

# Estimation of the Total Brain Volume Using Semi-Automatic Segmentation and Stereology of the Newborns' Brain MRI

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## ABSTRACT

Brain development begins in the early embryonic period and proceeds through the second decade of postnatal life. However information related to the development of the brain during this period is currently quite limited. The aim of the current study was to compare the total brain (cerebrum, cerebellum and brain stem, without the ventricular system) volumes in newborns using stereological (point-counting) and semi-automatic segmentation methods and Archimedes' principle. Seven newborn cadavers, aged 39.9 ( $\pm 1.2$ ) weeks, were included in the present study. Firstly, the total brain (TB) volume was determined by the use of the fluid displacement technique and then magnetic resonance images were analyzed by using two methods. The mean ( $\pm$ SD) TB volumes by fluid displacement, Cavalieri principle (point-counting), and semi-automated segmentation methods were  $288.70 \pm 76.18$ ,  $270.12 \pm 78.93$  and  $282.39 \pm 73.17$  cm<sup>3</sup> respectively. We did not find any significant differences among the three methods ( $p > 0.05$ ). From these results, it can be concluded that the semi-automated segmentation method and stereological technique can be used for reliable volume estimation of total brains in neonates. Based on these techniques we compared here in, the clinician may evaluate the growth of the brain in a more efficient and precise manner.

**Key Words:** MRI, brain, segmentation, stereology

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## Introduction

The development of the human brain and its compartments extends throughout, approximately, the first 1.5 years of life after conception. The rate of development is most rapid during in utero life and the first postnatal months. There is a significant

increase in whole brain size during these periods, with the brain reaching 80–90% of adult volume by age 2 (Pfefferbaum *et al.*, 1994; Caviness *et al.*, 1996). Measurement of brain volume is important and necessary for accurate or objective assessment of brain growth and of changes in the brain in normal subjects and patients. Early identification of neurodevelopmental disorders may lead to intervention programs and improved outcome (Resnick *et al.*, 1988; McCarton *et al.*, 1996). Magnetic resonance imaging (MRI) has made delineation of the border between the cerebral tissue and cerebrospinal fluid easier. This feature makes MRI suitable for the quantitative measurement of brain morphology and it helps physicians to diagnose and treat medical conditions.

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Some studies have focused on the volume estimation of the brain and its compartments in neonates using segmentation methods (Knickmeyer *et al.*, 2008; Prastawa *et al.*, 2005; Weisenfeld and Warfield, 2009; Despotovic *et al.*, 2010). Segmentation of the newborn brain is considerably more difficult than that of the adult brain. Also, there are some limitations in the segmentation of neonates' brains. The newborn brain exhibits reduced image contrast (due to the high water content and ongoing myelination in the white matter), lower spatial resolution (due to smaller head size), and lower signal-to-noise ratio (due to the short scanning period), (Prastawa *et al.*, 2005; Mewes *et al.*, 2006). However there are a few studies on estimating the brain volume of neonates using Cavalieri's principle (Iwasaki *et al.*, 1997; Nisari *et al.*, 2012).

The accurate volume estimation of the brain and its structures is an important process for the evaluation of brain disorders and abnormalities in neonates such as autism and schizophrenia (Knickmeyer *et al.*, 2008). To achieve this goal, the methods used for volume estimation of the brain in MRI need to be accurate and reliable.

The value of our study lies in the fact that only a few studies in the literature have investigated the total brain (TB) volumes in newborns using the stereological method; however no study has compared the stereological, segmentation and fluid displacement methods. This study was designed to fill this gap in knowledge; so we compared the volumes of the brain using stereological (point-counting), semi-automated segmentation methods and Archimedes' principle.

## Material and methods

### Subjects

Seven newborn cadavers were a mean age of 39.9 ( $\pm 1.2$ ) weeks, with 71.42% being male, and the ethnic composition was 100% Caucasian. The mean weight of the subjects was ( $\pm$ SD) 2.690  $\pm$  875 g. The supply of newborn cadavers is limited; therefore five of the brains from our previous study (Nisari *et al.*, 2012) were included in the present study.

All cadavers' neurodevelopmental histories were normal. The handling of the newborn specimens was conducted under the

appropriate ethical safeguards and protocols of the Local Ethics Committee of Erciyes University, Turkey. Firstly, the cadavers were fixed by neck vessel perfusion according to their sizes and then kept in a 10 % formalin solution for six months. The MRI was done after fixation. After the MR process, the brains were removed from the cranium. We didn't include the ventricular system in total brain (cerebrum, cerebellum and brain stem) volume estimations for all of the three methods.

### Magnetic resonance image data

We analyzed the neurologically intact cranial MR images of all the subjects. The MRI images were prepared using the following protocol. T1-weighted sagittal images using a 1.5 Tesla MR machine (Philips, Intera, and The Netherlands) were obtained. The following parameters were used for the imaging process: TR/TE: 25/5; two excitations; FOV: 130x160; 7-mm slice thickness without a gap between the slices and a 224 x 224 matrix.

To investigate the accuracy of our methods (point-counting and semi-automatic segmentation methods) prior to their practical application, we measured the volume of the seven cadaver brains using Archimedes' principle (measurement of real volume using water displacement).

### Real volume estimation

After fixation, the exact brain volumes were measured using Archimedes' principle, also known as the 'fluid displacement technique', in a measuring cylinder (Howard and Reed, 2005). For this purpose, we dissected the cranium to see the intracranial contents. After craniotomy, each brain was immersed in a measuring cylinder filled with distilled water at room temperature. The displaced water was measured volumetrically by means of a sensitive ruler, which was attached to the outer surface of the measuring cylinder. Each measurement was performed twice, and the average was accepted as the actual volume.

### Stereology

The Cavalieri principle has been used for volume estimations in the literature (Cruz-Orive, 1997; Gundersen *et al.*, 1999). Volume estimation using the Cavalieri method is



calculated as follows in the equation (Eq. 1), (Roberts *et al.*, 2000);

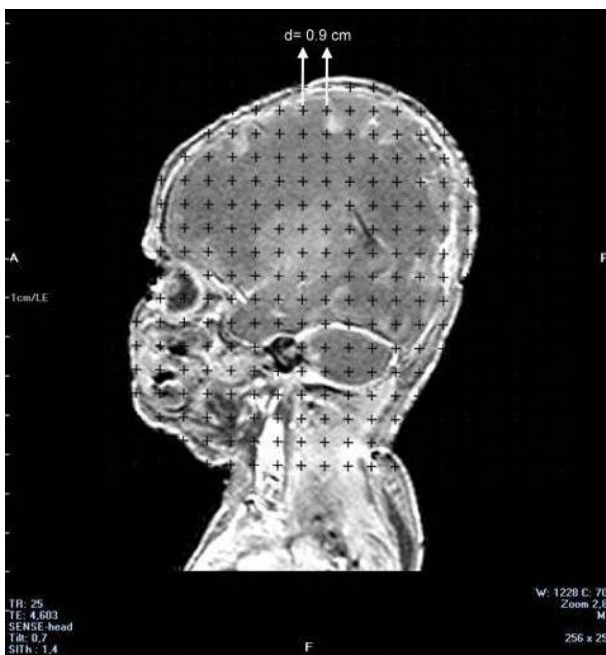
$$V = T \times \sum_{i=1}^n V_i \quad (1)$$

where  $V_i$  is the total volume of the tissue slice (which may comprise several slice profiles) in the  $i$ th slab and ‘T’ is the section thickness.

**Cavalieri Estimator:** The MRIs of a section series with 7 mm thickness were used for TB volume estimation. The images were saved on the computer and a transparent square grid test system with  $d = 0.9$  cm between the test points was superimposed on the brain images. The points hitting the structure’s sectioned surface area were counted for each section and the volume of the total brain was estimated using the formula (Eq. 2). The volume estimations of radiological images are shown below (Ertekin *et al.*, 2011; Acer *et al.*, 2007; 2008; Sahin and Ergur, 2006).

$$V = T \times [d]^2 \times \sum p \quad (2)$$

where ‘T’ is the section thickness, ‘d’ the distance between the test points of the grid and ‘ $\sum p$ ’ is the total number of points hitting the sectioned cut surface areas of the structure. According to this volumetric technique, a square grid of test points was positioned on each MRI, and all points hitting the structure were counted (Figure 1).



**Figure 1.** A sagittal MRI with a point-counting grid superimposed on it for the estimation of total brain volume.

### Error prediction for point counting

The error predictors given below come from the recent literature (Gundersen *et al.*, 1999; Garcia-Finana *et al.*, 2003).

The error of volume is computed as follows:

It can be shown that

$$CE^2(\tilde{V}) = CE^2_{CAV}(\hat{V}) + CE^2_{PC}(\tilde{V}) \quad (3)$$

where  $CE^2(\tilde{V})$  = coefficient of error of the volume estimate

$CE^2_{PC}(\tilde{V})$  = true mean variability due to point counting within sections

$CE^2_{CAV}(\hat{V})$  = true contribution of the variability among sections

In equation (3),  $CE^2(\tilde{V})$  is the square coefficient of error of the estimator of V when the areas are measured exactly.

A CE value lower than 5% is in the acceptable range according to the literature (Sahin and Ergur, 2006). It is also important to note that an appropriate grid size and the number of slices required for volume estimation of an object are crucial at the beginning.

### Segmentation of newborns’ brain

Nowadays, computer-aided systems are being continually developed to improve the quality of medical diagnosis and in order to make numerical evaluation. Another purpose of this study with a computer-aided system is to make a comparison with other methods by calculating the brain total area and volume. Brain areas and the total volume are calculated with the flow diagram shown in Figure 2. Figure 3 summarizes visually the process for finding the volume of the brain. The original brain image is converted to binary format. After defining a disc-shaped structural element, morphological erode and dilate operations are performed. Then, the edge detection process is made and the found edges are collected with the original brain image (Scott *et al.*, 2009). The arithmetic process is done by filling the area of the brain with white so that only the brain region is visible in the picture. The total brain volume is obtained by calculating the area of each brain cross-section. MRI-based volumetric analysis and calculation procedure generally was repeated



for the slice of images involved in the measurement process, and the individual per-slice volumes were summed to provide a single volume measure (O'Dwyer *et al.*, 2010).

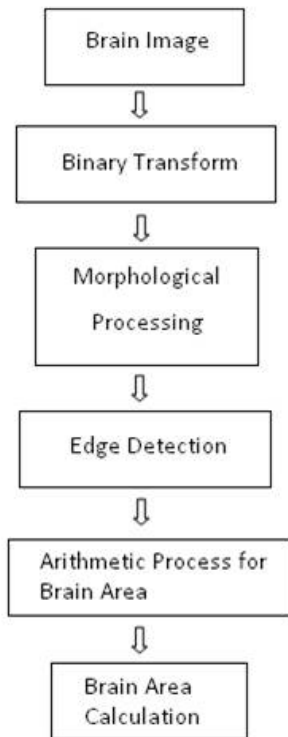


Figure 2. The flow diagram for brain areas.

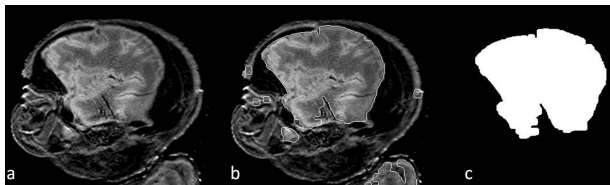


Figure 3. Process for finding volume of the brain. (a) original brain image, (b) edge detection of brain region, (c) brain area for this section.

### Statistical analysis

Statistical analysis was performed with SPSS 15.00 (SPSS, Chicago, IL). Mean values are presented with their standard deviations. Normality of distribution was tested using the Kolmogorov-Smirnov test and normal plot. The differences in the mean volumes of the brain, were measured using Cavalieri, semi-automated segmentation and fluid displacement methods, were tested using Kruskal-Wallis. A p value of < 0.05 was considered as statistically significant.

### Results and Discussion

We calculated the mean ( $\pm$ SD) TB volumes by fluid displacement, the Cavalieri principle (point-counting) and semi-automated segmentation methods and they were  $288.70 \pm 76.18$ ,  $270.12 \pm 78.93$  and  $282.39 \pm 73.17$  cm<sup>3</sup> respectively. There were no differences among the three methods ( $P = 0.875$ , Kruskal-Wallis).

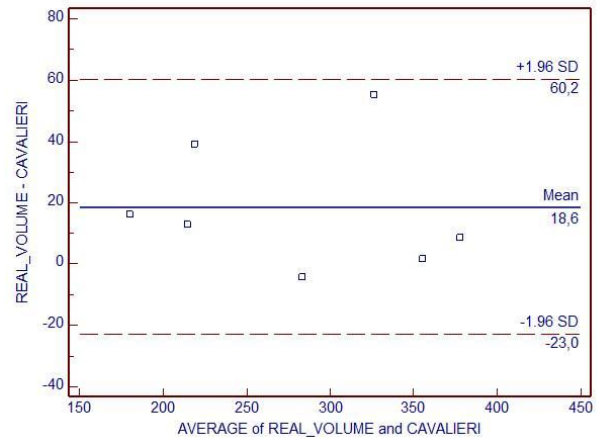


Figure 4. Bland-Altman analysis of the total brain volume as measured by Archimedean principle versus point-counting technique.

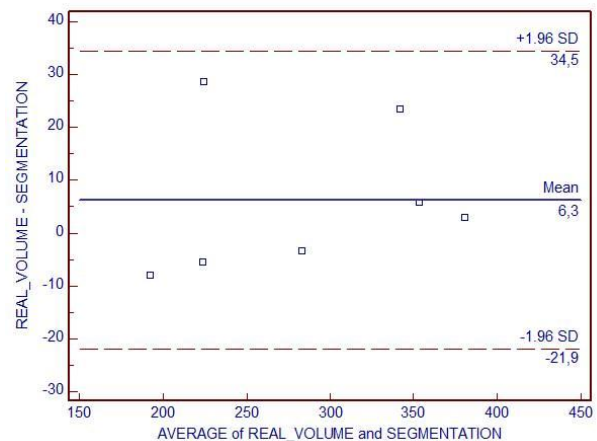


Figure 5. Bland-Altman analysis of the total brain volume as measured by Archimedean principle versus segmentation method.

We compared point-counting volume with fluid displacement, the gold standard. The difference of,  $-4.09$ - $55.2$  cm<sup>3</sup>, ( $-1.45$ - $16.48$  %) between the measurements is a 6.84 % underestimation of the TB volume by point counting (Table 1). The semi-automatic segmentation method compared with fluid displacement volume revealed a difference of  $-8$ - $28.60$  cm<sup>3</sup> ( $-4.25$ - $12$  %), 1.87 %



underestimation of the TB volume by the semi-automatic segmentation method (Table 2). The agreements between methods were subjected to Bland-Altman plots using volume differences of 95. According to Bland Altman analysis, there were no differences among the results of the three methods (Figures 4, 5).

**Table 1.** Differences between actual TB volume and volume obtained by point counting.

Case No	Actual volume (cm <sup>3</sup> )	Cavalieri (cm <sup>3</sup> )	Differences (cm <sup>3</sup> )	%
1	356.1	354.2	1.9	0.53
2	188.1	171.9	16.2	8.61
3	220.8	207.8	13	5.88
4	238.4	199.1	39.3	16.48
5	354.0	298.8	55.2	15.59
6	382	373.46	8.54	2,24
7	281.50	285.59	-4.09	-1,45
Min-Max	188.1-382	171.9-373.46	1.9-55.2	0.53-16.48
Mean±SD	288.70±76.18	270.12 ± 78.93	18.57±21.21	6.84±7.11

**Table 2.** Differences between actual TB volume and volume obtained by segmentation.

Case No	Actual volume (cm <sup>3</sup> )	Segmentation (cm <sup>3</sup> )	Differences (cm <sup>3</sup> )	%
1	356.1	350.3	5,8	1.63
2	188.1	196.1	-8	-4.25
3	220.8	226.2	-5,4	-2.45
4	238.4	209.8	28,6	12
5	354.0	330.5	23,5	6.64
6	382	378.92	3.08	0.81
7	281.50	284.92	-3.42	-1.21
Min-Max	188.1-382	196.1-378.92	3.08-28.6	0.81-12
Mean±SD	288.70±76.18	282.39 ± 73.17	6.3±14.37	1.87±5.65

Brain development in early childhood (birth to 2 years) probably plays a critical role in postnatal neurodevelopmental disorders. However, knowledge related to the development of brain during this period is currently quite limited (Pfefferbaum *et al.*, 1994; Benedetti *et al.*, 2006).

There are some studies which have focused on the volume estimation of the brain and brain compartments by means of segmentation methods using MRI in adults (Shen *et al.*, 2012; Allen *et al.*, 2003), children (Knickmeyer *et al.*, 2008; Jou *et al.*, 2009), and newborns (Anbeek *et al.*, 2008; Nishida *et al.*, 2006; Gui *et al.*, 2012).

Manual segmentation remains the current gold standard for region identification

but its application for large samples is impractical due to time constraints and it is subjective and error prone (Pham *et al.*, 2000; Zhou and Rajapakse, 2005). To eliminate the problems related to manual segmentation, several automated and semi-automated algorithms have been developed, including atlas-based methods (Fischl *et al.*, 2004; Haller *et al.*, 1997), voxel-based morphometry using Statistical Parametric Mapping (Ashburner and Friston, 2000) tensor-based morphometry (Leow *et al.*, 2005;), and boundary shift integral methods (Anderson *et al.*, 2007).

Anbeek *et al.* (2008) developed a fully automated method for the segmentation of neonatal brains. Their method uses a *K* nearest neighbor (KNN) classification technique with features derived from spatial information and voxel intensities. Their study investigated three methods for volume calculation: one derived from binary segmentation with the optimal threshold, one derived from majority segmentation, and one derived from probabilistic segmentation. They used manual segmentation results as a gold standard volume. In both binary segmentations, the volumes of the whole brain (358.2 and 357.1 mL), were significantly different from the gold standard volumes (319.0 mL), whereas the probabilistic segmentation volumes (326.0 mL) did not differ significantly from the gold standard for the whole brain.

Yu *et al.* (2010) examined the brain and its compartments' volumes in extremely low birth weight infants and reported that the mean total brain volumes were 269.838, 269.965 and 270.457 cm<sup>3</sup> calculated using automated, semi-automated and manual segmentation methods, respectively. Volumes determined by the automated program exhibited minimal differences from manually segmented volumes. The semi-automated approach that permits final correction, further reduced volume differences from 0.2 to 1.5 %.

Our results are different from those of other researchers; this discrepancy may be due to subjects groups used. They investigated living subjects in their studies but we used cadavers. However, there are some studies suggesting that human brain volume, measured by MR imaging, can change rapidly. Lack of fluid intake for 16 hours decreases brain volume by 0.55% and rehydration can



increase total cerebral volume by 0.72% (Duning *et al.*, 2005; Steen *et al.*, 2007) So these factors should be considered in comparisons of the studies. In addition to these, differences among our study and the others may be caused by different scanning protocols and measuring methods used.

Studies using segmentation methods for neonatal brains focus on the segmentation of gray matter, white matter, cerebrospinal fluid, central gray matter and cortical gray matter (Mewes *et al.*, 2006; Toft *et al.*, 1995; Huppi *et al.*, 1998; Tolsa *et al.*, 2004; Zacharia *et al.*, 2006; Warfield *et al.*, 2000). However, comparison of their methods with our method is difficult because some methods do not evaluate quantitatively or do not have a gold standard (Toft *et al.*, 1995; Huppi *et al.*, 1998); often only the visual inspection of the segmentation results is incorporated (Warfield *et al.*, 2000). Accurate MRI head segmentation into brain and non-brain tissue is an important step for a further neuroimaging and clinical analyses. Due to the lack of a gold standard, researchers have performed only a limited validation of their results (Knickmeyer *et al.*, 2008; Prastawa *et al.*, 2005; Yu *et al.*, 2010). In our study, quantitative validation of the segmentation results of the total brain, using the fluid displacement technique as a gold standard, was performed. The two methods were correlated with each other. There were no differences between the two methods.

Some studies have focused on the volume estimation of the brain and its compartments such as the ventricle, brain stem and cerebellum by using the point-counting method (Acer *et al.*, 2007, 2008; Ekinici *et al.*, 2008; Garcia-Finana *et al.*, 2009; Lee *et al.*, 2009). On the other hand, only one study which was our previous study has investigated the brain and brain compartments' volumes by the fluid displacement technique as a gold standard and point-counting method in newborns (Nisari *et al.*, 2012). The authors did not find any differences between the the fluid displacement and point-counting methods for all structures. Similarly, we did not find any significant difference among the three methods for total brain volume estimation.

For volume measurement, the accuracy of the methods is most important. Evaluation of the accuracy of the methods is more difficult

because of the lack of a gold standard. Few studies have used the estimations of cadaver human brains to assess the accuracy of their methods. Ashtari *et al.* (1990) reported that the error of calculated volumes compared with real volumes ranged from 0.0% to 3.4% for fresh cadaver brains using a semi-automated system on MRI. Hamano *et al.* (1990) also reported that the mean percentage error of calculated volumes compared with real volumes was 2.91% using cadaver brains. We evaluated the accuracy of our methods (semi-automatic segmentation and point-counting methods) comparing the volume calculated by the water displacement method (real volume) for cadaver brains and we determined no differences among the three methods.

In addition, the stereology method provides a coefficient of error (CE) of the measurement of the volume of the structure of interest. Therefore, it may be used to identify the optimal parameters of sampling needed to achieve a given precision, such as the number of MRI sections and the density of the point grid needed. Thus the stereological method provides an opportunity for the investigator to make appropriate changes in their sampling or estimating procedures (Sahin *et al.*, 2003).

## Conclusion

Ultimately, both of the methods evaluated in this study can be used for the assessment of total brain volume using MRI in living subjects. Excellent agreement was found among the three volumetric techniques. In addition we found that counting approximately 850-950 points on fourteen or fifteen systematically sampled MR sections of 7 mm section thickness enables total brain volume to be determined with a CE of 5%. Our methods for direct measurement of brain volume based on MRI may be important tools in determining neurodevelopmental disorders in newborns. Obviously, like all studies, our study also has some limitations, the most important of which to our mind is the sample size of our study. Further studies involving greater numbers of cadavers would be helpful to extend and support the findings in our study.

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