Ther Adv Neurol Disord
2021, Vol. 14: 1–12
DOI: 10.1177/17562864211007708

Hypoechogenicity of the midbrain raphe detected by transcranial sonography: an imaging biomarker for depression in migraine patients

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Abstract

Background: The high comorbidity of migraine and depression is suggestive of shared risk factors or common mechanisms between the two diseases. In individuals with a depressive disorder, there is a high prevalence of altered midbrain raphe (MBR) echogenicity, detectable *via* transcranial sonography (TCS), that is suggested to be linked with a dysfunction of the serotoninergic system. In patients with migraine, this alteration has seldom been explored in earlier studies, and conclusions are often lacking. Our study aimed to elucidate whether this alteration is specific to migraine and to determine whether it is related with depression.

Methods: This study enrolled patients with migraine ($n = 100$, 72% female) and patients with tension-type headache disorders (TTH) ($n = 62$, 78.5% female) from a headache clinic. In addition, 79 healthy subjects (79.7% female) were recruited as controls. All participants underwent a standard interview to evaluate headache information and an interview with psychiatrists for depression evaluation. TCS examinations were performed on all participants.

Results: Patients with migraine had a higher rate of MBR hypoechogenicity (28%) compared with that of healthy controls (15.2%) and that of patients with TTH (12.9%). In patients with migraine, reduced MBR echogenicity was associated with depressive symptoms assessed using the Hamilton Depression Rating Scale (HAM-D). No association between migraine self-medication and MBR echogenicity was found.

Conclusion: Reduced-echoic MBR detected by TCS is prevalent in migraine patients and is associated with depressive symptoms. TCS-detected hypoechogenic MBR abnormality could be an imaging biomarker of depressive symptoms in patients with migraine.

Keywords: depression, midbrain raphe, migraine, serotonin, tension-type headache, transcranial sonography

Received: 4 March 2021; revised manuscript accepted: 14 March 2021.

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Introduction

Migraine, a complex neurological disorder, ranks as the sixth most disabling disease worldwide.[1] The high comorbidity of depression with migraine, which further increases its disease burden and the challenges experienced in clinical care, has been widely reported in cross-sectional and longitudinal studies.[2–4] Patients with migraine are 1.6 times more likely to experience major depressive episodes compared with the general population, as reported by a 12-year longitudinal study.[4] Multiple studies have demonstrated that the comorbidity of migraine and depression is bi-directional[4,5]; therefore, various shared etiologic mechanisms associated with depression and migraine have been proposed.[6,7] While no single possibility has provided sufficient clarification, a dysfunctional serotoninergic system could be an explanation.[7–9]

The imbalance of serotonin in the brain has been implicated in both diseases. Pharmacological targeting of the serotoninergic system is most widely used in depression treatment.[10] Meanwhile, the serotoninergic system has been proven to play a critical role in the modulation of pain perception *via* descending inhibition of the nociceptive neuronal response.[11] Evidence exists suggesting that there is a dysfunction in serotonin synthesis, release, expression, transporting, and metabolism in patients with migraine.[12] Clinical studies have found low serotonin concentrations in migraineurs, as well as fluctuating plasma levels of serotonin during migraine attacks and interphases.[13] Furthermore, triptans and tricyclic antidepressants have been proven to be effective options for migraine treatment.[14] Changes in the serotoninergic system of both diseases can be partially explained by a shared genetic basis, such as the mutation in the serotonin transporter gene-linked polymorphic region (*5-HTTLPR*).[15]

Moreover, the two diseases have been observed to share overlapping activation mechanisms and structural changes in the brain areas involved in the serotoninergic system. Magnetic resonance imaging (MRI) studies have shown that both migraine and depression are associated with a reduced brain volume associated with aging.[16] Abnormal activity in the medial prefrontal cortex has been observed in both migraine and depression,[17] which can influence the activation of the dorsal raphe nucleus, leading to depressive symptoms and headaches.[7,18]

However, there is contrary evidence regarding the effect of serotonin (5-HT) in depression and migraine comorbidity, such as the lack of effect of selective serotonin reuptake inhibitors in migraine treatment.[19] Therefore, more evidence is needed to clarify the role of the serotoninergic system in migraine. Furthermore, there is currently no convenient biomarker for assessing changes in deep brain structures involved in the serotoninergic system.

Transcranial sonography (TCS) is a real-time imaging technique. By placing a probe through the temporal windows, a two-dimensional image of the deep parenchyma can be obtained. Due to its portability and low cost, TCS is widely used in clinical practice. For example, hyperechogenicity of the substantia nigra (SN) detected by TCS has been proven to be a risk factor for the incidence of Parkinson's disease in a population-based study,[20] and it has also been shown to be associated with disease progression.[21] The midbrain raphe (MBR) is graded as having normal or reduced echogenicity in TCS evaluations.[22] Reduced echogenicity in the MBR has been detected mainly in patients diagnosed with major depressive disorder and movement disorders paired with depression.[23] This has been attributed to structural changes or disruptions in the MBR and could be evidence supporting the hypothesis of monoamine deficiency in depressive disorder.[24]

TCS alterations in migraine patients have been reported in studies with low sample sizes in earlier research. These studies have indicated that MBR echogenicity might be associated with depression, migraine attack frequency, or analgesic usage among migraine patients.[25–27] However, the clinical implications of reduced-echoic MBR remain unclear, and it is unknown whether this change only occurs in migraine patients or also in patients with other headache disorders.

Therefore, by comparing patients with migraine with tension-type headaches (TTH) or healthy individuals, this study aimed to explore whether hypoechoic MBR was more prevalent among patients with migraine. Moreover, we aimed to investigate the feasibility of employing MBR assessments by TCS as an imaging biomarker of depression among migraine patients.

Methods and materials

Study design

We consecutively recruited patients diagnosed with migraine or TTH in the neurology department's headache clinic at the First Hospital of Jilin University, from 1 January 2019 to 1 January 2020. The healthy control patients, all from Changchun city, were recruited *via* social media and advertisements within the clinical department. All participants underwent a standard interview, performed by a neurologist, to collect headache information, as well as an interview with clinical psychiatrists and a TCS examination.

Participants

All patients who visited the headache clinic of the neurology department between 1 January 2019 and 1 January 2020 and who met the inclusion

criteria of this study were asked to participate in this observational study. The inclusion criteria for migraine patients were as follows: (1) age ranging from 18 to 55 years and (2) fulfilment of the diagnostic criteria for migraine or TTH, according to the International Classification of Headache Disorders (ICHD) III.[28]

Healthy controls were recruited either through social media, among hospital staff and university students, or through advertisements in the neurology clinical department, targeting patients' friends and family. The inclusion criteria for healthy controls were as follows: (1) age ranging from 18 to 55 years; (2) no history of primary headaches or other neurological disorders; and (3) no history of clinical depression, clinical anxiety, or consultation or treatment for psychiatric disorders.

The exclusion criteria of this study were as follows: (1) an insufficient temporal window, (2) a history of psychiatric disorders involving medical intervention (for healthy subjects) or current medical intervention for psychiatric disorders (for headache patients), and (3) a history of degenerative brain disorders.

A total of 100 patients diagnosed with migraine (72% female), 62 patients diagnosed with TTH (75.8% female), and 79 healthy controls (79.7% female) were included in the final analysis. To compare the characteristics of the two types of headache, 22 patients who met the diagnostic criteria of both migraine and TTH were excluded; 21 patients (13 migraineurs and 8 patients diagnosed with TTH) and 17 healthy controls were excluded due to the insufficiency of the temporal window on one or both sides. Ethical approval for this study was obtained from the ethics board of First Hospital Jilin University (Approval No. 19K007-001), and written informed consent was obtained from all participating patients and healthy controls.

Headache diagnosis

A neurologist experienced in the treatment of headache disorders interviewed patients recruited from the headache clinic. According to the ICHD III, patients fulfilling the diagnostic criteria for migraine were classified as the migraine group and patients fulfilling the diagnostic criteria for TTH (without any history of migraine) were classified as the TTH group. All participants completed a standard questionnaire to assess demographic and clinical data, while headache information (including headache history, symptoms, and medication usage) was collected from patients with headache disorders. The Migraine Disability Assessment (MIDAS) and Headache Impact Test (HIT-6) were used to rate the impact of patients' headaches.

Psychiatric interview

Two experienced clinical psychiatrists, blinded to the headache diagnosis, interviewed all participants, including the patients and healthy controls. Depression and anxiety were assessed using the Hamilton Depression Rating Scale-17 (HAM-D) and the Hamilton Anxiety Rating Scale (HAM-A). Depressive disorders were diagnosed according to the criteria of the Diagnostic and Statistical Manual of Mental Disorders V (DSM-V).

Transcranial sonography

On the same day, after the interviews, TCS examinations were performed by an investigator with experience in performing neurosonology and TCS (LY); the investigator was blinded to all clinical data. A TCS device (Aplio 500 US system, Toshiba Medical Systems, Tokyo, Japan) with a 2.5 MHz transducer was used to perform TCS. The dynamic range was set at 45–55 dB; the image depth started at 14–16 cm and was adjusted for each participant. The time gain compensation and image brightness were adapted manually, as needed. The participants were asked to assume a supine position while a transtemporal examination was performed, following the standard procedure.[22]

Subsequently, stored images of all participants were anonymized and re-evaluated offline by another experienced neurosonologist (XY). Conflicting image evaluations were discussed by the two evaluators to reach a consensus.

The MBR and substantia nigra (SN) were evaluated on a standard axial transection at the midbrain level.[22] SN hyperechogenicity was planimetrically evaluated from both sides. Based on the recommended cut-off value for SN hyperechogenicity and the data collected from our center, the SN was deemed abnormal if the hyperechogenic size was evaluated as being $>0.20\,cm^2$ from either one of the sides.

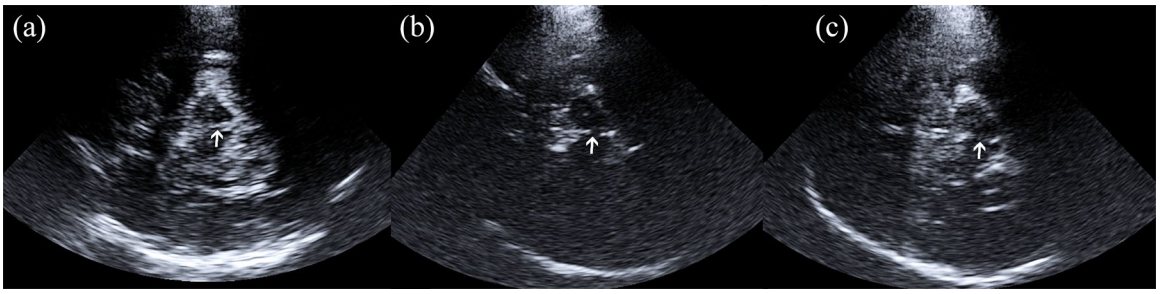

Figure 1. Examples of normal and hypoechogenic MBR on TCS. (a) Normal MBR on TCS image. The MBR is a clear and continuous line of high echogenicity (Grade 2; arrow). (b) Slightly reduced or interrupted echogenicity of the MBR on TCS image (Grade 1; arrow). (c) MBR with markedly reduced echogenicity on the TCS image, which is not visible despite the clear visibility of the red nuclei and basal cisterns (Grade 0; arrow).
MBR, midbrain raphe; TCS, transcranial sonography.

The assessment of the MBR was performed on both sides of the lower midbrain axial transection, where the basal cisterns, red nucleus, and the cerebral aqueduct are visible. We used a semi-quantitative scale comprising three grades to evaluate MBR echogenicity as follows: 0 = MBR not visible, 1 = light echogenicity or the appearance of an interrupted line, and 2 = a continuous line with an echogenicity similar to that of the basal cisterns or red nucleus. As recommended by the consensus guideline,[29] in this study, Grade 2 was considered to be normal, whereas Grade 1 and Grade 0 were considered to be indicative of MBR hypoechogenicity (Figure 1).

The widths of the third ventricle and frontal horns were measured along a standardised axial scanning plane at the thalamus level. The minimum width of the third ventricle was measured, while the bilateral frontal horns were measured at their most frontal positions.

Statistical analysis

In previous studies, MBR hypoechogenicity rates in the migraine population were reported to range from 20% to 53%,[25–27] whereas MBR hypoechogenicity has been detected in approximately 20% of the healthy population.[22] Therefore, to detect a 20% difference in the MBR hypoechogenicity rates with a power of 0.8 and an α value of 0.05, a total of 158 migraineurs and healthy controls were needed. In addition, 62 patients diagnosed with TTH were included in this study. We used Spearman's χ^2 test, the two-tailed Fisher's exact test, and the Mann–Whitney *U* test to assess differences in the distributions and means of the characteristics between groups, when appropriate. All analyses were conducted using IBM Statistical Package for the Social Sciences (SPSS) 24.0 software (Chicago, IL, USA), and the significance level was set at 0.05.

Results

Clinical findings

A total of 100 migraine patients, 79 healthy controls, and 62 TTH patients were included in the final analysis. The demographic and clinical characteristics of the participants are shown in Table 1.

Of the 100 migraine patients, 14% were diagnosed with migraine with aura (MwA) and 86% had migraines without aura (MwoA). Chronic migraine was diagnosed in 16% of the migraine patients, all of whom had MwoA; 79 (79%) patients with migraine reported the use of acute analgesics, and 68 (68%) patients with migraine reported using over-the-counter non-opioid analgesics, including non-steroidal anti-inflammatory drugs (NSAIDs), acetaminophen, or a combination of analgesics. Analgesics formulations comprised of aspirin with caffeine or pyrazolones with caffeine were most frequently used ($n = 49$, used on an average of 7 days/month). Ten patients used traditional Chinese medicine for acute treatment. Triptans were used by only two of the migraine participants. Nine patients reported using the calcium channel blocker flunarizine for preventive treatments.

The TTH patients in our study were older than the patients with migraine. The findings showed that the impact headaches of TTH patients were lower than those of the migraine patients (Table 1).

Table 1. Clinical findings of participants.

	Migraine $n=100$	Controls $n=79$	TTH $n=62$	p value
Age, years	35.5 (29.0–44.0)	39.0 (26.0–46.0)	44.5 (34.0–49.0)	0.75[a]
				0.002[b]
Sex, female	72 (72.0%)	63 (79.7%)	47 (75.8%)	0.23[a]
				0.59[b]
HAM-D score	7.0 (3.0–11.0)	1.0 (0–3.0)	6.0 (3.0–9.0)	<0.001[a]
				0.21[b]
HAM-A score	9.0 (5.0–12.5)	1.0 (1.0–3.0)	7.5 (5.0–12.0)	<0.001[a]
				0.25[b]
Disease duration, years	10.0 (5.0–15.0)	NA	6.0 (3.0–15.0)	0.12[b]
Attack frequency, days/month	3.0 (2.0–6.5)	NA	4.8 (2.0–16.0)	0.21[b]
HIT-6 score	62 (56–68)	NA	54 (45–60)	<0.001[b]
MIDAS score	20 (11–40)	NA	NA	NA
Use of analgesics	79 (79.0%)	NA	40 (64.5%)	0.092[b]

[a]Comparison of migraine and control groups.
[b]Comparison of migraine and TTH groups.
HAM-D, Hamilton depression rating scale (17 items); HAM-A, Hamilton anxiety rating scale; HIT-6, headache impact test; MIDAS, migraine disability assessment; NA, not applicable; TCS, transcranial sonography; TTH, tension-type headache.

Depression was diagnosed in 16 (16%) patients with migraine and in 6 (9.7%) patients with TTH. Higher scores on both HAM-D and HAM-A scales were detected in patients with migraine (Table 1). In addition, among migraineurs, women had a higher prevalence of depression (male:female = 1:7) and more severe depressive and anxious symptoms. The median (quartile) HAM-D score was 8 (5–13) for women with migraine *versus* 4 (1–7) for men with migraine ($p < 0.001$). The median (quartile) HAM-A score was 10 (7–16) for women with migraine *versus* 4 (6–9) for men with migraine ($p < 0.001$). None of the healthy controls were found to have depression, whereas six (7.6%) of them had a HAM-A score ≥ 8 (range, 8–11).

TCS findings

The TCS findings are listed in Table 2. No difference in SN echogenicity was found between groups, nor was there a difference between the migraine patients and healthy controls or between the migraine and the TTH patients. The areas of the SN (mean $\pm$ SD) were $0.11 \pm 0.05\,cm^2$, $0.13 \pm 0.03\,cm^2$, and $0.09 \pm 0.02\,cm^2$ in patients with migraine, patients with TTH, and healthy controls, respectively.

MBR echogenicity differed statistically between the migraineurs, TTH patients, and healthy controls ($\chi 2 = 7.115$, $p = 0.029$), with a higher prevalence of abnormal MBR in migraineurs compared with that in the healthy controls or TTH patients (Table 2). The MBR grades differed significantly between the migraineurs and controls, but not between the migraineurs and TTH patients (Figure 2) (for *post hoc* testing, the nominal significance level was adjusted to 0.025 using the Bonferroni method). Of the 241 participants, 11 were graded differently by the two evaluators. However, evaluation using Cohen's Kappa test revealed a high degree of inter-rater consistency [0.876; 95% confidence interval (CI): (0.805, 0.947), $p < 0.001$]. Among these 11 participants, 1 was rated as Grade 0 and Grade 1 by the two evaluators, whereas the other 10 were rated as Grade 1 and Grade 2.

Table 2. TCS findings of participants.

	Migraine	Controls	TTH	*p* value
SN hyperechogenicity (%)	4 (4.0)	4 (5.1)	7 (11.3)	0.50[a]
				0.15[b]
MBR hypoechogenicity (%)	28 (28)	12 (15.2)	8 (12.9)	0.040[a]
				0.025[b]
3V, mm	3.6 (3.0–4.2)	3.6 (3.0–4.2)	3.6 (2.9–3.9)	0.73[a]
				0.26[b]
LV, mm	7.1 (6.3–8.2)	8.0 (7.3–8.8)	7.5 (6.7–8.1)	<0.001[a]
				0.22[b]

[a]Comparison of migraine and control groups.
[b]Comparison of migraine and TTH groups.
3V, third ventricular; LV, lateral front horn; MBR, midbrain raphe; SN, substantia nigra; TCS, transcranial sonography; TTH, tension-type headache.

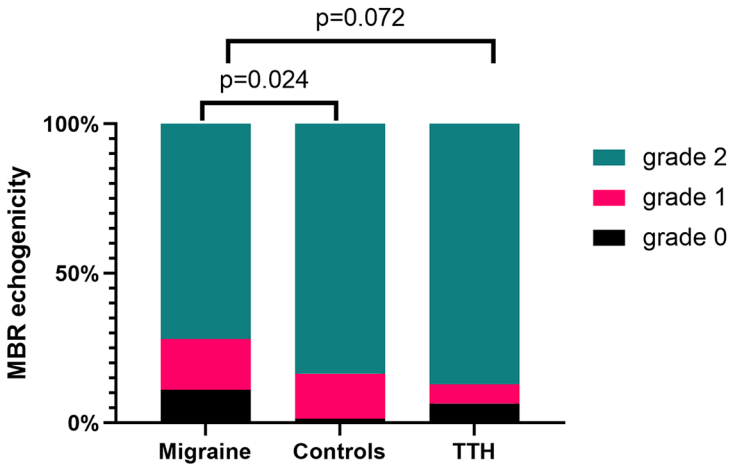

Figure 2. Distribution of MBR echogenicity in patients with migraine, patients with TTH, and healthy controls. The distribution of MBR grading differs between the migraine and control groups, but it does not differ between the migraine and TTH groups (significance level set at $\alpha = 0.025$).
MBR, midbrain raphe; TTH, tension-type headache.

All participants had normal widths of the third ventricle (<7 mm) and frontal horns (<17 mm). While no significant difference was discovered in the width of the third ventricle, migraine patients exhibited smaller frontal horn widths than the controls.

No significant difference was found in the TCS between the MwA and MwoA patients (Table 3).

MBR echogenicity and migraine features

In patients with migraine, an abnormal MBR echogenicity was associated with the female sex and the presence of depressive symptoms. Higher HAM-D and HAM-A scores were found in migraineurs exhibiting an abnormal MBR echogenicity (Table 4). An association between the hypoechogenicity of MBR and a higher HAM-D score was found only in patients with migraine, not in patients with TTH or healthy controls (Figure 3).

In addition, we found that patients with abnormal MBR echogenicity experienced a longer headache duration and a higher attack frequency, and they suffered from a more severe disabling impact of headaches (MIDAS). However, none of these findings significantly differed between groups. Moreover, the 11 migraineurs with MBR echogenicity of Grade 0 did not suffer from more severe migraines or more depression symptoms, statistically, compared with patients with MBR echogenicity of Grade 1.

In terms of self-medication, no association was found between medication use and MBR echogenicity in patients with migraine.

Discussion

The findings from our study suggest that a reduced echogenicity of the MBR is observed

Table 3. Clinical and TCS findings of patients with MwA and MwoA.

	MwA *n* = 14	MwoA *n* = 86	*p* value
Age, years	30.0 (23.0–40.0)	36.5 (30.0–45.0)	0.038
Sex, female	7 (50.0%)	65 (75.6%)	0.06
HAM-D score	7.0 (1.0–8.0)	7.0 (3.0–12.0)	0.22
HAM-A score	8.5 (4.0–12.0)	9.0 (5.0–14.0)	0.61
Disease duration, years	6.5 (3.0–14.0)	10.0 (5.0–15.0)	0.14
Attack frequency, days/month	1.5 (0.5–4.0)	4.0 (2.0–10.0)	0.026
HIT-6 score	64 (57–66)	62 (56–68)	0.75
MIDAS score	12 (3–20)	22 (13–42)	0.024
Use of analgesics	8 (57.1%)	60 (69.8%)	0.37
SN hyperechogenicity	0	4 (4.7%)	0.54
MBR hypoechogenicity	1 (7.1%)	27 (31.4%)	0.052
3V, mm	3.5 (3.0–4.5)	3.8 (3.3–4.2)	0.60
LV, mm	7.0 (6.3–8.7)	7.1 (6.3–8.1)	0.59

3V, third ventricular; HAM-A, Hamilton anxiety rating scale; HAM-D, Hamilton depression rating scale (17 items); HIT-6, headache impact test; LV, lateral front horn; MBR, midbrain raphe; MIDAS, migraine disability assessment; MwA, migraine with aura; MwoA, migraine without aura; SN, substantia nigra.

Table 4. Clinical characters of migraine patients with normal and hypoechoic MBR.

	Normal MBR *n* = 72	Hypoechoic MBR *n* = 28	*p* value
Age, years	34.5 (27.5–43)	38 (32–46.5)	0.095
Sex, female	47 (65.3%)	25 (89.3%)	0.016
Aura	13 (18.1%)	1 (3.6%)	0.12
Chronic migraine	11 (15.3%)	5 (17.9%)	0.99
Disease duration, years	10.0 (4.3–15.0)	11.0 (6.5–15.0)	0.75
Attack frequency, days/month	3.0 (2.0–5.5)	5.5 (2.0–15.0)	0.14
HIT-6 score	62 (56–68)	64 (54–69)	0.54
MIDAS score	18 (8–36)	25 (16–64)	0.089
Non-opioid analgesics	47 (65.3%)	21 (75.0%)	0.35
Non-opioid analgesics, days/month	4 (2–6)	5 (3–11)	0.21
HAM-D score	6.0 (2.0–9.0)	9.5 (6.0–15.0)	0.011
HAM-A score	8.0 (4.0–12.0)	11.0 (9.0–16.5)	0.032

HAM-A, Hamilton anxiety rating scale; HAM-D, Hamilton depression rating scale (17 items); HIT-6, headache impact test; MBR, midbrain raphe.

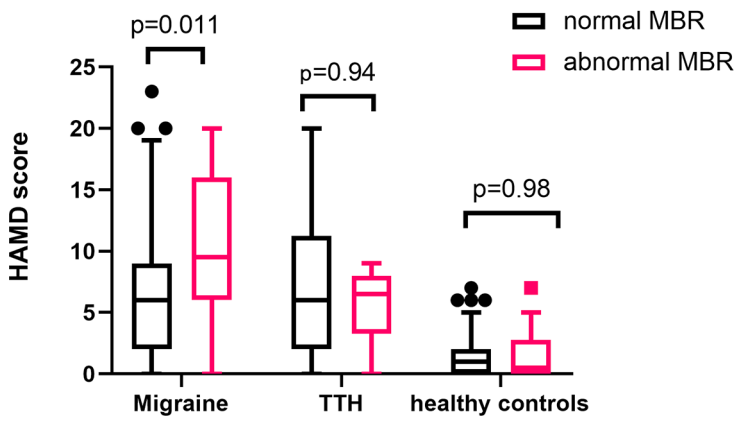

Figure 3. HAM-D scores of migraineurs, TTH patients, and healthy controls with normal and abnormal MBR echogenicity. A significantly higher HAM-D score was found only in patients with migraine.
HAM-D, Hamilton depression rating; MBR, midbrain raphe; TTH, tension-type headache.

more frequently in patients with migraine than in healthy individuals or patients with TTH. Furthermore, the data from our study indicate that, in patients with migraine, the reduced echogenicity of the MBR could be an imaging biomarker for clinical depressive symptoms.

MBR hypoechogenicity in migraine patients

The prevalence of an abnormal MBR in healthy controls was 15.2% in our study, which is consistent with the results of previous studies (10–25%).[30] Based on our results, the reduced-echo imaging of the MBR is not a prevalent characteristic finding in all primary headache disorders. The reductions were observed more commonly in a larger proportion of migraine patients (28%) than in either healthy controls or those with TTH (12.9%).

The literature investigating the correlation between MBR hypoechogenicity and migraine remains limited. We found three studies that focused primarily on the variables assessed in the present study. Two previous studies conducted by Ayzenberg *et al.* and Tao *et al.* reported reduced MBR echogenicity in 21% of episodic migraine patients and in 23.8% of MwoA patients, respectively[25,27]; these results led to the same conclusion that there was no differences in the prevalence of MBR abnormality between migraineurs and healthy controls. However, both studies included a limited sample of patients with migraine. In the study by Ayzenberg *et al.*, only patients diagnosed with episodic migraine were included,[25] and patients with Beck Depression Inventory scores $\geqslant$11 were excluded, thereby eliminating a population of migraine patients most likely to exhibit a change in MBR echogenicity, according to our findings. In Tao *et al.*'s study, only migraine patients without aura were included,[27] even though no evidence has suggested a potential difference in TCS images between migraine patients with and without aura. Furthermore, our participants were recruited consecutively from a headache clinic, including patients with chronic and episodic migraines, and patients with and without aura. We believe that the differences in the selection criteria account for the higher percentage of MBR abnormalities in our study.

Our primary conclusion is consistent with that of an earlier study conducted by Hamerla *et al.* with 51 migraine patients,[26] which reported a higher echo-reduced MBR prevalence (53%) in migraineurs compared with that in healthy controls (19%). Compared with our population of headache clinic visitors, Hamerla *et al.* recruited participants from a student population.[26] The higher prevalence of MBR in their study might be due to differences in the characteristics of the investigated population or the TCS evaluation methods. In our study, patients were examined from both sides of the temporal windows, and MBR echogenicity was classified as being abnormal only if a reduced-echoic image was observed on both sides. By contrast, although the earlier study used software-based analysis, it was unclear whether the examinations were performed on both sides.

No statistically significant differences in the MBR echogenicity were found between MwA and MwoA patients. In the previous study conducted by Hamerla *et al.*, although a much larger proportion of patients with aura (67%) were included, compared with only 14% in our study, they also did not observe a difference in MBR hypoechogenicity between migraineurs with and without aura.[26]

In the study conducted by Ayzenberg *et al.*, an association was observed between higher migraine attack frequency and reduced-echoic MBR imaging in migraineurs.[25] In our study, migraineurs with an abnormal MBR also tended toward a higher attack frequency and higher scores on the HIT-6 or MIDAS scales, although there were no statistically significant differences (Table 4).

Moreover, the 86 MwoA patients exhibited a higher prevalence of MBR abnormality, along with a higher headache attack frequency and higher MIDAS scores (Table 3). It is possible that in patients experiencing more frequent headache attacks and severe impacts, the abnormal MBR imaging could accompany the known trend in the prevalence of depressive symptoms.

MBR hypoechogenicity and self-medication

In addition, we analyzed the association between MBR hypoechogenicity and self-medication in migraine patients. In contrast to findings in Hamerla *et al.* of MBR hypoechogenicity as an indicator for analgesic use,[26] we found no association between analgesic use and MBR echogenicity, even though 68% of patients with migraine reported the use of non-opioid analgesics in our study. The self-medication choices of patients in our study differed significantly from those in the study of Hamerla *et al.*[26] Patients who suffered from more frequent migraine attacks and longer migraine-duration periods tended to use one or two kinds of combination analgesics at a higher frequency. Furthermore, likely owing to their relatively expensive cost (compared with that of OTC analgesics), the use of triptans as self-medication was uncommon in our outpatient centre. The fact that only two patients reported the use of triptans in our study prevented us from conducting a statistical analysis of the triptans users' MBR echogenicity. Moreover, the choices of self-medication could be significantly affected by income level, the migraine management education the patient might have received, and medication accessibility in our study. Therefore, it is difficult to draw conclusions based on the current evidence, and a prospective clinical study should be conducted for further investigation.

MBR hypoechogenicity and depressive symptoms in migraine patients

In migraine patients, a reduced-echoic MBR was found to be associated with depressive symptoms evaluated using the HAM-D scale. Consistent with previous studies,[31] we also found a higher prevalence of anxiety symptoms in patients with abnormal MBR.

Becker *et al.* were the first study to describe reduced MBR echogenicity in major depressive disorders detected by TCS.[23] Since then, the correlation between hypoechogenic MBR and depression has been identified in multiple studies that have investigated major depressive disorder and other neurological disorders.[32] In patients with major depressive disorder, the reduced echoic changes of the MBR were found to be associated with increased suicide attempts,[33] but with a better response to selective serotonin reuptake inhibitors.[34]

The nature of altered MBR echogenicity is currently poorly understood. The area of the MBR detected by TCS comprises various raphe nuclei, including the dorsal raphe nucleus and the median raphe nucleus (which are the main sources of serotonin in the brain), as well as the fiber complex of the basal limbic system, including the medial forebrain bundle and other pathways. Based on the evidence of fiber disruption in the post mortem research of a patient diagnosed with major depressive disorder and the increased intensity of the midline on T2-weighted MRI in patients with major depressive disorder, Becker *et al.* suggested that the change in echogenicity on TCS images was due to a distinct disruption of the fiber tracts of the basal limbic system.[24] However, despite ongoing studies with TCS and MRI on patients with depressive disorder, little evidence has been provided to support distinct fiber disruption.[35] By contrast, the involvement of the dorsal raphe nucleus and its related serotoninergic dysfunction in depression and migraine have been emphasized.

Neuroimaging studies with positron emission tomography (PET) have revealed increased binding of the 5-HT1A receptors in the pontine raphe nuclei, both in patients with depression and in patients with migraine.[36,37] Moreover, it has been suggested that damage to the raphe nuclei is associated with depressive symptoms in patients who have experienced brainstem strokes.[38] Furthermore, animal studies have implicated a neural circuit involving the dorsal raphe nucleus in depressive behaviors.[39] In animal models of primary and secondary depression, serotonergic projections from the dorsal raphe nucleus suppress the excitability of the amygdala and lateral habenula neurons.[39,40] Hypofunction of 5-HT transmission in animal models of depressive disorder and chronic nociceptive pain can cause depressive and anxiety-like behaviors.[40] Although there is a lack of direct evidence supporting a correlation between reduced-echoic MBR detected by TCS and neuronal

deactivation in the raphe nucleus, these clinical and laboratory studies could at least support the hypothesis that dysfunctional serotoninergic activation related to the raphe nucleus is a potential reason for primary and secondary depression.

Regarding the abnormal echogenicity of the MBR, a study of 53 patients with major depressive disorder found an association between MBR hypoechogenicity and the prevalence of short allele homozygosity of the *5-HTTLPR*.[31] This mutation affects the synthesis, transport, and binding of serotonin, which is also a prevalent genetic basis of migraine.[7]

According to our findings that higher HAM-D and HAM-A scores were associated with abnormal imaging of the MBR in migraine patients, we assume that a hypoechoic image of the MBR does not necessarily reflect a structural change in the brainstem, but rather represents a vulnerability to depressive symptoms. This imaging biomarker is probably related to serotoninergic system dysfunction; however, based on the current evidence, it is difficult to definitively conclude that it is the cause or result of a dysfunction of the serotoninergic system.

Other findings in TCS

Some results derived from this study lack clinical significance. For example, the discovery of similar SN hyperechogenicity rates in migraine patients and controls, which was consistent with the findings of previous studies. In addition, no difference was detected in the width of the third ventricle in the migraine patients compared with that of either TTH patients or the healthy controls. Although statistically significant reduction in the width of the lateral front horns was detected in patients with migraine compared with healthy controls, this discovery has little clinical significance. Additional structures detectable through TCS were deliberately excluded, as they did not indicate any significant change in the TCS images of patients with migraines, such as the lenticular nucleus and the caudate nucleus.[26] The red nucleus and the periaqueductal gray matter have been implicated in neuroimaging studies as potentially being involved in the pathophysiological mechanisms of migraine[41]; however, due to the limited accuracy of TCS in measuring these structures, they should be studied more extensively using MRI.

Clinical implications

Based on our results, TCS examinations could be a biomarker of depressive symptoms assessed by the HMA-D scale in migraine patients. As in our headache clinic, the evaluation of depression in cases of migraine is conducted widely in clinical practice. However, there is currently no valid indicator to determine whether and when patients should be recommended to undergo psychiatric consultation. Although this study provides no evidence that TCS examinations should be performed in all migraine patients, we believe that, in patients with a higher headache attack frequency and in those with more depressive symptoms on the HAM-D scale, a TCS examination of abnormal MBR imaging is another sign of depression vulnerability.

There are some limitations to our study. First, the ultrasound grading of the MBR was performed using semi-quantitative measurements, which may have been subject to operator-related bias. In this regard, we had two operators perform independent evaluations, and inter-operator agreement was reached. Second, the relationship between the alteration of MBR echogenicity and the functional changes of the serotoninergic system has not been demonstrated directly. In this regard, further research of TCS on animal models of depression and migraine might be illuminating. Moreover, in future clinical studies, a larger sample size is needed, including patients with headache who are also diagnosed with a depressive disorder, TTH patients recruited from the general population, and patients with mixed-type headaches; this could help determine whether MBR hypoechogenicity is an independent risk factor for depression in headache patients. A prospective clinical study with patients being treated with different medications could also help elucidate whether MBR hypoechogenicity is related to pharmacological treatments and whether it is reversible.

Conclusion

Our study found that reduced MBR echoic changes detected using TCS are more prevalent in migraine patients, and these changes are associated with depressive symptoms in migraine patients. Although further research is needed to deepen the understanding of this mechanism, we believe that our study adds new evidence to support the hypothesis that dysfunction of the

serotoninergic system is a shared pathophysiology in migraine and depression. Based on our findings, the use of TCS to detect echoic-reduced MBR could be a useful tool in the clinical diagnosis and management of patients with migraine and depression comorbidity.

Acknowledgements

The authors thank all staff from the headache clinic of neurology department at the First Hospital of Jilin University for their support in the recruitment of participants. We would also like to thank Dr. Jia ZF and Ms. Zheng Min from the Department of Clinical Research at Jilin University for their support in providing statistical advice on this manuscript.

Conflict of interest statement

The authors declare that there is no conflict of interest.

Funding

The authors received no financial support for the research, authorship, and/or publication of this article.

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