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Original Article

Brainstem Alteration in Migraine via 3D Volume Segmentation

Migrende Beyin Sapinin 3DHacim Segmentasyonu Aracliğiyla Değişimi

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Abstract

Background: Migraine is a multifaceted and complex disease that can be described as a mild headache or as a very severe headache lasting for days. It is a very common neurological disease in the society. Although migraine has a well-defined phenotype, some gaps in its pathophysiology can be partly filled by neuroimaging studies.

Materials and Methods: In this context, the present study aims to measure and compare the brainstem volumes of both patients diagnosed with migraine and healthy individuals by using 3D volume segmentation method. The study included 94 healthy participants (47 males and 47 females) with no illnesses or pathologies, and 98 participants (59 females and 39 males) with a migraine diagnosis who were between the ages of 18 and 50. Using ITK-SNAP software and 3D volume segmentation, brainstem volumes were assessed. To find differences, the brainstem volumes of the two groups were examined.

Results: The analyses showed that migraine patients and healthy people had significantly different brainstem volumes ($U = 2049.500$, $p < 0,01$). Compared to migraine patients, who had a median brainstem volume of 182.900 mm^3, healthy people had a median brainstem volume of 227.900 mm^3. Furthermore, healthy people had a broader range of brainstem volumes (117.800 mm^3 to 347.800 mm^3) than migraine patients (122.300 mm^3 to 259.300 mm^3). The group with migraines showed outliers. According to these findings, healthy people have bigger and more variable brainstem volumes than people who suffer from migraines.

Conclusions: The present study clearly showed the difference between brainstem volume of migraine patients and healthy individuals and also showed that this difference is statistically significant.

Keywords: Brainstem, Migraine, ITK- SNAP, Volume, Three-dimensional segmentation

ÖZ

Amaç: Migren, hafif baş ağrısından günlerce süren çok şiddetli baş ağrısına kadar çok yönlü ve karmaşık bir hastalıktır. Toplumda oldukça yaygın görülen bir nörolojik hastalıktır. Migrenin iyi tanımlanmış bir fenotipi olmasına rağmen, patofizyolojisindeki bazı boşluklar nörogörüntüleme çalışmalarıyla kısmen doldurulabilir. Bu bağlamda, mevcut çalışma, migren tanısı almış hastaların ve sağlıklı bireylerin beyin sapı hacimlerini 3B hacim segmentasyon yöntemi kullanarak ölçmeyi ve karşılaştırmayı amaçlamaktadır.

Gereç ve Yöntem: Bu çalışmada migren tanısı almış hastalar ile sağlıklı bireylerin beyin sapı hacimlerinin 3B hacim segmentasyon yöntemi kullanılarak ölçülmesi ve karşılaştırılması amaçlanmıştır. Çalışmaya herhangi bir hastalığı veya patolojisi olmayan 94 sağlıklı katılımcı (47 erkek ve 47 kadın) ve migren tanısı almış 18-50 yaşları arasındaki 98 katılımcı (59 kadın ve 39 erkek) dahil edilmiştir. ITK-SNAP yazılımı ve 3B hacim segmentasyonu kullanılarak beyin sapı hacimleri değerlendirilmiştir. Farklılıkları bulmak için iki grubun beyin sapı hacimleri incelenmiştir.

Bulgular: Analizler, migren hastalarının ve sağlıklı bireylerin beyin sapı hacimlerinin anlamlı derecede farklı olduğunu göstermiştir ($U = 2049.500$, $p < 0,01$). Ortanca beyin sapı hacmi 182.900 mm^3 olan migren hastalarıyla karşılaştırıldığında, sağlıklı bireylerin ortanca beyin sapı hacmi 227.900 mm^3'tür. Ayrıca, sağlıklı kişilerin beyin sapı hacimleri migren hastalarına (122.300 mm^3 ila 259.300 mm^3) göre daha geniş bir aralıktaydı (117.800 mm^3 ila 347.800 mm^3). Migren grubunda ise aykırı değerler gözlendi. Bu bulgulara göre, sağlıklı kişilerin beyin sapı hacimleri migren hastalarına göre daha büyük ve daha değişkendir.

Sonuç: Mevcut çalışma, migren hastalarının beyin sapı hacmi ile sağlıklı bireylerin beyin sapı hacmi arasındaki farkı açıkça ortaya koymuş ve bu farkın istatistiksel olarak anlamlı olduğunu göstermiştir.

Anahtar Kelimeler: Beyin sapı, Migren, ITK-SNAP, Hacim, Üç boyutlu segmentasyon

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Highlights

- This study shows that migraineurs have smaller brainstem volumes than healthy individuals.
- A novel method for volume calculation was utilized.
- Brainstem volume was calculated via segmentation, providing more accurate results.

Introduction

Migraine is a neurological disease that is thought to be caused as a result of the interaction of genetic and environmental factors, although the exact cause is unknown. A complex neurological condition, migraine is brought on by genetics, environmental circumstances, stress, hormone changes, dietary factors like caffeine and energy drinks, and changes in the pathways that the central nervous system uses to process pain. It is characterized by a throbbing headache usually felt on one side of the head, and may be accompanied by additional symptoms such as headache, nausea, vomiting, hypersensitivity to light or sound during attacks (1). Migraine can last for a few hours or a few days and can have a significant negative impact on the daily life of individuals (2).

This disease has a higher prevalence in women when compared with men and constitutes an important public health problem by affecting approximately one billion people worldwide. Studies for understanding the pathophysiology of migraine are carried out to develop accurate diagnosis and treatment methods and neuroimaging studies are also conducted in this context (3). According to previous studies, migraineurs may have volumetric alterations in both migraine-specific and pain-processing-related brain regions, including the brainstem. Reduced grey matter volume is frequently seen in these patients, especially in regions related to pain, cognition, and sensory integration. The entire brain volume, grey matter, brainstem, cerebellum, basal ganglia, thalamus, hippocampus, and amygdala are among the areas where volumetric losses have also been observed (4–6).

Since the brain stem is at the center of the pain pathways of migraine, it plays a critical role in understanding the pathophysiology of migraine. Structural changes in the brainstem have been observed in migraine patients and significant decreases in brainstem have been found especially in chronic migraine cases. Therefore, measuring the brainstem volume can make a significant contribution to better understanding the neurological effects and structural changes of migraine (7).

ITK-SNAP software, which has been used to measure brainstem volumes in the present study, is a software used for segmentation of three-dimensional medical images. In addition to providing semi-automatic and manual segmentation, it can simultaneously display images in three orthogonal planes. It is suitable for using many three-dimensional image formats, including DICOM and can make the segmentation of multiple images (8). In this study, we aimed to measure and compare the brainstem volumes of both patients diagnosed with migraine and healthy individuals by using ITK SNAP software.

Material and Methods

Study design

This retrospective study was conducted with the approval of the Bolu Abant Izzet Baysal University Non-Interventional Clinical Research Ethics Committee (2024/324). The study included MR images of 98 individuals (59 females and 39 males) diagnosed with migraine, as well as 94 individuals (47 males and 47 females) who did not have any diagnosis. These MR images were randomly selected from the Picture Archiving and Communication Systems (PACS) archive for analysis and evaluation.

Participants with a migraine diagnosis and MR images suitable for healthy brainstem volume measurement were included in the study. Individuals outside this age range, those without a migraine diagnosis, or those with brainstem images affected by surrounding pathological structures that hindered accurate measurement were excluded.

Inclusion criteria: Individuals aged 18–50 with a migraine diagnosis and suitable MR images for healthy brainstem volume measurement.

Exclusion criteria: Individuals under 18 or over 50, those without a migraine diagnosis, and those with MR images affected by surrounding pathological structures that prevented accurate measurement of brainstem volume. Volumetric measurements of the brainstem were performed using ITK-SNAP software.

Segmentation and measurements

MR images obtained from the PACS archive in DICOM format were transferred to ITK-SNAP software. After the brainstem images transferred to ITK-SNAP were enclosed using the 'Segmentation Mode' available in the 'Toolbox'. For the enclosed brainstem image, the 'Lower / Upper Threshold' adjustment was made on the right panel. The area intended for volumetric measurement was left in white, while surrounding structures were left in blue to prevent them from being segmented. To perform volumetric measurement, a ‘Bubble’ was added to the center of the brainstem from the ‘Add Bubble at Cursor’ section. When the 'Forward' and 'Play' buttons were clicked, the 'Bubble' was spread across the brainstem surface, facilitating the segmentation of the appropriate structures. The brainstem images with volumetric measurements are shown in the sagittal plane **(Figure 1).** ITK SNAP also allows users to visualize the structures the volumes of which have been measured in three dimensions. **(Figure 2)** shows the three-dimensional modelling of the brainstem image of an individual with migraine whose volume measurement has been completed. The volume value of the completed brainstem measurement can be accessed from the ‘Volume and Statistics’ tab under the ‘Segmentation’ section in the top-left corner of the screen **(Figure 3)**. The ITK-SNAP software provides users with the volumetric value of the respective structures in mm³ in a ready-to-use format.

Figure 1. Volumetric measurement of the brainstem of an individual with migraine by using ITK SNAP.

Statistical analysis

Conformity of the data to normal distribution was analysed with Shapiro-Wilk test. While descriptive statistics of the data which conformed to normal distribution were shown as minimum (min), maximum (max), mean (m) and standard deviation (sd), median and interquartile range (iqr) were used for variables which did not conform to normal distribution. The data used in the study were analysed with Paired Samples test conducted on brainstem volume measurements. Jamiovi Deskop software was used for analyses and Python 3.8.8 compiler and Jupyter Notebook 6.4.6 editor were used for drawing the figures.

Ethical approval

This study was conducted in accordance with the Declaration of Helsinki and institutional ethical guidelines. The study was approved by the Bolu Abant Izzet Baysal University Non-Interventional Clinical Research Ethics Committee (Number: 2024/324, Date: 03.12.2024). Since this study was retrospective, informed patient consent statement was not obtained.

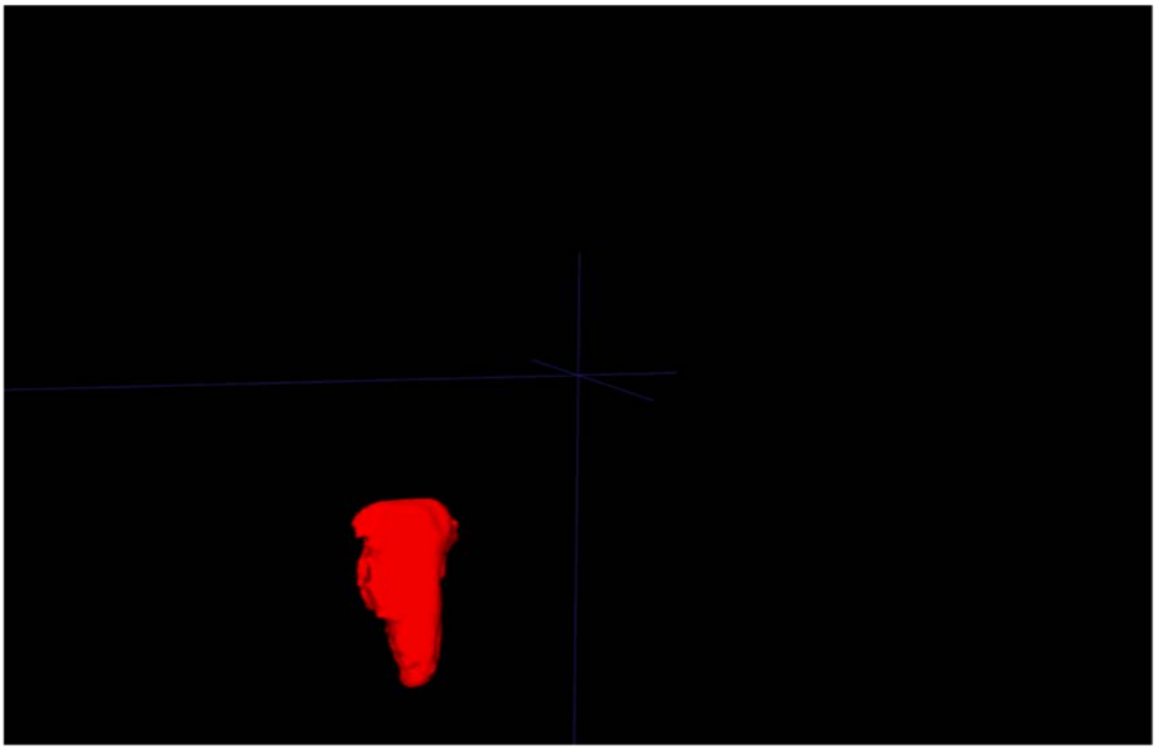

Figure 2. Three-dimensional imaging of the brainstem of an individual with migraine.

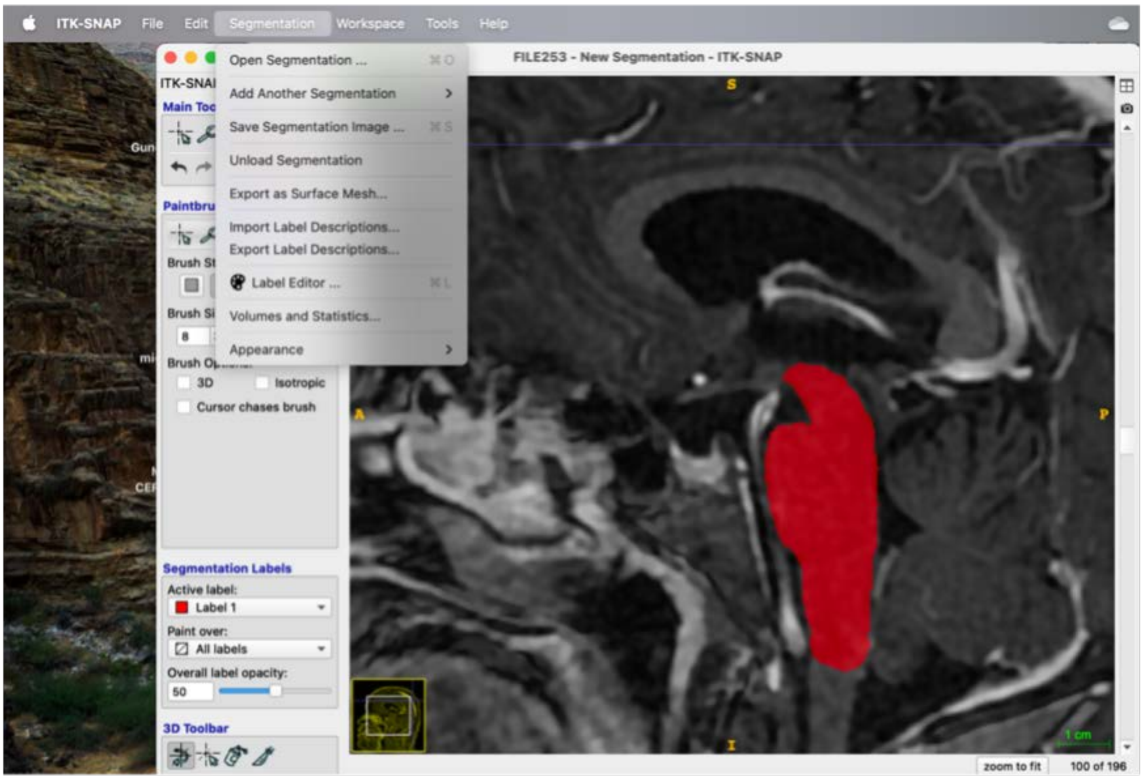

Figure 3. Section in ITK-SNAP where volume values of measured structures can be accessed.

Results

When the conformity of individuals to normal distribution was tested with Shapiro-Wilk Analysis, Shapiro-Wilk value was found as 0.905 and p value was found as 0.980 for healthy individuals. This result shows that the brainstem volume data of healthy individuals do not show a significant deviation from normal distribution ($p > 0.05$) and that the assumption of normal distribution is valid. Shapiro-Wilk value was found as 0.969 and p value was found as 0.021 for individuals with migraine. The brainstem volume data of individuals with migraine show a significant deviation from normal distribution and that the assumption of normal distribution is not valid ($p < 0.05$). These data show that the data obtained from healthy individuals are normally distributed, while there is a deviation from normal distribution in the data obtained from individuals with migraine. Analysis results are shown in **(Table 1)**.

Table 1. Comparison of Brainstem Volume Normality Between Healthy Individuals and Migraine Patients

	W	p
Healthy Group	0.995	0.980
Migraine Patients	0.969	0.021

Abbreviations: **W: Shapiro-Wilk test statistic, which measures the normality of data distribution. **p: Probability value indicating whether the data significantly deviate from a normal distribution (p > 0.05 suggests normality).*

Mann-Whitney U test was conducted to determine whether there was a significant difference between healthy individuals and individuals with migraine in terms of brainstem volume. As a result of the analysis, a statistically significant difference was found between healthy individuals and individuals with migraine in terms of brainstem volume ($U = 2049.500$, $p < 0.01$). U value was calculated as 2049.500 and the p-value indicating that this difference is significant was found to be less than 0.05. These data show that the volumetric differences between groups are not random. A p value less than 0.05 proves that there is a significant difference between groups. The U value obtained as a result of the test shows that the volumetric differences between the two groups are statistically significant. These results show that the brainstem volume differs between groups. Analysis results are shown in **(Table 2)**.

Table 2. Comparison of Brainstem Volumes Between Healthy Individuals and Migraine Patients

	U	p
Brainstem volume	2049.500	<0.01

Abbreviations: **U: Mann-Whitney U test statistic, which compares the distributions of two independent groups. **p: Probability value indicating whether there is a statistically significant difference between the groups (p <0.05 suggests a significant difference).*

Analysis results show statistically significant differences between healthy individuals and individuals with migraine in terms of brainstem volume. The median value of the brainstem volumes of healthy individuals (227.900) was found to be significantly higher than that of individuals with migraine (182.900). This shows that brainstem volumes of healthy individuals are larger than those of patients with migraine in general. It was found that the brainstem volumes of healthy individuals are larger than those of individuals with migraine and the distributions are larger, while the brainstem volumes of individuals with migraine are smaller and they show less variation. In addition to these results, it was found that minimum and maximum values showed a larger range in healthy individuals. While the minimum value of brainstem volume was 117.800 in healthy individuals, maximum value was measured as 347.800. In individuals with migraine, the minimum value of brainstem volume was found as 122.300, while the maximum value was found as 259.300. The brainstem volumes of individuals with migraine were found to be both lower and in a narrower range (137), while the volumes of healthy individuals were found to have a larger range (230). Analysis results are shown in **(Table 3)**.

Table 3. Descriptive Statistics of Brainstem Volumes Data

Variables	**Median**	**Min.-Max.**	**Range**
Healthy Group	227.900	117.800-347.800	230
Migraine Patients	182.900	122.300-259.300	137

Discussion

In the current study, difference between these two groups were statistically volumetric alterations of the brainstem were assessed using ITK-SNAP software in 98 individuals. The analysis revealed a significant difference in brainstem volume between migraine patients and healthy individuals. Specifically, the brainstem volume of healthy individuals was found to be significantly larger than that of migraine patients.

Migraine patients between the ages of 15 and 60 who were admitted to the neurology outpatient clinic of a tertiary hospital between January and July 2017 were included in a study examining the relationship between migraine and brainstem excitability. Migraine diagnosis was made according to International Headache Disorder Classification (IHS, 2013). Hearing threshold and brainstem excitability were measured in individuals with migraine and compared with the control group. As a result of the study, individuals with migraine were found to have both lower hearing threshold and increased sensitivity of brainstem auditory neurons (9).

In a study examining the cyclic neurophysiological characteristics of migraine attacks, neuronal excitability in the brainstem and primary somato-sensory region was compared between migraine stages for 30 consecutive days in two patients with episodic migraine. Electroencephalography (EEG) recordings of potentials associated with somatosensory and paired pulse paradigms were recorded for 30 days in both patients. Normalized current density in the brainstem and somatosensory region was recorded and analysed.

In both patients, increased excitability was found both in the brainstem and in the somatosensory region. As a result of the study, it was emphasized that migraine involves a system starting in the brainstem extending to the somatosensory region and that it is a cyclical excitatory disorder (10).

In a thesis published in 2012, brain volume changes in migraine patients were examined and compared with healthy individuals. The volumes of total intracranial and subcortical structures were measured with Freesurfer method on cranial MRs of all groups. As a result of the study, it was concluded that migraine may cause permanent central nervous system dysfunction and also atrophy in neocortical structures in individuals. It was also emphasized that atrophy level may be associated with the number of days or the frequency of attacks (11).

In another study, a case of chronic migraine-type chronic daily headache was presented in a patient with vascular malformation haemorrhage in midbrain and it was emphasized that the structural lesions in the brainstem could cause headache (12).

In a study conducted in 2019, magnetic resonance images of 28 childhood migraine (CHM) patients and 41 control patients with similar age and gender distribution were analysed. Migraine patients were also classified as migraine with aura (8 patients) and migraine without aura (20 patients) group. ‘volBrain’ program was used from T1 weighted images and subcortical grey matter volumes were measured and compared. As a result of the study, putamen volumes of CHM patients were found to be significantly increased when compared with the healthy control group. In addition, putamen volume increase was found to be higher in aura migraine group when compared with migraine without aura and control groups. It was pointed out that it may be easier to clarify the pathogenesis of migraine with the increase in imaging methods and the relationship between childhood migraine and subcortical grey matter structure volumes were also emphasized (13).

In a study which suggested that the changes in the anatomy and functioning of the brainstem may contribute to the onset and duration of headaches during migraine attacks, voxel-based morphometry of T1 weighted anatomic images (57 control, 24 migraine) and diffusion tensor images (22 control, 24 migraine) were used to evaluate the brainstem anatomy of individuals. A decrease was found in the grey matter volumes in spinal trigeminal core and dorsomedial pons of individuals with migraine. In addition, decreased grey matter was found in the regions of pain modulating systems such as midbrain periaqueductal grey matter and dorsolateral pons. The data found clearly showed that the structure of the brainstem can change during migraine attacks. It was concluded that migraine cycle can increase the probability of creating dynamic changes in the structure and functioning of the brainstem, which may trigger and change brainstem sensitivity. It was stated that it may be in a position to modify these changes that may occur in the functioning and structure of the brainstem and potentially prevent the triggering of migraine crisis (14).

In another study summarizing studies on grey matter changes in patients with migraine, the harmony between structural changes and functional changes which occur in the related brain sections during migraine attacks was

examined. Articles published between January 1985 and November 2015 were searched in 'PubMed' and 'Embase' and the references in the related primary articles were examined. Meta-analysis was conducted by using activation likelihood estimation (ALE). Eight clinical studies including a total of 390 subjects (191 patients and 199 controls) were analysed for structural changes. Five functional studies including 93 patients and 96 controls were also analysed. A decrease was shown in the grey matter volumes of migraine patients in bilateral inferior frontal gyrus, right precentral gyrus, left mid frontal gyrus and left cingulate gyrus.
On the contrary, an increase in activation was found in somatosensory, cingulate, limbic lobe and brainstem in patients with migraine. It was concluded as a result of the study that the changes in grey matter in migraine patients may show pain processing mechanism and related symptoms, while increased activation may suggest dysregulation of these areas or increased effort due to the use of compensatory strategies involving pain processing in migraine. It was emphasized that being informed about these structural and functional changes may also be useful for the follow-up and treatment of migraine (15).
In a study including 24 chronic migraine patients and 24 healthy controls, all of whom were women, MRI of the participants were processed with Freesurfer (automatic segmentation) method. As a result of the study, cerebellum and brainstem volumes of women with chronic migraine were found to be smaller when compared with the control group. Structural white matter anomalies were also found in the bilateral parieto-occipital regions of women with migraine. Although it is still unclear whether these structural brain changes are a cause or a consequence of migraine, it was emphasized in the study that there is a need for volumetric neuroimaging studies with larger groups especially on the chronification of migraine to shed light on this issue (16).

Study limitations

One of the major limitations of our research is the incapacity to evaluate the causal relationship between variations in brainstem volume and migraine. The study design's retrospective nature, which only permits the observation of associations rather than causal interactions, is the source of this shortcoming. To ascertain if anatomical alterations in the brainstem cause migraines or if migraines cause these alterations, a prospective study design in which individuals are monitored over time would be required. The very small sample size is another drawback that could limit how far the results can be applied. A bigger cohort would increase the study's statistical power and enable more reliable findings, even though our sample of 98 migraine patients and 94 healthy people offers insightful information. Furthermore, a sample that is more varied in terms of age, ethnicity, and migraine subtypes may offer a more comprehensive understanding of the potential variations in brainstem volumes among other groups. Additionally, even though ITK-SNAP software is an effective tool for 3D volumetric analysis, the quality of the results depends on the consistency and resolution of the imaging data because it relies on MRI scans. Measurement variability may be introduced by differences in MRI scanning hardware or techniques between institutions, which could compromise the validity of the results.
Finally, confounding variables that can affect brainstem volumes, such as medication use, comorbidities, or the intensity and duration of migraines, were not taken into consideration. To provide a more thorough knowledge of the connection between migraine and brainstem structure, future research should consider these parameters.

Conclusion

Analyses showed a significant difference between migraine patients and healthy individuals in terms of brainstem volume. According to independent samples t-test results, brainstem volumes of healthy individuals were found to be significantly higher than those of migraine patients. Confirmatory analyses with Mann-Whitney U test also showed that the difference was statistically significant. Lower brainstem volume in individuals with migraine indicate structural differences regarding the neurological basis of migraine. Considering the role of brainstem in pain regulation mechanisms in the literature, the results found emphasize the relationship between migraine pathophysiology and brainstem structure. The fact that healthy individuals have a wider range of volume suggests that the neurological structures of this group may show more variations.
In addition, the fact that individuals with migraine have both low and narrower range of brainstem volume shows the limiting effects of migraine to brainstem structure. This is completely different from the wider and more varied range of volume in healthy individuals. This difference can be considered as an important finding supporting the

potential pathological effects of migraine on the brainstem. This study provides an important basis to better elucidate the effects of migraine on the brainstem. However, there is a need for further research to determine whether these differences in the brainstem volume are a cause or a consequence of migraine. Future studies may contribute to answering this question by examining the effects of especially structural changes in the brainstem on migraine development.

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Ethical Approval: *The study was approved by the Bolu Abant Izzet Baysal University Non-Interventional Clinical Research Ethics Committee (Number: 2024/324, Date: 03.12.2024). Since this study was retrospective, informed patient consent statement was not collected.*

Author Contributions: *Concept: GTS, EZB, CA. Literature Review: GTS, EZB. Design: GTS, EZB, CA. Data acquisition: EZB, CA. Analysis and interpretation: EZB. Writing manuscript: GTS, EZB. Critical revision of manuscript: GTS. These authors contributed equally to this work.*

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Data Availability: *The data used to support the findings of this study are available from the corresponding author upon request.*

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