com

INTRODUCTION

Since femur that is the longest bone in the body binds many muscles and tendon on itself and joins in the structure of many joints and it is the biggest bone in the body, it's quite important. For these reasons, the studies related with femur were focused on presenting the biometric differences between races and sexes ^[1].

Many morphometric studies were performed on femur in human and veterinary medicine. The measurements were taken from computerized tomography (CT) images of femurs belonging to Caucasus adults in Anatolia and their morphometric variations depending on sex and age were presented ^[2]. Some authors studied on the morphometric measurements of tibia and femur belonging to African and European societies ^[3]. Sex and right-left differences of femoral condyles in Greek society were also measured by digital caliper and then compared ^[1]. Whether there was a difference between left and right sides as well as parameters such as gender and age or not was presented by 3D model formed via CT images belonging to femur and radius of human ^[4].

Morphometric measurements of hind limb bones of New Zealand rabbits were carried out to compare the right and left sides ^[5]. After taking several morphometric measurements belonging to the thoracic and hind limb bones of New Zealand rabbits and guinea pigs, whether there was a difference between sexes as well as right and left sides was presented as a result of measurements performed by caliper ^[6,7]. The measurements were taken on X-ray images of the whole skeleton of chinchilla and the differences between sexes were determined ^[8]. The hind limb skeleton anatomy was presented by X-ray images of chinchilla ^[9].

Recently, some anatomic and morphometric studies have been based on comparison of the data obtained via many methods instead of just a single method. Related with this, in a study, 3D reconstruction obtained from CT images of human femur was stated both manually and automatically, then the data were compared ^[10]. Moreover, the morphologies of hind limb skeleton of rodents of different races were presented by using X-ray images of and 3D reconstruction ^[11].

Analysis and morphometry of human or animal bones are quite difficult especially irregular surfaces. In order to obtain 3D models of substances having different shapes and properties, different approaches are present. Photogrammetry, which has low cost, no exposure to radiation for patients and is an appropriate model to get measurement values from the formed model, is considered as a reliable method that can be preferred for the evaluation and investigation of bones ^[12,13]. Photogrammetry is a branch of technology and science where reliable information can be obtained about substances and environment as a result

of recording, measurement and interpretation processes of photographic images shaped by radiating beams from substances and their environment as well as their radiated electromagnetic energy. This method is used to determine, measure and interpret the form and characteristic properties of living or non-living things with high accuracy ^[12].

In addition to photogrammetric methods, 3D reconstruction was carried out in anatomic studies by using technology in terms of some software programs of 2D MDCT images. Thus, anatomic structures can be clearly displayed by this model and morphometric measurements can be taken ^[14]. Except 2D CT images, reconstructions obtained by X-ray radiography and laser scanners were same as the data obtained from the models formed via CT images and therefore they are trusted methods in morphological studies ^[15].

In the literature survey, the studies related with chinchilla femur were limited just with 2D images and they were not transferred to 3D structure. In this study which was performed by taking the advantage of computer technology and photograph technology, the morphometric measurements were taken from models belonging to chinchilla femur obtained by mimics program and photogrammetric methods, the data of both methods were compared and determination of whether there was a difference between genders as well as right and left sides was carried out.

MATERIAL and METHODS

This study was accepted by the ethics committee of the Veterinary Faculty of Selcuk University on 29.01.2016 (Decision number: 2016/15).

In the study, a total of 12 adult chinchillas (*Chinchilla lanigera*) of both sexes weighing from 500 to 600 g. were used. Throughout this study, 3D models of femur were obtained one by one with mimics program and photogrammetric methods.

In order to obtain 3D reconstruction via mimics program, MDCT images of femur were obtained at high resolution. The animals of which the images would be taken were anesthetized with a mixture of 60 mg/kg ketamin (ketalar) and 6 mg/kg xylazine (Rompun) intravenously ^[16]. Under anesthesia, MDCT images of animals in prone position were taken. The parameters of MDCT instrument (Somatom Sensation 64; Siemens Medical Solutions, Germany) were adjusted as; physical detector collimation, 32 x 0.6 mm; final section collimation, 64 x 0.6 mm; section thickness, 0.50 mm; gantry rotation time; 330 msec; kVp; 120; mA, 300; resolution, 512 x 512 pixel; resolution range, 0.92 x 0.92. Dosage parameters and scannings were performed by taking standard protocols and literature ^[17,18] into consideration. Thus, radiometric resolution (MONOCHROME2; 16 bits) was obtained at the lowest radiation level and with optimum

image quality. The axial images were stored in the format of DICOM and then they were transferred to a personal computer having 3D modeling program Mimics 13.1 (Multimodal Immersive Motion rehabilitation with Interactive Cognitive Systems).

In the first stage of automatic segmentation process, the limits of femur were determined. Section cleaning process was applied one by one to places outside these limits with computer mouse and the femur was colored after cleaning these places. The images the limits of which were determined were overlapped and reconstruction of femur was carried out by 3D transformer component of Mimics 13.1 program.

In 3D model formation via photo-grammetric method, first of all, identification of objects to be evaluated with photogrammetric evaluation should be done in a known coordination system during taking photos or coordinates should be given to some points on the object. Since measuring 3D coordinates of points on the object by traditional methods is almost impossible, a new setup was designed and coded targets were used for control points (Fig. 1).

Coded targets can be used to identify a local coordinate system and model scale to make correct match-up picture alignment process. Determination and match-up of coded targets in the pictures were carried out automatically by the software.

Picture taking geometry has great importance in taking photogrammetric images. In the study, a picture scale depending on the size of the object was determined by taking the digital camera into consideration and B/H (Base/Height) ratio suitable for this picture scale as well as picture shooting plan. The pictures belonging to object surfaces

were taken with Sony DSC-W570 digital camera having 16 megapixels optical resolution, equal distances to the object with 70% overlapping ratio, Base/Height ratio of 1/3 and by raising the camera with approximately 30° angle (Fig. 2).

In our study, Photoscan software was used in order to make photogrammetric evaluation. Photogrammetric evaluation process stages of photoscan software constituted of picture alignment, formation of dense point cloud, formation of 3D model grid network, model tissue covering and place referencing.

During picture alignment process, the software automatically perceives coded target points on pictures and moreover finds common points outside these coded target points after match-up between pictures and forms connection points. By means of these points, the location



Fig 1. Setup used for taking photos and coded target points

Şekil 1. Resim çekiminde kullanılan düzenek ve kodlu hedef noktaları

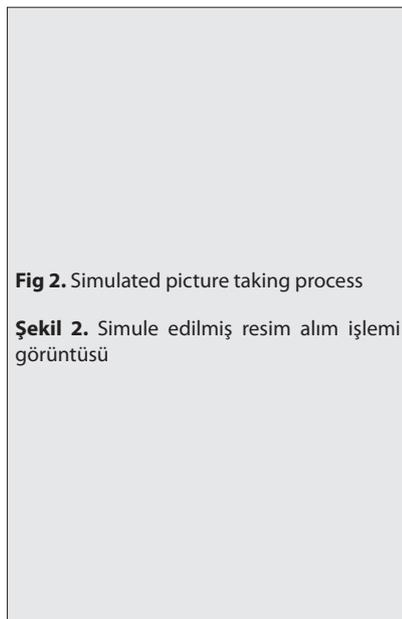


Fig 2. Simulated picture taking process

Şekil 2. Simule edilmiş resim alım işlemi görüntüsü

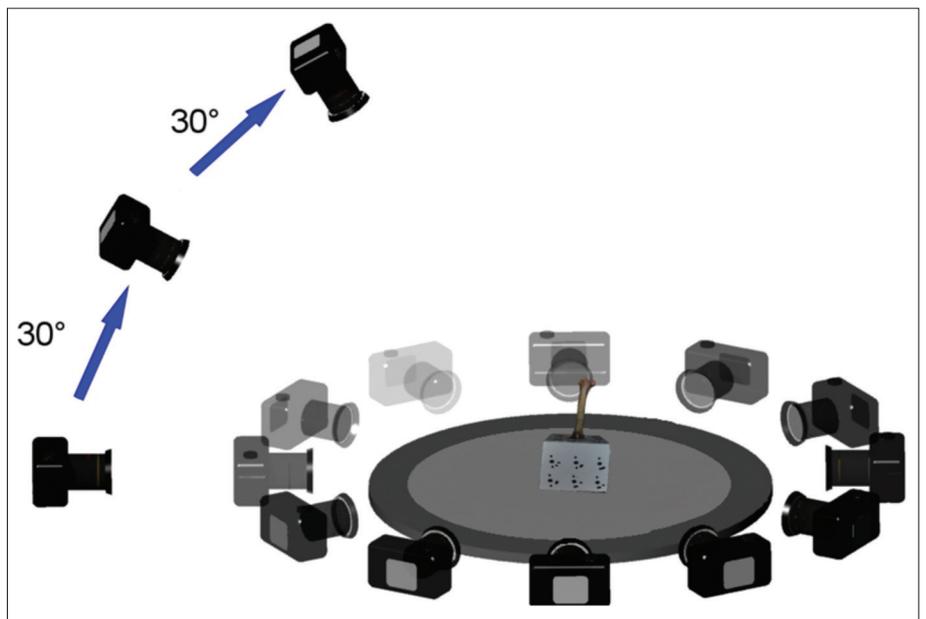




Fig 3. 3D model of femur obtained by photogrammetric methods

a. Caudal view, b. Lateral view, c. Cranial view, d. Medial view

Şekil 3. Fotogrametrik yöntemlerle elde edilen femur'un 3B modeli

a. Kaudal görünüm, b. Lateral görünüm, c. Kranial görünüm, d. Medial görünüm

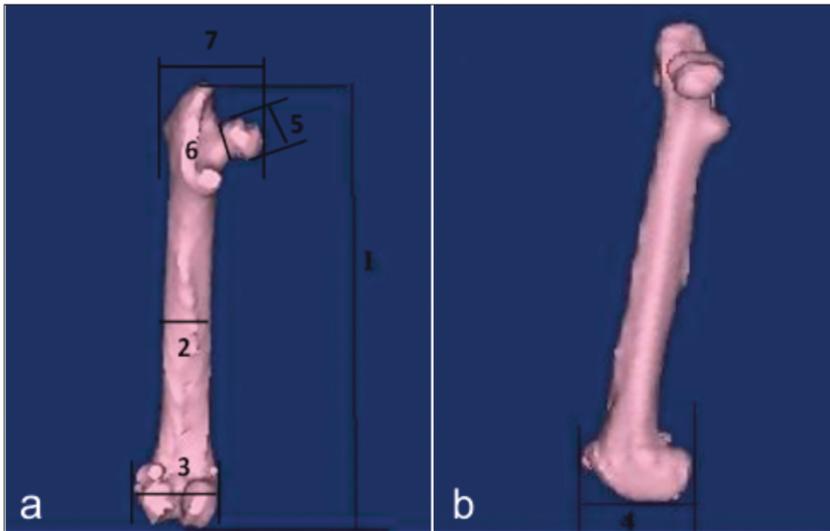


Fig 4. Measurement points taken from femur

a. Caudal view of femur, b. Medial view of femur

1. Maximum femoral length, 2. Mid-shaft transverse diameter, 3. Distal width, 4. Medial condylar depth, 5. Femoral head diameter, 6. Narrowest neck width, 7. Proximal width

Şekil 4. Femur'dan alınan ölçüm noktaları

a. Femur'un kaudal görünümü, b. Femur'un medial görünümü

1. Femur'un maksimum uzunluğu, 2. Gövde ortasının transvers çapı, 3. Distal genişlik, 4. Medial kondiler derinlik, 5. Femur başının çapı, 6. Boynun en dar kısmının genişliği, 7. Proksimal genişlik

of camera and directions can be calculated during every picture taking process. After this process, dense point match-up was carried out between stereo picture pairs and point cloud model was formed.

Georeferencing of the model was performed by at least three points whose real earth coordinates were known by transforming with 7 parameters (3 parameters of translation, 3 parameters of version, 1 parameter of scale). Since just real dimensions of the model were required in our study, transformation was carried out by scaling the model after using the distances between coded target points.

After georeferencing of the model, 3D grid network was formed from dense point cloud, after formation of numerical surface model, 3D real model of the object was obtained by covering this model picture tissue (Fig. 3). These processes were carried out for each bone and 3D of all bones were obtained.

Various morphometric measurement values, surface areas and volumes were taken from 3D models belonging to femur of chinchilla formed by photogrammetric and mimics programs. While taking measurements of femur, literatures such as ^[1,2,19] were taken into consideration (Fig. 4).

Measurements

1. *Maximum femoral length*: Maximum distance from the uppermost margin of the head of the femur to the lowest margin of the medial condyle.
2. *Mid-shaft transverse diameter*: Transverse diameter at the middle of the shaft.
3. *Distal width*: The maximum distance across the femoral condyles in the transverse plane.
4. *Medial condylar depth*: The maximum anteroposterior diameter of the medial femoral condyle.

5. *Femoral head diameter*: Width of caput ossis femoris.
6. *Narrowest neck width*: Narrowest width of the collum ossis femoris.
7. *Proximal width*: The maximum distance between the head of femur to greater trochanter.
8. *Volume*: Volume of the femur.
9. *Surface area*: Surface area of the femur.
10. *Index*: (Mid-shaft transverse diameter/Maximum femoral length) x100

The average values and standard deviations of morphometric measurements obtained by mimics program and photogrammetric methods in terms of right and left sides as well as in terms of sexes were calculated. The materiality control of differences between average values was carried out via SPSS 16.00 software program and independent t- test.

RESULTS

Several morphometric measurement values were obtained from 3D reconstruction formed by using mimics program of MDCT images belonging to chinchilla femur and from the model obtained by photogrammetric methods. The statistical results of volume and surface area were found significant at the level of $P<0.05$ (Table 1, Table 2).

In Table 1, it was observed that some measurement values belonging to chinchilla femur obtained by mimics program and photogrammetric methods (maximum femoral length, mid-shaft transverse diameter, distal width, medial condylar depth, femoral head diameter, narrowest neck width, proximal width and index) were not different statistically in both right and left sides. However,

Table 1. Statistical results of morphometric measurements of chinchilla femur obtained by mimics program and photogrammetric methods (Mean±SD)

Tablo 1. Şinşilla femur'unun mimics programı ve fotogrametrik yöntemlerle elde edilen morfometrik ölçüm değerlerinin istatistikî sonuçları (Ortalama±SS)

Measurements	Right		Left	
	Mimics (n=12)	Photogrammetry (n=12)	Mimics (n=12)	Photogrammetry (n=12)
Maximum femoral length (mm)	55.64±2.31	55.00±2.16	55.50±2.21	55.01±2.17
Mid-shaft transverse diameter (mm)	5.54±0.43	5.32±0.35	5.55±0.43	5.24±0.41
Distal width (mm)	9.33±0.34	9.13±0.36	9.38±0.36	9.13±0.36
Medial condylar depth (mm)	8.74±0.32	8.58±0.39	8.70±0.31	8.62±0.33
Femoral head diameter (mm)	5.57±0.50	5.35±0.34	5.63±0.42	5.35±0.27
Narrowest neck width (mm)	3.52±0.25	3.36±0.22	3.52±0.27	3.31±0.25
Proximal width (mm)	12.01±0.57	11.89±0.72	12.09±0.61	11.90±0.66
Volume (cm ³)	1471.54±158.65 ^a	1105.83±144.50 ^b	1456.38±153.30 ^a	1107.50±135.31 ^b
Surface (cm ²)	1698.03±144.88 ^a	1106.08±145.15 ^b	1685.44±141.33 ^a	1109.75±123.34 ^b
Index	9.94±0.55	9.67±0.53	10.00±0.62	9.51±0.55

^{a,b} Different letters in the same line are statistically significant ($P<0.05$)

Table 2. Statistical results of morphometric measurements of chinchilla femur obtained by mimics program and photogrammetric methods for females and males (Mean±SD)

Tablo 2. Şinşilla femur'unun mimics programı ve fotogrametrik yöntemlerle elde edilen morfometrik ölçüm değerlerinin birlikte erkek ve dişilerde istatistikî sonuçları (Ortalama±SS)

Measurements	Right		Left	
	Male (n=12)	Female (n=12)	Male (n=12)	Female (n=12)
Maximum femoral length (mm)	54.04±1.04 ^a	56.60±2.36 ^b	54.04±0.99 ^a	56.48±2.36 ^b
Mid-shaft transverse diameter (mm)	5.25±0.24 ^a	5.61±0.45 ^b	5.20±0.25 ^a	5.59±0.51 ^b
Distal width (mm)	9.04±0.24 ^a	9.42±0.37 ^b	9.03±0.28 ^a	9.48±0.33 ^b
Medial condylar depth (mm)	8.43±0.35 ^a	8.90±0.17 ^b	8.47±0.28 ^a	8.85±0.23 ^b
Femoral head diameter (mm)	5.21±0.23 ^a	5.71±0.46 ^b	5.30±0.21 ^a	5.68±0.42 ^b
Narrowest neck width (mm)	3.29±0.12 ^a	3.59±0.25 ^b	3.24±0.17 ^a	3.58±0.25 ^b
Proximal width (mm)	11.52±0.31 ^a	12.38±0.60 ^b	11.64±0.34 ^a	12.35±0.67 ^b
Volume (cm ³)	1191.16±212.57 ^a	1386.21±230.35 ^b	1176.91±188.83 ^a	1386.96±220.18 ^b
Surface (cm ²)	1287.18±310.02 ^a	1516.93±329.07 ^a	1288.66±292.23 ^a	1506.52±323.53 ^a
Index	9.71±0.55 ^a	9.91±0.55 ^a	9.63±0.53 ^a	9.89±0.70 ^a

^{a,b} Different letters in the same line are statistically significant ($P<0.05$)

the measurement values of volume and surface areas belonging to both right and left femur were statistically different between two methods ($P < 0.05$).

In Table 2, it was observed that a statistical difference was found in the values of maximum femoral length, mid-shaft transverse diameter, distal width, medial condylar depth, femoral head diameter, narrowest neck width, proximal width and volume of chinchilla in both right and left femur between genders. However, there wasn't a significant difference in terms of statistics in surface and index values between sexes. Moreover, a statistically difference was not found between right and left femur of both male and female chinchillas.

DISCUSSION

The values of maximum femoral length, mid-shaft transverse diameter, distal width, medial condylar depth, femoral head diameter, narrowest neck width, proximal width and volume of chinchilla indicated a statistically significant difference between sexes and they were higher in females than males. According to the results obtained from rontgen images of chinchilla femur performed by Lammers et al.^[8], the determination of femur length that was longer in females than males was in accordance with our results obtained via photogrammetric and Mimics program. The rate of femur volume to body height in human beings is higher in males than in females^[10].

It was observed that there wasn't a difference in surface and index values of chinchilla between sexes. A statistical difference was also not indicated in any morphometric measurement values obtained from right and left femurs of both female and male chinchillas. Pazvant and Kahvecioğlu^[7] stated that right and left morphometric values (maximum femoral length, mid-shaft transverse diameter, distal width, proximal width, femoral head diameter, index) of long bones of thoracic and hind limbs in guinea pigs were very close to each other and did not have homotypical variation in terms of statistics, however, they indicated that sex was an important factor. A statistical difference was not found between right and left sides of maximum femoral length, mid-shaft transverse diameter, distal width, proximal width, femoral head diameter values of New Zealand rabbits^[5]. Moreover, there was not also a difference in New Zealand rabbits between sexes and it was observed that maximum femoral length was longer in males than in females^[6]. But in broilers statistical difference was found between the sexes in femoral morphology^[20].

In some biomedical researches, it was stated that a statistical difference was not found between sexes in terms of the bone sizes of permanently used *Callithrix jacchus*^[21].

In human beings, while maximum femoral length was statistically different between females and males, there was no difference in mid-shaft transverse diameter. Moreover,

these values were higher in males than in females in contrast to chinchilla^[2]. In the morphometric study of Terzidis et al.^[1] which was performed on femur of Greek people, while a statistical difference was recorded between women and men, a difference was not found between right and left sides in both men and women. According to the measurements of the model obtained from CT images, it was determined that while there was a statistical difference between sexes in terms of femoral head diameter and narrowest neck width values, a difference was not found between right and left sides. Moreover, it was observed that the values belonging to men were higher than those of women^[4].

It's typical that there are differences in surface area and volumes of 3D model obtained via mimics software and 3D model obtained via photogrammetric method. In mimics software, the limits of femur are determined, 3D model of the object is formed by overlapping the images the limits of which are determined. In photogrammetric method, on the other hand, the camera is calibrated, after the errors caused by the camera (e.g. distortion) are corrected, dense point cloud is formed by means of common points in the images. 3D models are obtained from this point cloud. The density of this formed point cloud is approximately 300 points per square millimeter. Moreover, the measurement sensitivity of used control points is 0.05mm.

In the production of numerical surface model aiming to represent the object with a surface that is defined mathematically, distribution and frequency of points are the most effective factors in providing the suitability of the model for the real shape of the object. From so many points (so much point density it has) a 3D surface is produced, so much it approaches to the real surface. As the number (density) of points increases, the accuracy obtained from numerical surface model also increases.

As a consequence, according to the values obtained by both methods, sexual dimorphism was observed in femur of chinchilla. The measurement values obtained by mimics program and photogrammetric methods were not statistically different except volume and surface area. This indicated that both methods can be used in anatomic and morphometric studies confidently. Moreover, 3D reconstruction of rigid materials such as bones and cartilages transferred to anatomy laboratory via photogrammetric techniques may be useful for forensic medicine and/or diagnostic purposes.

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