

Normative Volumetric Values of the Brainstem in Healthy Adults: Study of 3D Segmentation Technique

Sağlıklı Yetişkinlerde Beyin Sapı Hacminin Normatif Değerleri: 3B Segmentasyon Tekniği Çalışması

Ece Zeliha Büyükuysal¹ , Gamze Taşkın Şenol¹ , Sıddıka Halıcıoğlu² 

¹Bolu Abant İzzet Baysal University Faculty of Medicine, Department of Anatomy, Bolu, TÜRKİYE

²Bolu Abant İzzet Baysal University, Faculty of Medicine, Department of Radiology, Bolu, TÜRKİYE

Abstract

Background: The brainstem is a critical center that connects the spinal cord with the brain and governs essential life functions. Its subdivisions-mesencephalon, pons, and medulla oblongata-play vital roles in regulating motor, sensory, and autonomic system functions. The morphological characteristics of these structures are of great importance for both neuroscientific research and clinical diagnostic and follow-up processes. The aim of this study is to compare the volumes of brainstem subregions in healthy individuals and to establish normative reference values.

Materials and Methods: This retrospective study evaluated magnetic resonance images of 191 healthy individuals (105 females, 86 males) aged between 18 and 65 years, with no neurological disorders. The volumes of the mesencephalon, pons, and medulla oblongata were measured using manual segmentation via the ITK-SNAP software. The volumetric data obtained were analyzed both as absolute values and as proportions relative to the total brainstem volume, and normative reference ranges were determined.

Results: The Friedman ranking test used for volumetric comparisons among the structures revealed statistically significant differences between the volumes of the mesencephalon, pons, and medulla oblongata ($p < .001$). Post hoc analyses indicated that the volume of the pons was significantly larger than that of both the mesencephalon and medulla oblongata. Analyses conducted using the Mann-Whitney U test showed no statistically significant differences between sexes in terms of mesencephalon, pons, and medulla oblongata volumes ($p > .05$). According to the Shapiro-Wilk test, the volumes of the mesencephalon and pons did not show normal distribution, whereas the medulla oblongata volume met the normality assumption.

Conclusions: This study provides normative volumetric values and reference ranges for brainstem subregions in healthy individuals. The data obtained may serve as a comparative reference for understanding individual variation in brainstem anatomy and for aiding early diagnosis and monitoring of neurological disorders. Additionally, no significant sex-related differences were observed in the regional volume proportions within the total brainstem. These findings offer valuable reference data for neuroimaging-based clinical evaluations and make important contributions to basic science research.

Keywords: Mesencephalon, Pons, Medulla oblongata, ITK-SNAP

Öz

Amaç: Beyin sapı, omurilik ile beyin arasındaki bağlantıyı sağlayarak temel yaşam fonksiyonlarını yöneten kritik bir merkezdir. Alt bölümleri olan mesencephalon, pons ve medulla oblongata; motor, duyu ve otonom sistem işlevlerinin düzenlenmesinde hayati roller üstlenmektedir. Bu yapıların morfolojik özellikleri, hem nörobilimsel araştırmalar hem de klinik tanı ve takip süreçleri açısından büyük önem taşımaktadır. Bu çalışmanın amacı, sağlıklı bireylerde beyin sapı alt bölümlerinin hacimlerini karşılaştırarak normatif referans değerler oluşturmaktır.

Materyal ve metod: Bu çalışmada, 18-65 yaş aralığında ve herhangi bir nörolojik patolojisi bulunmayan 191 sağlıklı bireyin (105 kadın, 86 erkek) manyetik rezonans görüntüleri retrospektif olarak değerlendirilmiştir. Mesencephalon, pons ve medulla oblongata hacimleri ITK-SNAP yazılımı aracılığıyla manuel segmentasyon yöntemiyle ölçülmüştür. Elde edilen hacimsel veriler, hem mutlak değerleriyle hem de toplam beyin sapı hacmi içindeki oranlarına göre analiz edilmiş ve normatif referans aralıkları belirlenmiştir.

Bulgular: Yapılar arası hacimsel karşılaştırmalarda Friedman sıralama testi, mesencephalon, pons ve medulla oblongata hacimleri arasında istatistiksel olarak anlamlı farklar olduğunu ortaya koymuştur ($p < .001$). Post-hoc analizlerde pons hacminin, hem mesencephalon hem de medulla oblongata'ya göre anlamlı düzeyde daha büyük olduğu belirlenmiştir. Mann-Whitney U testi ile yapılan analizler, cinsiyetler arasında mesencephalon, pons ve medulla oblongata hacimleri açısından istatistiksel olarak anlamlı bir fark bulunmadığını göstermiştir ($p > .05$). Shapiro-Wilk testine göre, mesencephalon ve pons hacimleri normal dağılım göstermemiş, medulla oblongata hacimleri ise normal dağılım varsayımını karşılamıştır.

Sonuç: Bu çalışma, sağlıklı bireylerde beyin sapı alt bölümlerine ait normatif hacim değerlerini ve referans aralıklarını ortaya koymuştur. Elde edilen veriler, beyin sapı yapılarındaki bireysel varyasyonların anlaşılması ve nörolojik hastalıkların erken tanı ve takip süreçlerinde karşılaştırmalı veri kaynağı olarak kullanılabilir. Ayrıca, toplam beyin sapı hacmi içindeki bölgesel oranların cinsiyete göre anlamlı farklılık göstermediği belirlenmiştir. Bu bulgular, nöro görüntüleme tabanlı klinik değerlendirmelerde başvuru verisi niteliğinde olup, temel bilim araştırmalarına da önemli katkılar sunmaktadır.

Anahtar Kelimeler: Mesencephalon, Pons, Medulla oblongata, ITK-SNAP

Corresponding Author: Gamze Taşkın Şenol, Bolu Abant İzzet Baysal University Faculty of Medicine, Department of Anatomy, Bolu, TÜRKİYE

E-mail: rumeysagamzetaskin@ibu.edu.tr / **ORCID ID:** 0000-0001-5587-1055

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Introduction

The brainstem plays a critical role in maintaining the connection between the spinal cord and the brain and in regulating vital life functions. Through its complex neural networks and nuclei, it enables the harmonious functioning of the sensory, motor, and autonomic nervous systems (1).

Comprising three main sections, the brainstem occupies a central position in the integrated operation of the nervous system due to its anatomical and functional characteristics. Normative morphological data regarding the subregions of the brainstem provide fundamental references for both neuroimaging studies and the early diagnosis of neurological disorders.

The mesencephalon, the most superior part of the brainstem, is involved in visual and auditory reflexes as well as motor control mechanisms. It also plays a significant role in the regulation of the sleep–wake cycle and pain modulation (2).

The pons, located between the mesencephalon and the medulla oblongata, contributes to balance and coordination through its connections with the cerebellum. It also contains the nuclei of cranial nerves V, VI, VII, and VIII, which are responsible for essential functions such as facial movements, sensory processing, hearing, and balance. Numerous motor and sensory pathways between the brain and the spinal cord pass through the pons, and the integrity of these pathways is crucial for proper movement and sensory perception (3).

The medulla oblongata, positioned at the lowest part of the brainstem, contains the cardiac, vasomotor, and respiratory centers. These centers regulate heart rate, blood pressure, and respiratory rhythm. It also houses the nuclei responsible for reflex actions such as swallowing, vomiting, and coughing. Each region of the brainstem is of vital importance for sustaining neurological functions (4).

The brainstem, with its subregions including the mesencephalon, pons, and medulla oblongata, regulates motor, sensory, and autonomic functions. Furthermore, it plays a central role in maintaining consciousness, organizing reflexive movements, and supporting vital life functions. Therefore, investigating the volumetric characteristics of the brainstem is of great importance for both basic neuroscience research and clinical practice (5).

The aim of this study is to determine the volume values of the brainstem subregions in healthy individuals, to establish normative reference values, and to evaluate the applicability of these values in neurological assessments. In this context, the use of ITK-SNAP software provides a reliable manual segmentation framework that enables precise volumetric delineation of brainstem subregions, thereby enhancing the accuracy of morphometric measurements.

Material and Methods

This study was approved by the Bolu Abant İzzet Baysal University Non-Interventional Clinical Research Ethics Committee (date: March 04, 2025; no: 2025/79) and carried out in compliance with the Declaration of Helsinki's principles.

This study retrospectively analyzed magnetic resonance imaging (MRI) data from 191 healthy individuals (105 females and 86 males), aged 18 to 65 years, who presented to Bolu Abant İzzet Baysal University Training and Research Hospital and had no known neurological disease. The MRI data were obtained in DICOM format.

The MRI scans were acquired using a 1.5 Tesla system (GE Healthcare, Milwaukee, WI, USA), and the following imaging parameters were applied: T1-weighted 3D sequence (Fast Spoiled Gradient Recalled), repetition time (TR): 8.5 ms, echo time (TE): 3.2 ms, voxel size: 1x1x1 mm³, slice thickness: 1.0 mm, flip angle: 12°, field of view (FOV): 256 mm. These parameters were included to ensure standardization and to support the reliability of volumetric measurements.

Volumetric assessments of the brainstem subregions—mesencephalon, pons, and medulla oblongata—were performed using three-dimensional manual segmentation via ITK-SNAP software. To enhance methodological transparency, the anatomical boundaries used in the segmentation process were explicitly defined.

Mesencephalon, delineated superiorly at the level of the mammillary bodies and inferiorly at the pontomesencephalic sulcus. Pons, segmented between the pontomesencephalic sulcus superiorly and the pontomedullary sulcus inferiorly. Medulla oblongata, delineated superiorly at the pontomedullary sulcus and inferiorly at the level of the foramen magnum.

To evaluate the reliability of the manual segmentation process, intra-rater agreement analyses were conducted on a random subset of 30 participants using intraclass correlation coefficients (ICC). Intra-rater ICC value exceeded 0.90, indicating excellent segmentation reliability.

Each participant's volumetric data were recorded in Microsoft Excel and included in the analysis process. During the 3D manual segmentation performed on ITK-SNAP, example volume labelings of the mesencephalon (red), pons (green), and medulla oblongata (blue) structures on sagittal sections are presented in Figure 1, and the completed 3D brainstem model from a sample participant is shown in Figure 2.

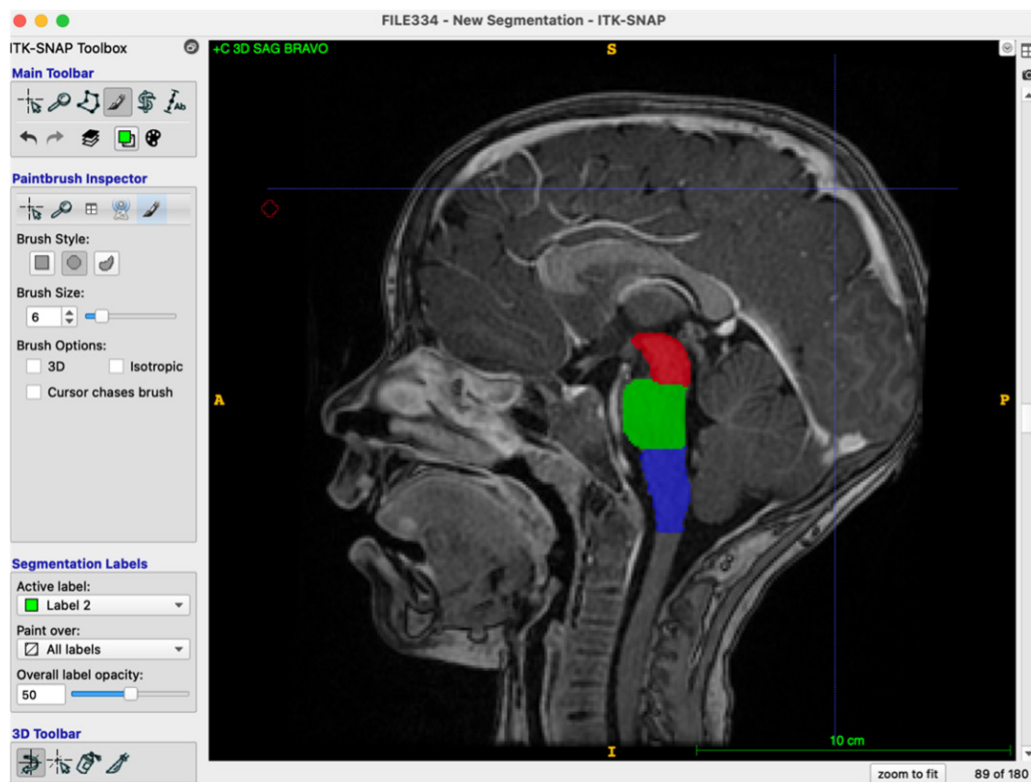


Figure 1. Segmentation of brainstem subdivisions using ITK-SNAP

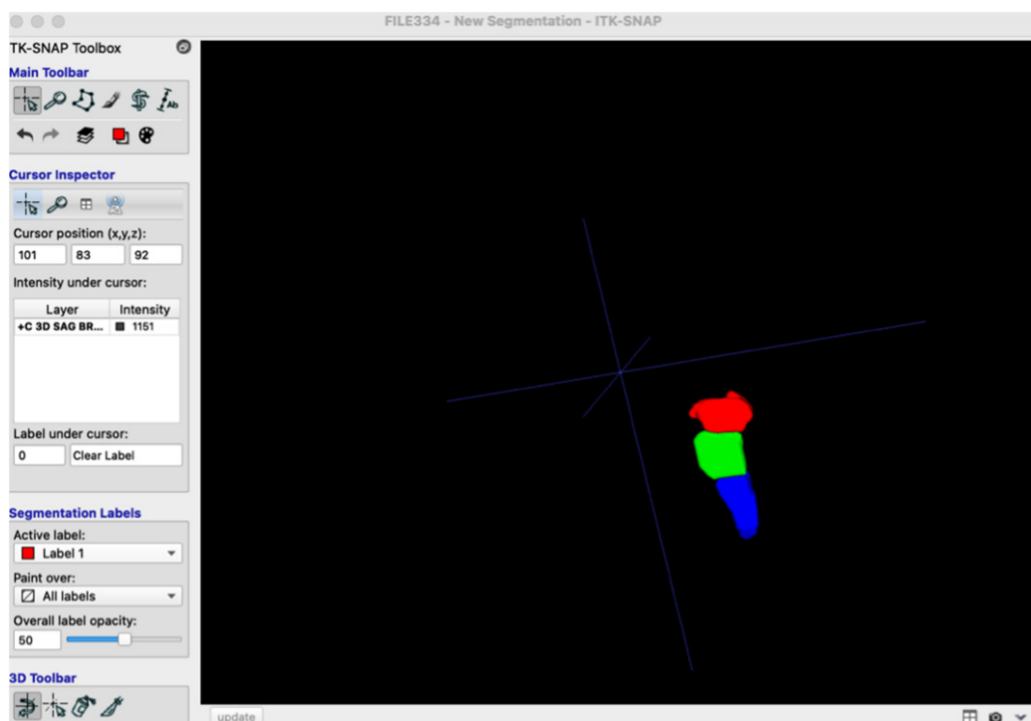


Figure 2. Three-dimensional visualization of brainstem subdivisions

Statistical Analysis

The normality of the data distribution was assessed using the Shapiro-Wilk test. For variables with a normal distribution, descriptive statistics were presented as minimum (min), maximum (max), mean, and standard deviation (SD). For variables that did not meet the assumption of normality, the median and interquartile range (IQR) were used. To compare the volume values of brainstem subdivisions, the paired sample t-test was applied when parametric assumptions were met; otherwise, non-parametric methods were preferred. Gender comparisons were performed using the Mann-Whitney U test. For regional volume differences among brainstem subdivisions, the Friedman rank test followed by Siegel-Castellan post-hoc analysis was used. Statistical analyses were conducted using JASP software, and graphical visualizations were generated using Python version 3.8.8 in the Jupyter Notebook 6.4.6 interface.

Results

This study shows that the pons has a substantially larger volume than the mesencephalon and medulla oblongata, with no sex-

related differences found. It also offers normative volumetric reference values for brainstem subregions in healthy people. These results offer solid reference data that could improve neuroimaging study monitoring, interpretation, and early diagnosis. They also add important information to neuroscientific studies on brainstem morphology.

All descriptive statistical findings are presented in Table 1 and Table 2. Figure 3 visualizes the mean volume values of the medulla oblongata, mesencephalon, and pons using a profile graph. When examining the average volumes, the mesencephalon has the lowest mean value at 1,700.26 mm³, whereas the pons shows the highest average at 2,874.08 mm³. The medulla oblongata, with a mean volume of 1,956.36 mm³, lies between these two structures. The graph clearly illustrates that the pons volume is significantly higher than both the mesencephalon and medulla oblongata. Furthermore, the trend observed in the graph also suggests a significant difference between the mesencephalon and medulla oblongata volumes.

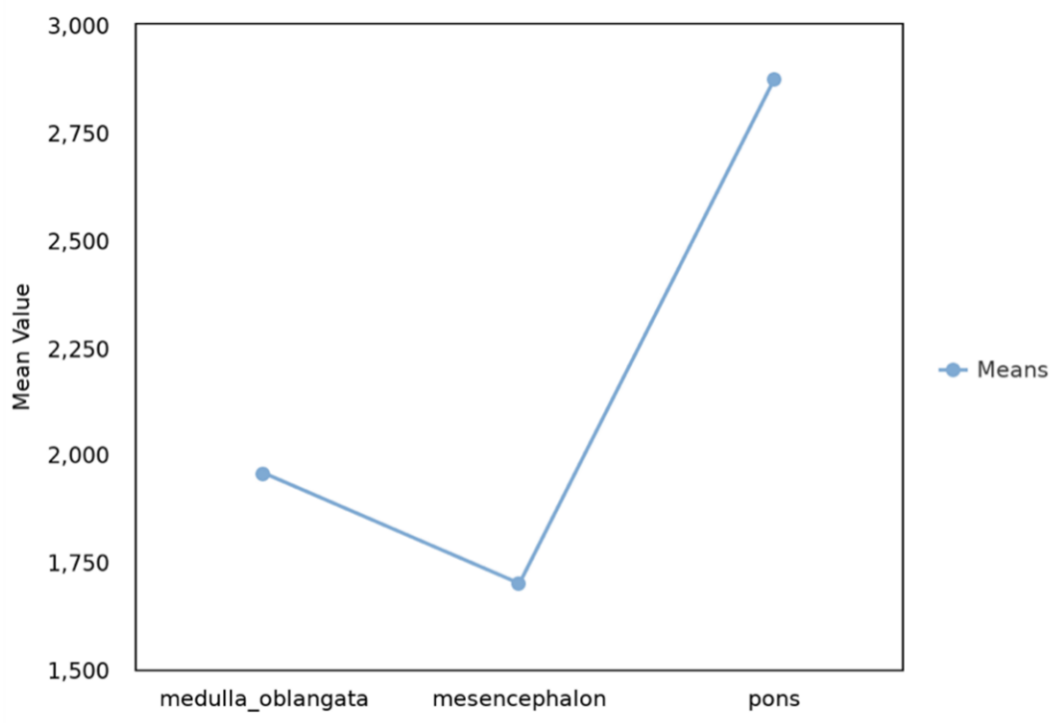


Figure 3. Graph of mean volume values of brainstem subregions

Table 1. Descriptive statistics for brainstem subdivisions*

	M	SD	N	SE _m	Min	Max
Medulla oblongata	1,956.36	393.48	190	28.55	852.00	2,934.00
Mesencephalon	1,700.26	417.73	190	30.31	638.00	3,416.00
Pons	2,874.08	571.99	190	41.50	1,125.00	4,262.00

M: Mean (mm³), SD: Standart deviation, SE_m: Standard error of the mean, Min: Minimum, Max: Maximum. *A dash (–) indicates that the statistic is undefined due to constant values or insufficient sample size

Table 2. Descriptive statistics of brainstem subdivisions by gender

	Gender	M	SD	N	SE _m	Min	Max
Medulla Oblongata	m	1,999.84	450.22	85	48.83	852.00	2,934.00
	f	1,921.17	338.89	105	33.07	1,070.00	2,680.00
Mesencephalon	m	1,716.33	481.62	85	52.24	638.00	3,416.00
	f	1,687.25	359.71	105	35.10	841.00	2,873.00
Pons	m	2,936.48	620.74	85	67.33	1,269.00	4,262.00
	f	2,823.57	526.87	105	51.42	1,125.00	3,821.00

m: Male, f: Female, M: Mean (mm³), SD: Standart deviation, SE_m: Standard error of the mean, Min: Minimum, Max: Maximum

To evaluate the normality of the volume distributions of the brainstem subcomponents-mesencephalon, pons, and medulla oblongata-Shapiro-Wilk tests were applied. The results revealed whether the volumes of these regions statistically deviated from normal distribution at the 5% significance level. For the mesencephalon volume data, the analysis yielded $W=0.96$ and $p<.001$, indicating a statistically significant deviation from normality. Similarly, for the pons volume data, $W=0.94$ and $p<.001$, again suggesting a significant deviation from a normal distribution. In contrast, the medulla oblongata volume data yielded $W=0.99$ and $p=.099$, which indicates that the assumption of normality was not violated at the 5% significance level. This suggests that the volume of the medulla oblongata is normally distributed.

According to the results of the Levene's test, the assumption of variance homogeneity was violated for medulla oblongata volumes ($F(1,188)=6.12$, $p=.014$). Similarly, for mesencephalon volumes, the variances differed significantly ($F(1,188)=5.37$, $p=.022$), indicating a lack of homogeneity of variances. In contrast, the Levene's test result for pons volumes was not statistically significant ($F(1,188)=1.43$, $p=.233$), suggesting that the assumption of homogeneity of variances was met for the pons structure.

To compare groups, the median values of the volumes of the medulla oblongata, mesencephalon, and pons structures were analyzed using the Friedman test for ranked sums. The analysis revealed a statistically significant test statistic ($\chi^2(2)=297.95$, $p<.001$). This finding indicates that there are

statistically significant differences among the median volume values of the three brainstem structures. Detailed ranking information is provided in Figure 4 presents the box plots. To further examine the significant differences observed in the Friedman test, post hoc pairwise comparisons were conducted using Siegel and Castellan's all-pairs test ($\alpha=.05$). The analyses revealed statistically significant differences between each pair of structures. Specifically:

- The difference between mesencephalon and pons volumes was found to be significant ($z=16.77$, $p<1 \times 10^{-63}$),
- A significant difference was also identified between mesencephalon and medulla oblongata volumes ($z=4.83$, $p=1 \times 10^{-6}$),
- The difference between pons and medulla oblongata volumes was also statistically significant ($z=11.93$, $p<8 \times 10^{-33}$).

These findings demonstrate that there are statistically significant volumetric differences among the mesencephalon, pons, and medulla oblongata. In order to examine whether the volumes of the brainstem subregions differ by sex, the Mann-Whitney U test was applied. The analysis included data from 85 male and 105 female participants. For mesencephalon volumes, the mean rank was 97.49 for males and 93.89 for females. The Mann-Whitney U test yielded $U = 4,631.5$, $z=-0.45$, $p=.654$. This result indicates that there is no statistically significant difference in mesencephalon volume distribution between male and female participants (male $mdn=1,744.00$ mm³; female $mdn=1,711.00$ mm³). Regarding pons volumes, the average rank was 104.08

for males and 88.55 for females. Although the difference approached statistical significance, it was not found to be significant ($p=.053$). Therefore, no significant difference was detected in pons volumes between male ($mdn=3,078.00 \text{ mm}^3$) and female ($mdn=2,897.00 \text{ mm}^3$) participants. For medulla oblongata volumes, the mean rank was 101.62 for males

and 90.54 for females. The analysis revealed no statistically significant sex-related difference in medulla oblongata volumes (male $mdn=2,000.00 \text{ mm}^3$; female $mdn=1,936.00 \text{ mm}^3$). Boxplots for sex comparisons of mesencephalon, pons, and medulla oblongata volumes are presented in Figure 5, Figure 6, and Figure 7, respectively.

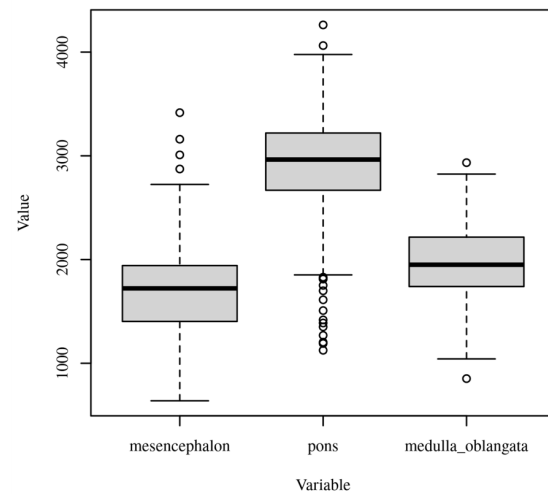


Figure 4. Boxplot of mesencephalon, pons, and medulla oblongata volumes

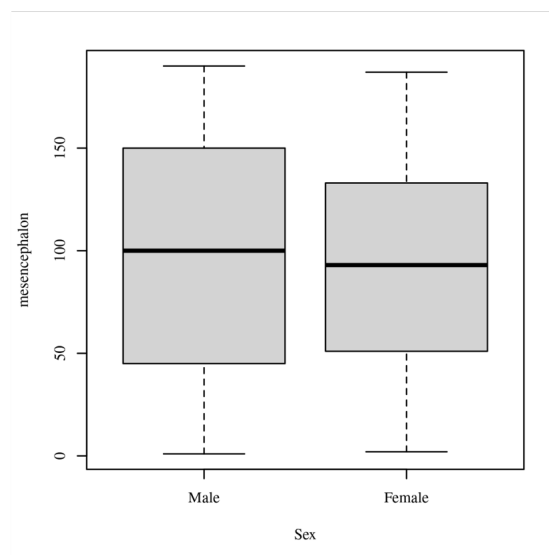


Figure 5. Distribution of rank values for mesencephalon volumes by sex

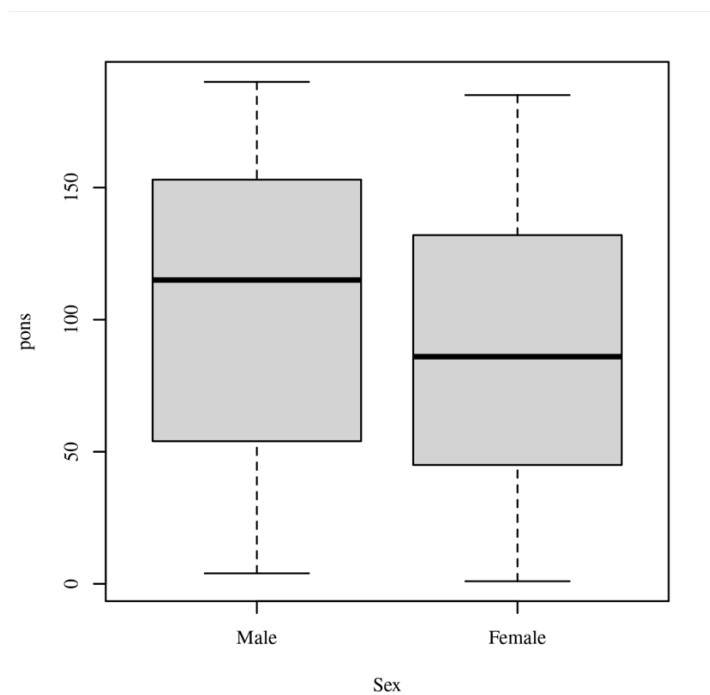


Figure 6. Distribution of rank values for pons volumes by sex

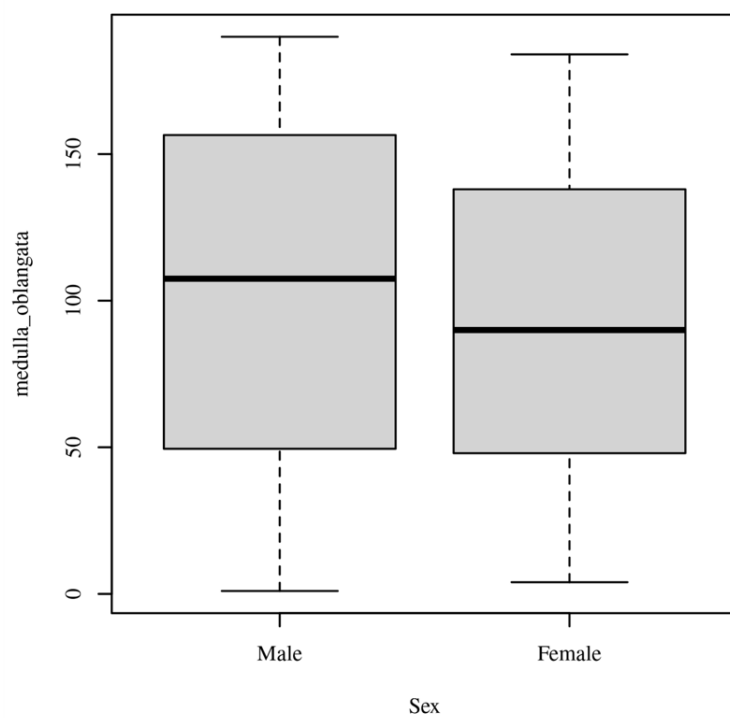


Figure 7. Distribution of rank values for medulla oblongata volumes by sex

Discussion

A review of the literature reveals that there is no specific study in which the volumes of the mesencephalon, pons, and medulla oblongata in healthy individuals were measured using ITK-SNAP software. Existing research generally focuses on total brainstem volume or structural changes associated with specific neurological disorders. However, the lack of detailed volumetric assessments targeting the subregions of the brainstem indicates that this area remains open to further investigation, and that our study may contribute significantly to the literature in this regard.

In a study that reviewed structural and functional neuroimaging research examining neuroanatomical and pathophysiological abnormalities in autism spectrum disorders (ASD), structural imaging findings highlighted increased gray and white matter volumes in the frontal, temporal, and parietal lobes, as well as an overall increase in total brain volume. This was interpreted as a reflection of widespread neuronal network abnormalities during early developmental periods. Functional imaging revealed abnormal activity in the temporal lobe and amygdala, along with increased activation in posterior cortical regions. While the study proposed several hypotheses concerning the neurobiology of autism, it emphasized the need for further research to draw definitive conclusions. The study aimed to provide a comprehensive perspective on neuroanatomical impairments associated with autism (6).

A 2019 master's thesis investigated changes in brain volume in children with Down syndrome (DS) using MRI and the VolBrain segmentation method. The study included 14 DS participants and 14 healthy controls aged 13–18 years. It evaluated total brain volume, gray and white matter volumes, as well as specific areas such as the brainstem. In the DS group, volumes in the cerebellum, brainstem, and other regions were significantly lower compared to healthy individuals. Motor functions, balance, and levels of independence were assessed using the Pediatric Berg Balance Scale and the Gross Motor Function Measure. The DS group demonstrated lower motor and balance performance compared to controls. The thesis emphasized that volumetric reductions in DS affect motor skills and daily functioning (7).

Another study aimed to assess gray and white matter volumes in healthy individuals aged 20–25 using magnetic resonance imaging (MRI) and a planimetric method. Twelve participants (6 female, 6 male) were included, and volumes of the total brain, gray matter, and white matter were calculated using semi-automated software. While no statistically significant difference was found between sexes in terms of white matter volume, white matter was observed to be larger in males than females. These findings suggest that gray and white matter volume ratios may be useful in evaluating diseases such as brain atrophy. Similarly,

the present study aimed to determine normative reference values for brainstem subregions—namely, the mesencephalon, pons, and medulla oblongata—by measuring their volumes in healthy individuals. In both studies, the measurement of regional brain volumes provides valuable information for both basic neuroscience research and clinical applications. Furthermore, the reliability of the imaging and analysis methods used enhances the scientific validity of such studies (8).

A study aimed at determining normative values of the global brain atrophy index (BV/CSF) in the pediatric age group and identifying threshold values for atrophic brains retrospectively examined 655 healthy individuals aged 0–16 and 46 children with brain atrophy due to various conditions. Normative data analyses showed that the BV/CSF index was higher in the 0–7 age group compared to the 8–16 age group. A pediatric threshold value of 25.61 was established for global brain atrophy, and sex was found to have no significant effect. These findings suggest that this index may serve as a biomarker for the diagnosis of pediatric global brain atrophy (9).

Another study evaluated the effects of age and sex on brainstem morphology using simple linear MRI measurements. The results indicated that aging leads to significant reductions in the dimensions of the mesencephalon, pons, and medulla oblongata. In particular, the reduction in the mesencephalon was thought to be associated with degeneration of the substantia nigra and loss of superior cerebellar fiber tracts. The age-related reductions in pons and medulla oblongata measurements contradicted some previous findings in the literature, highlighting the influence of different methodological approaches. Additionally, no significant effect of sex on brainstem measurements was observed. The authors emphasized that normative brainstem data could be used as a reference in the diagnosis and follow-up of neurodegenerative diseases. They also suggested that future studies with larger sample sizes in diverse ethnic groups would be valuable for more comprehensive evaluations of the effects of aging and sex on the brainstem (10).

In a further study, morphometric measurements were conducted using MRI to determine age-related changes in the brainstem and related structures. The results showed that many brainstem regions undergo growth from birth through young adulthood, followed by gradual shrinkage with aging. Male participants were observed to have significantly higher values than females in many of the measured brainstem parameters. However, in some measurements, sex differences were not prominent, suggesting that genetic and environmental factors may also play a role. High variability in parameters such as the pontomesencephalic angle (PMA) and interpeduncular angle (IPA) indicated that caution is needed when interpreting these variables in clinical practice. The study also found that certain parameters—such as the mamillopontine distance (MPM), medulla anteroposterior thickness (MedAP), and pons anteroposterior thickness (PAP)—

remained relatively stable throughout the aging process. These findings are valuable for understanding changes in the brainstem during normal aging and for contributing to the early diagnosis of neurodegenerative diseases. The authors recommended that future studies conducted in different ethnic groups and with larger sample sizes are needed to evaluate these findings in broader populations. Furthermore, they emphasized the importance of advanced imaging techniques to investigate microstructural changes in the brainstem in more detail (11).

Moreover, a 2021 MRI-based morphometric study that generated reference measurements for the brainstem and related posterior fossa structures underscored the necessity of robust normative datasets by demonstrating substantial anatomical variability even among healthy individuals. These findings further support the clinical value of establishing population-specific benchmarks, aligning closely with the aims of the present research (12).

In this study, the distribution of medulla oblongata, mesencephalon, and pons volumes in healthy individuals was descriptively examined. Based on the average volume values, the pons (2,874 mm³) was identified as the largest subregion, while the mesencephalon (1,700 mm³) had the lowest volume, and the medulla oblongata (1,956 mm³) fell in between. Results of the Shapiro–Wilk test indicated that the volumes of the mesencephalon and pons did not meet the assumption of normal distribution, whereas the medulla oblongata volumes were normally distributed. Levene’s test revealed that the assumption of homogeneity of variance was violated for the medulla oblongata and mesencephalon, but was met for the pons.

The Friedman ranking test demonstrated statistically significant differences among the volume values of brainstem structures. Subsequent Siegel–Castellan post-hoc analysis revealed significant differences in all pairwise comparisons (pons-mesencephalon, pons-medulla oblongata, mesencephalon-medulla oblongata). These findings indicate that the volume of the pons is statistically significantly larger than those of the other two structures.

In comparisons between sexes, no statistically significant differences were found in the volumes of the mesencephalon, pons, or medulla oblongata. These results suggest that there are regionally significant structural differences among brainstem subregions; however, these differences appear to be independent of sex.

The normative volume data obtained in this study may serve as valuable reference information for neuroimaging-based clinical assessments. Furthermore, future imaging-based studies involving populations with broader age ranges and diverse ethnic backgrounds will contribute to the more comprehensive determination of normative parameters related to brainstem morphology.

This study was presented as an oral presentation at the 25th National Anatomy Congress, Ordu University, Ordu, on 25-28 August 2025.

Ethical Approval: *This study was approved by the Bolu Abant İzzet Baysal University Non-Interventional Clinical Research Ethics Committee (approval no: 2025/79, date: March 04, 2025).*

Author Contributions:

Concept: E.Z.B., G.T.Ş., S.H.

Literature Review: E.Z.B., G.T.Ş.

Design: E.Z.B., G.T.Ş.

Data acquisition: E.Z.B., S.H.

Analysis and interpretation: E.Z.B., S.H.

Writing manuscript: E.Z.B., G.T.Ş., S.H.

Critical revision of manuscript: E.Z.B., G.T.Ş.

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