Title: Raphe Nuclei Echogenicity and Diameter of Third Ventricle in Schizophrenia Measured by Transcranial Sonography

Running title: Raphe Nuclei and Third Ventricle in Schizophrenia

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Abstract

Introduction: Serotonergic system hyperactivity at 5-HT_{2A} receptors on glutamate neurons in the cerebral cortex is one of the pathways that is theoretically linked to psychosis.

In addition to neurotransmitter dysfunction, volumetric studies revealed loss of cortical gray matter and ventricular enlargement in patients with schizophrenia, although there is no case-control research on patients with schizophrenia in order to evaluate echogenicity of RN or DTV.

To address these issues, the present study assessed midbrain raphe nuclei (RN) as the main source of brain serotonin and diameter of third ventricle (DTV) as an index of atrophy by transcranial sonography (TCS) in a group of patients with schizophrenia.

Methods: 30 patients with schizophrenia and 30 controls were assessed by TCS for RN echogenicity and DTV. TCS was done through temporal bone window via a phased-array ultrasound using 2.5 MHz transducer in depth of 14-16 cm. RN echogenicity assessed by a semi-quantitative visual scale and DTV was measured in thalamic plane.

Results: 23 patients (76.5%) and 15 (50 %) controls showed hypoechogenicity of RN which was marginally significant ($p=0.06$). DTV was in average larger in the patient's group (0.388 cm vs 0.234 cm, $p<0.001$).

Conclusion: Increased DTV in the patients with schizophrenia is consistent with previous neuroimaging findings. However, marginally lower echogenicity of midbrain RN on TCS in schizophrenia is a new finding that supports the serotonin hypothesis of schizophrenia.

Keywords: Raphe nuclei, Third ventricle, Schizophrenia, Serotonin

Introduction:

Schizophrenia is one of the most disabling psychiatric disorders, with the prevalence of 1% worldwide. (Charlson et al., 2018). It is characterized by positive (e.g. delusion) and negative symptoms (e.g. social withdrawal), as well as cognitive deficits(Świtaj et al., 2012).

Research has implicated dysfunction of various neurotransmitters in the pathophysiology of schizophrenia. Serotonin and dopamine are the major neurotransmitters that have been implicated in schizophrenia. Numerous studies have revealed alterations in GABA, Glutamate and NE neurotransmission in several brain regions in schizophrenia(Yang & Tsai, 2017).

According to serotonin theory of psychosis hyper-functioning of cortical serotonin/5-hydroxytryptamine 5-HT_{2A} is associated with psychosis, which leads to hyper-activation of 5-HT_{2A} receptors on glutamate neurons. This over-activity can result in increased release of serotonin and/or increased expression of 5-HT_{2A} receptors, which leads to glutamate release. Release of glutamate in the Ventral Tegmental Area (VTA) might trigger the mesolimbic pathway, leading to extra dopamine in the ventral striatum which eventually results in psychotic features (Stahl, 2018).

The main brain morphological abnormalities noticed in patients with schizophrenia include loss of cortical gray matter, decreased volume of the amygdala, hippocampus, the frontal and temporal lobes and ventricular enlargement suggesting that in addition to neurotransmitters dysregulation a neurodegenerative process is present in a subset of patients with schizophrenia, probably those with severe cognitive impairment(Barkataki, Kumari, Das, Taylor, & Sharma, 2006; Lauer & Krieg, 1998; Lieberman et al., 2001; Pérez-Neri, Ramírez-Bermúdez, Montes, & Ríos, 2006).

Transcranial parenchymal sonography (TCS) displays echogenicity of brain tissue through the intact skull and is a noninvasive, easily accessible, cost-effective, quickly applicable and practical method for evaluation of basal ganglia, and midbrain structures including raphe nuclei and diameter of third ventricle (DTV) and frontal horns of the lateral ventricles with a comparable resolution to magnetic resonance imaging(Uwe Walter et al., 2008).

Former TCS studies indicated reduced echogenicity of brainstem raphe (BR) in patients with major depression (G. Becker, Struck, Bogdahn, & Becker, 1994; Ghourchian, Zamani, Poorkosary, Malakouti, & Rohani, 2014; Zhang et al., 2016), and depression associated with Parkinson's disease (T. Becker et al., 1997) and Wilson's disease (U Walter et al., 2005) but not in healthy adults, bipolar affective disorder (Krogias et al., 2011) multiple sclerosis with depression or geriatric depression (Berg et al., 2000; Şenel, Özel-Kızıl, Sorgun, Tezcan-Aydemir, & Kırıcı, 2020). A previous study investigated echogenicity of the substantia nigra (SN) in different schizophrenic subforms by TCS amongst patients with schizophrenic spectrum psychoses treated by neuroleptic drugs, although there is no case-control research on patients with schizophrenia in order to evaluate echogenicity of RN or DTV (Jabs, Berg, Merschdorf, Bartsch, & Pfuhlmann, 2001). Moreover, a review article suggested more investigations by this tool among patients with psychiatric disorders (Drepper et al., 2018). According to the serotonin hypothesis and the relatively similar pathogenesis of depression and schizophrenia, it can be considered to have same findings on raphe echogenicity.

In the present study we evaluated the echogenicity of BR nuclei, and DTV in a group of schizophrenia patients and compared them with healthy controls.

Material and methods:

Subjects

30 patients with schizophrenia were selected from Iran psychiatric Hospital and 30 control persons of the same age and sex were enrolled from the hospital staff. Exclusion criteria for case group were acute phase of illness and other psychiatric comorbidities like major depressive disorder, as well as poor temporal window for TCS. Control group was evaluated by a psychiatrist to rule out possible psychiatric disorders. Schizophrenia was diagnosed based on the Diagnostic and Statistical Manual of Mental Disorders, fifth edition (DSM-5) (Edition, 2013).

We performed the study according to the Declaration of Helsinki and the Ethics Committee of the Iran University of Medical Sciences (IRB: IR.IUMS.REC1394.8821215225) approved the study. We received a written informed consent from all of the participants.

Transcranial Sonography

An experienced neurologist performed the TCS, who was blind to the diagnosis. We used a phased-array ultrasound system (the Osnos 5500 ultrasound system, Sonosite), with a 2.5 MHz probe (S3 probe) and penetration depth of 14-16 cm, through the temporal bone window in the midbrain plane for assessment of BR and thalamic plane for measuring DTV. For the latter, we assessed the distance between the leading edges of the brain-ventricle interfaces in axial imaging planes. In the mesencephalic plane, the BR was recognized within the borders of the midbrain surrounded by the basal cisterns. Raphe nucleus echogenicity was graded as normal (continuous) and abnormal (non-continuous and absent)

Statistics:

SPSS software (version 18) was used in order to analyze the data. We calculated mean $\pm$ standard deviation (SD) for quantitative and frequency percentages for qualitative variables. We used Chi-square test to compare decreased echogenicity between two groups. In this study, we considered a type II error of less than 0.05 as significant.

Results:

A total of 30 patients with schizophrenia and 30 controls participated in the study. The mean age of patients with schizophrenia and controls were 37.37 (SD= 8.8) and 37.20 (SD= 9.8) years, respectively. In the patients group, 23 patients showed decreased echogenicity of brainstem RN (76.5%), whereas in the control group only 15 individuals (50%) had a decreased echogenicity. There was marginally significant difference between the two groups in terms of echogenicity of Raphe Nucleus (p-value = 0.06).

Comparing the two groups for DTV, it was significantly higher in the patients group, (0.388 cm vs 0.234 cm, p -value<0.001). Also, there was a significant correlation between age and diameter of third ventricle, with older participants having larger ventricles (p =0.003) (Figure 1).

Overall, DTV and age had a statistically significant correlation (p =0.003), however when we investigated this correlation separately in patients and controls, although there still was a significant correlation between age and DTV in patients group (p =0.001), this correlation was not statistically significant in controls (p =0.091). However as it is evident with the fitlines in the figures 1, in both groups older people tended to have larger DTVs.

Discussion:

Some evidence reveals that ultrasound (US) imaging may provide biomarkers and therapeutic options in psychiatric disorders. TCS is useful in determining vascular, structural and functional brain changes in mental disorders. Some studies showed changes in BR echogenicity with TCS in psychiatric disorders(Siragusa et al., 2020).. This study was aimed for assessment of BR nuclei and DTV in patients with schizophrenia as representatives of serotonin system and brain atrophy respectively.

As far as we know, this is the first study evaluating raphe nuclei echogenicity and third ventricle diameter in patients with schizophrenia by using TCS. Results of this study revealed no significant BR echogenicity change in patients with schizophrenia compared to healthy control group but increased DTV.

However, in this study group size was small and the p -value was borderline (p -value = 0.06), so the results may not be representative for patients with schizophrenia and it declare the necessity of further investigations with larger sample sizes.

It should be noted that several studies have revealed decreased echogenicity of BR nuclei in depressive disorders(Ghourchian et al., 2014). It seems that hypoechogenicity of BR in major depression is related to basal limbic system dysfunction although the exact etiology of altered echogenicity of brainstem raphe nuclei is not still clear. Decreased echogenicity of the brainstem raphe nuclei in TCS may reflect decreased level of serotonin in major depressed patients

(Ghourchian et al., 2014). The better response of depressed patients with hypoechogenicity of the brainstem raphe nuclei to SSRIs may support this hypothesis too (Uwe Walter, Prudente-Morrissey, Herpertz, Benecke, & Hoepfner, 2007).

The serotonin hypothesis of psychosis in schizophrenia is somewhat different from that of major depressive disorder. Psychosis can be associated with hyperactivation of 5-HT_{2A} receptors on glutamate neurons. This hyperactivation may be caused by various factors including increased serotonin level, upregulation of 5-HT_{2A} receptors, or a 5-HT_{2A} agonist (Carhart-Harris et al., 2016), all of them could result in a decrease in glutamate. Release of glutamate in the ventral tegmental area activates mesolimbic dopamine pathway in its turn and results in psychosis (Ghajar et al., 2018; Stahl, 2018). Regarding the different serotonergic dysfunction in depression and schizophrenia, it makes sense to obtain distinct results from the ultrasound examination of raphe nuclei in these two entities.

Positive symptoms of psychosis are attributable to dopamine excess in the mesolimbic pathway. Therefore, we expect that blockade of dopamine D₂ receptors should result in treatment. However, antagonism of 5HT_{2A} (without D₂ antagonism) has shown acceptable antipsychotic effects in the patients with Parkinson's disease, and some preliminary evidence for efficacy exists in the patients with psychosis and dementia (Stahl, 2016).

In addition, dysregulation of serotonin system is known to play a major role in the etiology of obsessive-compulsive disorder (OCD). Changes in the serotonergic brainstem RN have been shown in major depressive disorder and depressed patients with Parkinson's and Huntington's disease. Although decreased echogenicity of midbrain RN is characteristic in patients with major depressive disorder, it was not shown in patients with OCD, which can be described by the involvement of RN projections rather than RN serotonergic neurons (Mohammadzade et al., 2018).

Another finding of this study was larger third ventricular diameter of patients with schizophrenia. Şenel and colleagues reported increased diameter of third ventricle measured by TCS in geriatric depression (Şenel et al., 2020). Ventricular enlargement is apparent in patients with chronic schizophrenia but is not a feature at the earliest stages of the illness (Berger et al., 2017).

Regarding the pathophysiology of schizophrenia, neurodevelopmental and neurodegenerative theories are the most popular and there are studies support both mechanisms are plying role in combination(Berger et al., 2017). Longitudinal cohort studies on patients with schizophrenia revealed progressive loss of cerebral grey matter, more apparent in the frontal and temporal lobes (Dell’Osso, Palazzo, & Altamura, 2018).

The main brain morphological abnormalities observed in patients with schizophrenia are loss of cortical gray matter, decreased volume of the amygdala, hippocampus, frontal and temporal lobes and ventricular enlargement (Pérez-Neri et al., 2006). In addition, our findings show that DTV is, significantly, correlated with age in both groups that is similar to some other studies which used TCS among patients with other neurodegenerative neuropsychiatric disorders(Almasi-Dooghaee, Rohani, Imani, Nadjafi, & Zamani, 2021). Therefore, our results are compatible with previous findings reflecting brain atrophy some probable neurodegenerative processes in schizophrenia.

Limitations of the present study are the lack of other neuroimaging techniques such as MRI and the small sample size. We recommend considering these points for future studies.

Conclusion:

Increased DTV in the patients with schizophrenia is consistent with previous neuroimaging findings. However, marginally lower echogenicity of midbrain RN on TCS in schizophrenia is a new finding that supports the serotonin hypothesis of schizophrenia. Further studies are required in this regards.

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None

Conflict of interests

None

References:

- Almasi-Dooghaee, Mostafa, Rohani, Mohammad, Imani, Atefeh, Nadjafi, Shabnam, & Zamani, Babak. (2021). The role of transcranial sonography in differentiation of dementia subtypes: an introduction of a new diagnostic method. *Neurological Sciences*, 42(1), 275-283.
- Barkataki, Ian, Kumari, Veena, Das, Mrigendra, Taylor, Pamela, & Sharma, Tonmoy. (2006). Volumetric structural brain abnormalities in men with schizophrenia or antisocial personality disorder. *Behavioural brain research*, 169(2), 239-247.
- Becker, Georg, Struck, Michael, Bogdahn, Ulrich, & Becker, Thomas. (1994). Echogenicity of the brainstem raphe in patients with major depression. *Psychiatry Research: Neuroimaging*, 55(2), 75-84.
- Becker, Thomas, Becker, Georg, Seufert, Jochen, Hofmann, Erich, Lange, Klaus W, Naumann, Markus, . . . Beckmann, Helmut. (1997). Parkinson's disease and depression: evidence for an alteration of the basal limbic system detected by transcranial sonography. *Journal of Neurology, Neurosurgery & Psychiatry*, 63(5), 590-595.
- Berg, D, Supprian, T, Thomae, J, Warmuth-Metz, M, Horowski, A, Zeiler, B, . . . Becker, G. (2000). Lesion pattern in patients with multiple sclerosis and depression. *Multiple Sclerosis Journal*, 6(3), 156-162.
- Berger, Gregor E, Bartholomeusz, Cali F, Wood, Stephen J, Ang, Anthony, Phillips, Lisa J, Proffitt, Tina, . . . Lin, Ashleigh. (2017). Ventricular volumes across stages of schizophrenia and other psychoses. *Australian & New Zealand Journal of Psychiatry*, 51(10), 1041-1051.
- Carhart-Harris, Robin L, Muthukumaraswamy, Suresh, Roseman, Leor, Kaelen, Mendel, Droog, Wouter, Murphy, Kevin, . . . Orban, Csaba. (2016). Neural correlates of the LSD experience revealed by multimodal neuroimaging. *Proceedings of the National Academy of Sciences*, 113(17), 4853-4858.
- Charlson, Fiona J, Ferrari, Alize J, Santomauro, Damian F, Diminic, Sandra, Stockings, Emily, Scott, James G, . . . Whiteford, Harvey A. (2018). Global epidemiology and burden of schizophrenia: findings from the global burden of disease study 2016. *Schizophrenia bulletin*, 44(6), 1195-1203.

- Dell’Osso, Bernardo, Palazzo, M Carlotta, & Altamura, A Carlo. (2018). Neurodevelopmental and Neurodegenerative Alterations in the Pathophysiology of Schizophrenia: Focus on Neuro-Immuno-Inflammation *Neurodegenerative Diseases* (pp. 337-348): Springer.
- Drepper, C., Geißler, J., Pastura, G., Yilmaz, R., Berg, D., Romanos, M., & Gerlach, M. (2018). Transcranial sonography in psychiatry as a potential tool in diagnosis and research. *World J Biol Psychiatry*, 19(7), 484-496. doi: 10.1080/15622975.2017.1386325
- Edition, Fifth. (2013). Diagnostic and statistical manual of mental disorders. *Am Psychiatric Assoc*, 21.
- Ghajar, Alireza, Khoae-Ardakani, Mohammad-Reza, Shahmoradi, Zahara, Alavi, Amir-Reza, Afarideh, Mohsen, Shalbafan, Mohammad-Reza, . . . Akhondzadeh, Shahin. (2018). L-carnosine as an add-on to risperidone for treatment of negative symptoms in patients with stable schizophrenia: A double-blind, randomized placebo-controlled trial. *Psychiatry Research*, 262, 94-101.
- Ghourchian, Shadi, Zamani, Babak, Poorkosary, Kosar, Malakouti, Seyed Kazem, & Rohani, Mohammad. (2014). Raphe nuclei echogenicity changes in major depression. *Medical journal of the Islamic Republic of Iran*, 28, 9.
- Jabs, B. E., Berg, D., Merschorf, U., Bartsch, A. J., & Pfuhlmann, B. (2001). Differences in substantia nigra echogenicity of nosological subtypes within the schizophrenic spectrum. A preliminary transcranial ultrasound study. *Neuropsychobiology*, 44(4), 183-186. doi: 10.1159/000054940
- Krogias, Christos, Hoffmann, Kija, Eyding, Jens, Scheele, Dirk, Norra, Christine, Gold, Ralf, . . . Assion, Hans-Jörg. (2011). Evaluation of basal ganglia, brainstem raphe and ventricles in bipolar disorder by transcranial sonography. *Psychiatry Research: Neuroimaging*, 194(2), 190-197.
- Lauer, Christoph J, & Krieg, Jürgen-Christian. (1998). Slow-wave sleep and ventricular size: a comparative study in schizophrenia and major depression. *Biological psychiatry*, 44(2), 121-128.
- Lieberman, Jeffrey, Chakos, Miranda, Wu, Huwei, Alvir, Jose, Hoffman, Elaine, Robinson, Delbert, & Bilder, Robert. (2001). Longitudinal study of brain morphology in first episode schizophrenia. *Biological psychiatry*, 49(6), 487-499.

- Mohammadzade, Nahid, Shalbafan, Mohammad Reza, Shariat, Seyed Vahid, Zamani, Babak, Shariati, Behnam, Omrani, Fateme, & Rohani, Mohammad. (2018). Raphe nuclei echogenicity changes in obsessive-compulsive disorder. *Medical journal of the Islamic Republic of Iran*, 32, 91.
- Pérez-Neri, Iván, Ramírez-Bermúdez, Jesús, Montes, Sergio, & Ríos, Camilo. (2006). Possible mechanisms of neurodegeneration in schizophrenia. *Neurochemical research*, 31(10), 1279-1294.
- Şenel, Başak, Özel-Kızıl, Erguvan T, Sorgun, Mine H, Tezcan-Aydemir, Sabiha, & Kırıcı, Sevinç. (2020). Transcranial sonography imaging of brainstem raphe, substantia nigra and cerebral ventricles in patients with geriatric depression. *International Journal of Geriatric Psychiatry*.
- Siragusa, Marta, Rémenieras, Jean-Pierre, Bouakaz, Ayache, Escoffre, Jean-Michel, Patat, Frédéric, Dujardin, Paul-Armand, . . . El-Hage, Wissam. (2020). A systematic review of ultrasound imaging and therapy in mental disorders. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, 109919.
- Stahl, Stephen M. (2016). Parkinson's disease psychosis as a serotonin-dopamine imbalance syndrome. *CNS spectrums*, 21(5), 355-359.
- Stahl, Stephen M. (2018). Beyond the dopamine hypothesis of schizophrenia to three neural networks of psychosis: dopamine, serotonin, and glutamate. *CNS spectrums*, 23(3), 187-191.
- Świtaj, Piotr, Anczewska, Marta, Chrostek, Anna, Sabariego, Carla, Cieza, Alarcos, Bickenbach, Jerome, & Chatterji, Somnath. (2012). Disability and schizophrenia: a systematic review of experienced psychosocial difficulties. *BMC psychiatry*, 12(1), 193.
- Walter, U, Krolkowski, K, Tarnacka, B, Benecke, R, Czlonkowska, A, & Dressler, D. (2005). Sonographic detection of basal ganglia lesions in asymptomatic and symptomatic Wilson disease. *Neurology*, 64(10), 1726-1732.
- Walter, Uwe, Kanowski, Martin, Kaufmann, Jörn, Grossmann, Annette, Benecke, Reiner, & Niehaus, Ludwig. (2008). Contemporary ultrasound systems allow high-resolution transcranial imaging of small echogenic deep intracranial structures similarly as MRI: a phantom study. *Neuroimage*, 40(2), 551-558.

- Walter, Uwe, Prudente-Morrissey, Lara, Herpertz, Sabine C, Benecke, Reiner, & Hoeppner, Jacqueline. (2007). Relationship of brainstem raphe echogenicity and clinical findings in depressive states. *Psychiatry Research: Neuroimaging*, 155(1), 67-73.
- Yang, Albert C, & Tsai, Shih-Jen. (2017). New targets for schizophrenia treatment beyond the dopamine hypothesis. *International journal of molecular sciences*, 18(8), 1689.
- Zhang, Ying Chun, Hu, Hua, Luo, Wei Feng, Sheng, Yu Jing, Chen, Xiao Fang, Mao, Cheng Jie, . . . Liu, Chun Feng. (2016). Alteration of brainstem raphe measured by transcranial sonography in depression patients with or without Parkinson's disease. *Neurological Sciences*, 37(1), 45-50.

TABLE 1. Comparison of TCS findings in schizophrenia patients and control group

Variable	SCZ (n = 30)	HC (n = 30)	P value
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Sex			
Male			
Female			
Age (year)	37.3±8.8	37.2±9.8	0.945
Brainstem Raphe Echogenicity			
Normal	7 (23.3%)	15 (50%)	0.06
Reduced	23 (26.6%)	15 (50%)	
Third Ventricle ()	0.38	0.23	<0.001
Abbreviations: SCZ, schizophrenia ; HC, healthy control .			

Figure 1. Simple scatter with Fit Line of Diameter of Third Ventricle by Age