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**ARAŞTIRMA / RESEARCH**

# Stereological measurement of liver volume in newborn cadavers

## Yenidoğan kadavralarında karaciğer hacminin stereolojik olarak hesaplanması

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**Abstract**

**Purpose:** The aim of our study was to compare different techniques for the estimation of liver volume using cadaveric sample, magnetic resonance imaging (MRI) and ultrasonography (USG) images, and to measure errors associated with volume estimation techniques based on fluid displacement (the Archimedes' principle).
**Materials and Methods:** Our study was formed of five newborn cadavers aged 39.7±1.5 weeks and weighted 2.220±1.056 g. We used three different methods for calculate the liver volume.
**Results:** Liver volume (LV) measured by the Archimedes' principle was 70.00±49.96 cm$^3$. There was no significant difference between the methods.
**Conclusion:** MR and USG images provide easy, applicable and reproducible estimates in calculating volume of normal and anomalous liver with the Cavalieri's principle. We think that our study may be a reference for similar studies to be done in the upcoming years.
**Keywords:**. Liver volume, stereology, Cavalieri's principle, newborn cadavers

**Öz**

**Amaç:** Bu çalışmada yenidoğan kadavralarına ait karaciğerler üzerinde, Ultrasonografi (USG), Manyetik Rezonans Görüntüleme (MRG), Arşimet prensibi ve dilimleme yöntemleri kullanarak, bu yöntemler arasındaki farklılıkların ortaya çıkarılması amaçlanmıştır.
**Gereç ve Yöntem:** Çalışmamız 39.7 ± 1.5 hafta yaşları ve 2.220 ± 1.056 g ağırlıklı beş yenidoğan kadavradan oluşmuştur. Karaciğer hacmini hesaplamak için üç farklı yöntem kullanılmıştır.
**Bulgular:** Arşimed prensibi ile ölçülen karaciğer hacmi (LV) 70.00 ± 49.96 cm$^3$ idi. Metodlar arasında anlamlı fark bulunmadı.
**Sonuç:** MR ve USG görüntüleri, Cavalieri prensibi ile normal ve anormal karaciğer hacminin hesaplanmasında kolay, uygulanabilir ve tekrarlanabilir tahminler sağlar. Çalışmamızın önümüzdeki yıllarda yapılacak benzer çalışmalara referans olabileceğini düşünüyoruz.

**Anahtar kelimeler**: Karaciğer hacmi, stereoloji, Cavalieri prensibi, yenidoğan kadavrası.

## INTRODUCTION

The liver is the largest of the intraabdominal organs and occupies a significant portion of the abdominal cavity. It has a brown, elastic and delicate structure[1]. In many diseases, there is a change in the size and morphology of the liver. Because of this; liver volume estimation is important in clinical practice. Liver volume measurements can be used in the planning of the radiotherapy dose, in evaluating the response to treatment, and in making surgical decisions about liver resection[2].

In recent years, there are many studies done about the calculating organ volumes with using imaging techniques. All of this studies used the Cavalieri's principle from the stereological methods to calculate organ volumes[3-7]. Using this method, which has become widespread in recent years, it has been shown that the volume can be calculated in an objective and efficient manner for each structure, which is actually

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3 dimensional (3D) and whose images that can be taken parallel to each other by the image analysis systems[8].

In this study, we aimed to determine the normal values of liver volume with ultrasonography(USG), magnetic resonance imaging (MRI), the Archimedes' principle, and slicing methods in newborn cadavers and to calculate the differences that may arise from the methods we use. Meanwhile, we believe that our study will be useful in clinical assessment of many diseases related with the dimension and morphology of the liver and in planning liver transplantations.

## MATERIALS AND METHODS

This study was carried out on a total of 5 newborn cadavers, which were kept and used for training purposes in the Department of Anatomy Laboratory of Erciyes University Faculty of Medicine. It was approved by the Ethics Committee of the Erciyes University Medical Faculty before starting to the study (2011/79). Newborn cadavers without any morphological defect and pathology of the liver were included in our work.

All abdominal USG and upper abdominal MRI were made to image the livers of newborn cadavers. Length, width, and thickness were conservatived on USG and caliper. Volume calculations were performed by the point-counting method using MRI with the Cavalieri's principle. Then, these livers extracted by dissection were immersed in water according to the Archimedes' principle and volume calculation was made. The estimated values in the computer environment were compared with this measurement which is accepted as gold standard. Finally, the livers were sliced at 0.5 cm space by a special slicing tool and it was studied in liver sections. Volume calculations were performed using a cycloid probe and the transparent square grid test system (Cavalieri's principle) for each section.

### Volume calculation with USG measurements

The liver sizes were measured while in the supine or in the slightly right lateral decubitus position by USG images. The length, width, and thickness of each liver were measured (Fig. 1). Finally, the liver volumes were determined according to the ellipsoid formula using these values measured for the livers of newborn cadavers[9, 10] The ellipsoid formula (Formula 1) is shown below:

**Formula 1: Volume = Length × Width × Thickness × 0.52**

### Archimedes' volume as a reference volume

The livers removed by dissection from newborn cadavers were left in a container with a certain amount of water and the amount of water they displaced were observed. These values were considered as the actual liver volumes. Our measurement results were estimated to the other procedures and the mean and standard deviation (± SD) of the measurements were determined.

### Volume calculation using stereological method

In stereological studies, volume calculation is based on the Cavalieri's principle. Serial sections parallel to each other are taken for this (the distance between each section will be equal) and they are counted with dots on these sections[11].

In this study, the liver volumes were calculated stereologically in two different ways. The first volume calculation was made using the transparent square grid test system on the liver MR images, the other volume calculation was made using the transparent square grid test system on the cadaveric liver slices. The point-counting method is based on the Cavalieri's principle. According to this method, the MR slices with a cross-sectional thickness of 1.6 mm in the three plans including the axial, coronal, and sagittal were examined one by one. While estimating the volume, the transparent square grid test system with d=2.5 mm was placed 3 times on each section and the points corresponding on the liver were counted and the mean of these numbers were taken (Fig. 2).

After volume calculation was made according to the Archimedes' principle in the dissected livers, they were sliced at 0.5 cm intervals by a special slicing tool. The points corresponding on the liver were counted using the transparent square grid test system (d=0.5 cm) for each section as applied on liver sections in the same MR images (Fig. 2). The following formula was used in volume calculation.

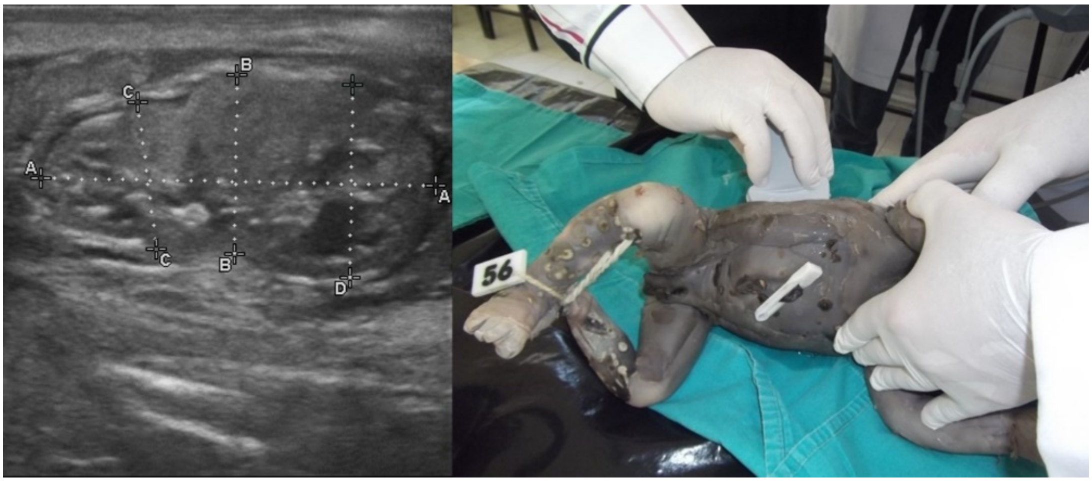


**Figure 1. Measurement of the thickness and length of the liver with USG (A-A: Length, B-B: Thickness)**

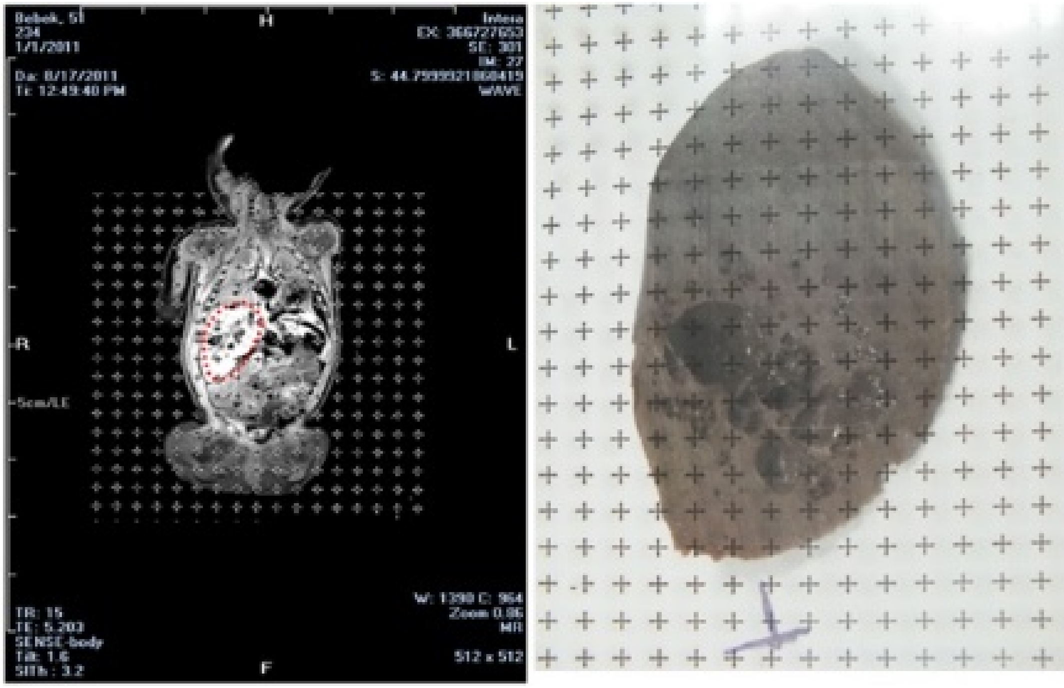


**Figure 2. Placement of the transparent square grid test system on sliced section and on MRI**

In the following formula (Formula 2), 't' is the distance between two sections (cross-sectional area), 'd' is the distance between two points, 'SL' is the length of the scale in the image measured by a ruler or caliper, 'SU' is the length that is represented by the scale indicating the magnification of the image, and '$\sum P$' is the sum of the numbers of the points obtained from all sections. Volume is obtained by multiplying them.

**Formula 2:** $V = t \times [((SU) \times d)/SL]^2 \times \sum P$

The error coefficient (CE) for the volume is calculated as follows (Formula 3)[5, 12]:

**Formula 3:** $CE^2 (V) = CE^2_{CAV} (V) + CE^2_{PC}$

The obtained CE value is the last data of the calculation. If it is less than or equal to 0.05, the sampling used in the study is considered sufficient[13]. If the value of the error coefficient found is high, the number of sections obtained or the point frequency of the transparent square grid test system is changed[6, 14, 15]. In the literature, there are studies that use these formulas to calculate the error coefficient[5, 16-18]. In this study, we calculated the CE values as a predictor using the R program. Firstly, we developed codes to calculate the contribution to the predictive CE using the statistical package R.

## Statistical analysis

In order to evaluate the agreement between the volume measurements of the Archimedes' method and the volume measurements of other methods, some statistical measures, including the concordance correlation coefficient (CCC), Intraclass Correlation Coefficient (ICC), Pearson's correlation coefficient (r), statistical agreement values including p values obtained from paired-t test, least squares analysis (ppt, pls), and coefficient of variation, were calculated. For the entire study, CCC, ICC and r values should be maximized and ppt, pls and coefficient of variation (CV) values should be minimized[19]. The analyses were performed using the SPSS 22.0 Syntax Module and the R 2.15.3 software.

## RESULTS

In our study, the length, width and thickness of the liver were measured by two different methods. The USG and caliper measurements are shown in Table 1. Two of these measurements were performed on images taken from the entire cadavers. Other three measurements were performed after the livers were removed from the cadavers.

The liver volumes obtained by the point-counting method using axial, sagittal, and coronal MR images were compared with those obtained by the Archimedes' principle (gold standard), they differed between -13.85 and 3.73 cm$^3$, -30.02 and -1.44 cm$^3$, and -36.57 and -3.19 cm$^3$, respectively (Table 2). The liver volumes obtained from coronal and sagittal MR images were found to be higher than those obtained from axial MR images (Table 2).

The mean liver volumes calculated using the ellipsoid formula for the USG and caliper measurements (lengtxwidhtxheightx1/2) were 115.86±102.51 and 92.36±47.62 cm$^3$, respectively (Table 3). When all liver volumes were examined, the mean±SD difference in liver volume between the Archimedes' principle and the physical slicing method was found to be 15.88±18.26 cm$^3$ (Table 3).

We observed that the liver volumes measured using caliper gave better estimates than those measured using USG. The mean±SD differences in liver volume between the Archimedes' principle and USG and caliper were found to be 45.86±52.55 cm$^3$ and 22.36±2.34 cm$^3$, respectively (Table 3, Fig. 3).

We observed that the volumes calculated using MR images were closest to the Archimedes' principle (gold standard) and that the volumes calculated in the axial plane among these images showed the closest values to the Archimedes' principle (ICC=0.98).

Moreover, we observed that the values measured with the help of calipers were closest to the Archimedes principle (ICC=0.94). Pearson's correlation and p values also support our results. The results of all methods are shown in Table 4.

**Table 1. Measurement of the liver dimensions using USG and caliper (length-thickness-width(cm))**

| | Length | | Width | | Thickness | |
|---|---|---|---|---|---|---|
| Baby no | USG | Caliper | USG | Caliper | USG | Caliper |
| 1 | 10.30 | 10.00 | 9.87 | 6.90 | 4.93 | 4.80 |
| 2 | 5.05 | 5.40 | 5.76 | 7.70 | 2.81 | 3.30 |
| 3 | 4.97 | 6.60 | 5.49 | 6.60 | 3.52 | 4.20 |
| 4 | 4.58 | 3.90 | 5.07 | 6.50 | 3.22 | 2.90 |
| 5 | 7.52 | 6.70 | 9.65 | 6.90 | 5.58 | 4.30 |
| Mean ± SD | 6.48±2.42 | 6.52±2.25 | 7.16±2.38 | 6.92±0.47 | 4.01±1.18 | 3.90±0.77 |

**Table 2. Volume values measured in the axial, coronal, and sagittal planes and comparison of the mean volume values of these plans with the gold standard volume values (cm³)**

| Baby no | Archimedes Volume | MRI (axial) Volume | MRI (coronal) Volume | MRI (sagittal) Volume | MRI (mean) Volume |
|---|---|---|---|---|---|
| 1 | 142.00 | 128.15 (-13.85) | 111.98 (-30.02) | 105.43 (-36.57) | 115.18 (-26.82) |
| 2 | 44.00 | 43.75 (-0.25) | 36.88 (-7.12) | 34.30 (-9.7) | 38.31 (-5.69) |
| 3 | 45.00 | 44.01 (-0.99) | 39.15 (-5.85) | 23.73 (-21.27) | 35.63 (-9.37) |
| 4 | 19.00 | 22.73 (3.73) | 17.56 (-1.44) | 15.81 (-3.19) | 18.70 (-0.3) |
| 5 | 100.00 | 80.68 (-19.32) | 91.12 (-8.88) | 89.83 (-10.17) | 87.21 (-12.79) |
| Mean ± SD | 70.00±49.96 | 63.86±41.55 (-6.14±8.41) | 59.33±40.12 (-10.67±9.84) | 53.82±40.90 (-16.18±9.06) | 59.00±40.46 (-11.00±9.5) |

**Table 3. Comparison of the values obtained by the Archimedes' principle with the values obtained by other methods.**

| Baby no | Archimedes Volume | Caliper Volume | USG Volume | MRI (mean) Volume | Slicing Volume |
|---|---|---|---|---|---|
| 1 | 142.00 | 165.60 (23.60) | 250.59 (108.59) | 115.18 (-26.82) | 200.83 (58.83) |
| 2 | 44.00 | 68.60 (24.60) | 40.86 (-3.14) | 38.31 (-5.69) | 55.38 (11,38) |
| 3 | 45.00 | 91.47 (46.46) | 48.02 (3.02) | 35.63 (-9.37) | 49.83 (4.83) |
| 4 | 19.00 | 36.75 (17.75) | 37.38 (18.38) | 18.70 (-0.30) | 30.04 (11.04) |
| 5 | 100.00 | 99.39 (0.61) | 202.46 (102.46) | 87.21 (-12.79) | 93.33 (6.67) |
| Mean ± SD | 70.00±49.96 | 92.36±47.62 (22.36±2.34) | 115.86±102.51 (45.86±52.55) | 59.00±40.46 (11.00±9.5) | 85.88±68.22 (15.88±18.26) |

Values in parentheses indicate the differences between the values measured by the Archimedes principle (gold standard) and the other methods (MRI, USG, physical slicing, and caliper).

**Table 4. Volume values obtained from axial, sagittal and coronal MR images, mean volume values obtained from MR images and volume values obtained from ultrasonography (USG), physical slicing, and caliper**

| | USG (cm³) | Caliper (cm³) | Physical Section (cm³) | MRI Mean (cm³) | Axial | Sagittal | Coronal |
|---|---|---|---|---|---|---|---|
| CCC(95% CI) | 0.64(0.25-0.85) | 0.83(0.33-0.97) | 0.87(0.52-0.97) | 0.94(0.80-0.98) | 0.97(0.87-0.99) | 0.89(0.57-0.98) | 0.94(0.77-0.98) |
| ICC | 0.77(0.12-0.97) | 0.94(0.54-0.99) | 0.91(0.39-0.99) | 0.98(0.79-1.00) | 0.98(0.80-1.00) | 0.96(0.66-1.00) | 0.97(0.74-1.00) |
| R | 0.97(0.65-1.00) | 0.94(0.35-1.00) | 0.96(0.48-1.00) | 0.99(0.96-1.00) | 0.99(0.90-1.00) | 0.98(0.70-1.00) | 0.99(0.89-1.00) |
| Paired t-test | 0.136 | 0.041* | 0.230 | 0,070 | 0.238 | 0.051 | 0.100 |
| Least Square | 0,005* | 0,017* | 0,011* | <0,001* | 0.001* | 0.004* | 0,001* |
| CV | 88,50 | 51,60 | 79,40 | 68,6 | 65,10 | 76,00 | 67,60 |

CCC — concordance correlation coefficient; CI — confidence interval; ICC — Intraclass correlation coefficient; r — Pearson's correlation coefficient; ppt — p value obtained from paired-t test; pls — p value obtained from least square analysis; CV — coefficient of variation; maximum CCC, ICC and r values and minimum ppt, pls and CV values indicate best agreement between physical section and gold standard volume

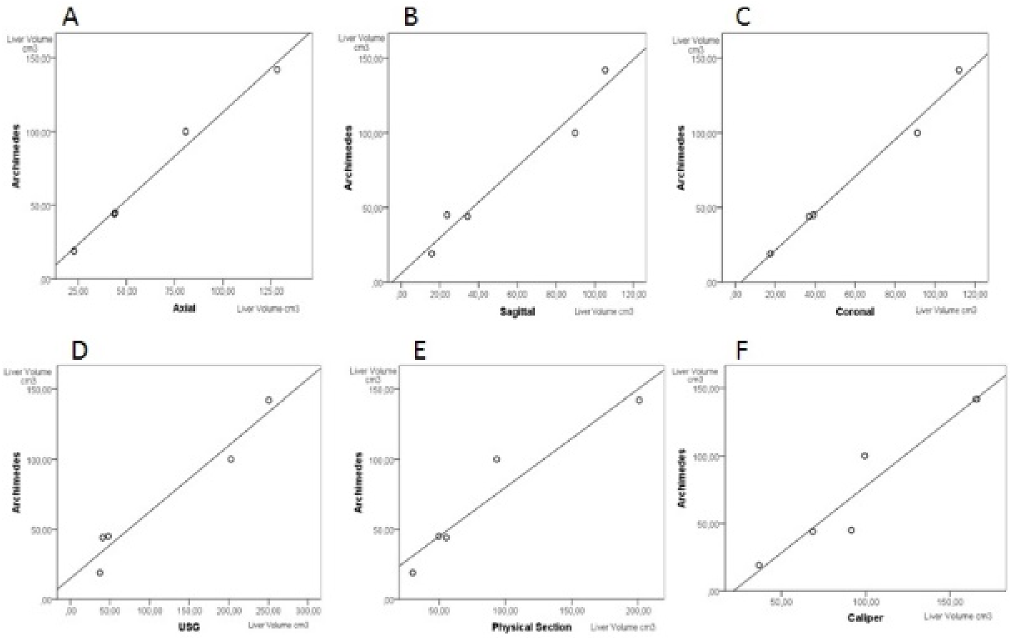


**Figure 3. Scatter plots displaying the volume estimates of Archimedes principle versus (A) axial magnetic resonance imaging (MRI), (B) sagittal MRI, (C) coronal MRI, (D) ultrasonography, (E) physical section, (F) caliper.**

## DISCUSSION

In many diseases, there are the changes in the sizes and morphologies of these organ. For this reason, it is very important to determine the normal parameters of these organs in order to accurately evaluate the changes.

There are numerous studies in the literature about the calculation of organ volumes by computed tomography (CT) and MRI[3-5, 20]. In these studies, the Cavalieri's principle from the stereological methods was used as volume calculation method[3, 4, 7]. Using the Cavalieri's principle on MRI, the volumes of the organs such as brain[21], fetus[7, 14], bile duct[22], heart[23], kidney[24] and prostate[25] were calculated.

In clinical practice, it may require a simple formula that can be used for subsequent monitoring of treatment and for rapid calculation of liver volume. Although different methods have been evaluated for USG and CT, there is no standard for measuring liver size in cross-sectional images[26, 27].

When studies on organ sizes and volumes in the literature are examined, it is seen that organ sizes and volumes can be measured by many different methods (Percussion, USG, MRI, CT, Stereology, Cavalieri, etc. that are performed directly on cadavers). Moreover, the presence of different correlations between organ sizes and other body parameters is also emphasized. The same is true for the liver.

In the literature, as in the studies of Yuan et al.[28] and Yoshizumi et al.[29], the age, height, weight, body mass index, and body surface area of the patients were calculated and it was tried to calculate the standard liver volume with the help of computerized tomography.

Urata et al.[30] create a formula to determine the standard liver volume by identifying age, weight, height, and body surface area in 47 male and 45 female healthy donors in order to determine the standard liver volume in adults.

In the newborn period, ultrasonography is a highly-preferred imaging method. With this technique, a rapid assessment of the internal organs is achieved without any anesthesia and without the risk of radiation[24, 31-33]. The normal ranges of spleen, liver and kidney sizes identified by ultrasonography in children and adults have been described previously. However, the available data on the term and preterm neonates is limited[24, 32].

In a study conducted with ultrasound in 194 children by Dittrich et al.[34] and in a study conducted with ultrasound in 307 children by Konus et al.[24], the organ dimensions showed the best correlation with the body lengths. In a study performed in 584 healthy children under the age of 7 by Rocha et al.[35], they reported that liver dimensions measured by USG showed the best correlation with the children's body lengths and that there was no difference in the girls and boys.

Another study, Sureyya et al. performed in a total of 253 (99 preterm and 154 term infants) healthy newborns with gestational ages of 24-41 weeks and weights of 638-4800 g., they evaluated the proportions of the liver, spleen and kidney dimensions with the gestational age, weight and height by ultrasonography within the first week following birth. And found that the length of liver was 4.61±0.95 cm in preterm infants and 5.45±0.87 cm in term infants, respectively[32]. These organ dimensions showed the best correlation with the lengths of the newborns. As seen in the literature, it was found that organ dimensions had no any relationship with gender[24, 32].

Different study conducted in 281 Thai children (148 boys and 133 girls) under 2 years of age by Weerakul et al.[31], they calculated the body surface areas by taking age, weight, and size. The liver lengths were also measured by ultrasonography and physical examination. It was reported that the liver size was found to be 5.4±1.0 cm by physical examination and 5.1±1.1 cm by ultrasound, respectively. They reported that the liver size obtained by physical examination was 0.3 cm larger than those obtained by ultrasound. They reported that the liver size obtained by ultrasound showed the best correlation with the body surface area according to other variables (age, weight, and height). In a study performed with ultrasound in Indian children from the same age groups by Dhingra et al.[36], they found that the mean liver length was 9.59±1.98 cm (9.63 cm in boys, 9.54 cm in girls) and reported that the liver size was larger in Indian children than Thai children.

Sahin et al[6], conducted on 5 normal livers obtained from cadavers MR images were scanned in the horizontal and sagittal plane and the consecutive sections at 10 mm thickness were taken. There was no difference between the liver volumes obtained by 3 different investigators in order to evaluate the agreement of the volume estimates.

Sahin and Ergur[20], examined the effect of section thickness in estimating the liver volume. 5 normal cadaveric livers were scanned in the horizontal plane and the sections were taken at 10 mm, 7.5 mm, 5 mm and 2.5 mm thickness. It was reported that there was no significant difference between the planimetry and the point-counting method and the optimal cross-sectional thickness was 4-5 mm.

There are studies in which the ellipsoid formula is used to measure the volume of organs. It is reported that the volume measurements obtained with this method are reported to be similar to the actual volume obtained according to the Archimedes' principle[25].

Acer et al.[25] performed physical fragmentation, USG and volume calculations according to the Archimedes' principle in 5 adult prostate glands obtained after radical prostatectomy. As a result, they considered the Archimedes' principle as the gold standard and found that the values obtained with USG were calculated to be 25% less.

It has been observed that the volume of the liver may be measured with automatic volume measurement methods in recent studies[37-39]. In these methods, the measurements may be made by using various measurements. The biggest advantage of these methods is the fast volume calculations. However, the superiority of our study is the fact that the application is made on cadaver livers and forms a golden standard. In addition, automatic volume measurement methods are not user-friendly and require complex mathematical operations and codes, which poses a second disadvantage.

In the literature, there was no study in which the liver volume was calculated using MRI and CT in newborns. Although MRI is non-invasive method, these methods are not preferred because they can be applied under anesthesia in newborns. Moreover, we think that CT is not preferred due to radiation exposure. Some negligible differences between organ volumes calculated using different methods are considered as natural.

Limitation: In our study, volume measurements were made on 5 cadaver livers with various methods. Since it is difficult to obtain baby cadavers, we believe that the study is superior, valid and reliable. Moreover, the fact that our studies were performed with MR images is a superior aspect of MR applications, as it does not contain radiation, such as CT, and is a non-invasive procedure. In the literature we see that many such measurement operations are performed on CT images.

As a result of our study, it was seen that the stereological methods can give very close results with MR from the clinical and routine diagnostic methods and stereological methods can also be used in volume calculations. In conclusion, we believe that the results of our study will contribute to the clinical evaluation and preoperative surgical planning of diseases that would change the liver size.

**Yazar Katkıları:** Çalışma konsepti/Tasarımı: SA, EU, NA, TE, AD; Veri toplama: SA,.EU, NA, TE; Veri analizi ve yorumlama: SA, EU; Yazı taslağı: SA; İçeriğin eleştirel incelenmesi: SA, EU, NA, TE, AD; Son onay ve sorumluluk: SA, EU, NA, TE, AD; Teknik ve malzeme desteği: SA; Süpervizyon: SA, EU, NA, TE, AD; Fon sağlama (mevcut ise): yok.
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**Ethical Approval:** This study has been approved by the Ethics Committee of the Erciyes University Medical Faculty (2011/79).
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