Journal of Affective Disorders 209 (2017) 66–70

Contents lists available at ScienceDirect

Journal of Affective Disorders

journal homepage: www.elsevier.com/locate/jad

Research paper

A pilot study on predictors of brainstem raphe abnormality in patients with major depressive disorder

Milutin Kostić[a,*], Ana Munjiza[a], Danilo Pesic[a], Amir Peljto[a], Ivana Novakovic[a,b], Valerija Dobricic[b], Dusica Lecic Tosevski[a,c], Milija Mijajlovic[b,c]

[a] Institute of Mental Health, Belgrade, Serbia
[b] Neurology Clinic, Clinical Center of Serbia, Belgrade, Serbia
[c] School of Medicine, University of Belgrade, Belgrade, Serbia

ARTICLE INFO

Keywords:
Transcranial sonography
Serotonin transporter gene
Anxiety
Depression
5-HTTLPR

ABSTRACT

Background: Hypo/anechogenicity of the brainstem raphe (BR) structures has been suggested as a possible transcranial parenchymal sonography (TCS) marker associated with depression.
Aim: The aim of this study was to analyze possible association of the abnormal BR echogenicity in patients with major depression when compared to healthy controls, and to evaluate its clinical and genetic correlates.
Methods: TCS was performed in 53 patients diagnosed as major depressive disorder (MDD) without psychotic symptoms and in 54 healthy matched controls. Results: The TCS detected BR abnormalities were significantly more frequent in MDD patients (35 out of 53; 66%) in comparison to matched controls (5 out of 56; 9%). The prevalence of short allele (*s*) homozygocity in the length polymorphism of the promoter region of the serotonin transporter gene (5-HTTLPR) was significantly higher in MDD patients relative to those with normal BR echogenicity. A stepwise statistical discriminant analysis revealed statistically significant separation between MDD patients with and without BR abnormalities groups based on the four predictors combined: the Hamilton Anxiety Rating Scale item 5 („*difficulty in concentration, poor memory*"), presence of social phobia, *s* allele homozygocity of the 5-HTTLPR polymorphism, and presence of generalized anxiety disorder.
Limitations: Cross-sectional design and heterogenous treatment of depressed patients.
Conclusions: Reduced BR echogenicity in at least a subgroup of MDD patients may reflect a particular phenotype, characterized by more prevalent comorbid anxiety disorders, associated with particular genetic polymorphisms and neurotransmitter(s) deficits, most probably altered serotonergic mechanisms.

1. Introduction

Major depressive disorder accounts for significant global morbidity, including medical comorbidities, mortality, and disability (Belmaker and Agam, 2008), but our understanding of its pathophysiology is still limited. Genetic factors conferred a moderate contribution of 37% to unipolar depression (Sullivan et al., 2000). One of the most studied genes in depression, and the first gene to show gene-environment interaction in this disorder was the serotonin transporter gene, namely the polymorphism of its promoter region (5-HTTLPR) (Caspi et al., 2003).

Transcranial parenchymal sonography (TCS), which displays brain tissue echogenicity through the intact skull, gathered increased attention, since it enabled studies of not only midbrain structures, but also echogenicity of the basal ganglia, as well as the transverse diameter of the third and of the frontal horn of the lateral ventricle (Berg et al., 2008). It has been explored as a diagnostic method for neuropsychiatric disorders for more than two decades (Becker et al., 1994). In particular, hypo/anechogenicity of the brainstem raphe (BR) structures has been suggested as a possible TCS marker associated with depression (Berg et al., 2008). Brainstem raphe (BR) was normally depicted as a highly echogenic line, with an echogenicity identical to that of the nucleus ruber (Mijajlović, 2010). Although hypoechogenicity of the BR was present in 8–28% of healthy controls (Becker et al., 1995; Walter et al., 2007a; Mijajlović, 2010), such abnormality (hypo/anechogenicity) was particularly prevalent in patients with unipolar depression (50–70%), with some studies showing abnormal BR echogenicity in up to 90% of patients (Becker et al., 1995; Walter et al., 2007a; Budisic et al., 2010; Ghourchian et al., 2014; Krogias and Walter, 2016). Hypo/anechogenicity was hypothesized to reflect structural disruption of the BR, resulting in impaired serotonergic innervations (Becker et al., 2001). This abnormality was also frequent in depressed patients with

* Corresponding author.

http://dx.doi.org/10.1016/j.jad.2016.11.034
Received 29 August 2016; Received in revised form 26 October 2016; Accepted 15 November 2016
Available online 22 November 2016

Parkinson's disease (PD; 40–60%) or Huntington's disease (Walter et al., 2007b; Krogias et al., 2011a). However, BR alterations were not found in patients with bipolar affective disorder (10–36%) or in patients with schizophrenia (Mijajlović, 2010; Krogias et al., 2011b).

The aim of this study was to analyze possible association of the abnormal BR echogenicity in patients with major depression when compared to healthy controls, and to evaluate clinical and genetic correlates of such an association.

2. Methods

2.1. Patients

Our study comprised 58 consecutive inpatients treated for Major depressive disorder (MDD) without psychotic symptoms (both first episode and recurrent), recruited at the Institute of Mental Health (Belgrade, Serbia). The diagnosis was established using the Structured Clinical Interview for Axis–I (SCID-I) for DSM-IV (First et al., 2002). Patients were excluded if they were (a) < 18 and > 65 years old; (b) had any other central nervous system disease or other causes of focal or diffuse brain damage, including signs of small vessel disease of the brain on MRI examination; (c) had any psychiatric comorbidity within the Axis I, except for panic disorder, social anxiety disorder and generalized anxiety disorder (GAD), as well as personality disorders; and (d) the Mini Mental State Examination score < 27 (Folstein et al., 1975). All patients were treated with antidepressant drugs and adjunct therapy such as stabilizers, antipsychotics, benzodiazepines or hypnotic drugs (Table 1). Fifty-four age- and sex-matched healthy controls (HC) were also included in the study.

The study was done in accordance with the latest version of the Declaration of Helsinki and approved by the Ethics Committee of the Institute of Mental Health. Patients and HC were included in the study after signing an informed written consent.

Table 1
Demographic and clinical features of patients with major depressive disorder (MDD) with (MDD-BR+) and without (MDD-BR-) brainstem raphe abnormality on the transcranial sonography.

	MDD-BR-	MDD-BR+	p
Number of patients[a]	18	35	–
Age (years)[*]	45.6 ± 10.3	42.1 ± 9.8	0.238
Male/female ratio[a]	3/15	11/24	0.248
Education[*]	3.2 ± 0.9	3.0 ± 0.8	0.500
Age at onset (years)[*]	37.8 ± 12.1	34.6 ± 10.9	0.332
HDRS[*]	22.8 ± 3.5	23.3 ± 5.8	0.762
BDI[*]	33.6 ± 13.5	30.8 ± 13.3	0.479
HARS[*]	22.6 ± 8.7	23.9 ± 6.9	0.544
Duration of problems (months)[*]	92.3 ± 88.2	89.8 ± 86.8	0.902
Duration of psychiatric treatment (months)[*]	59.4 ± 73.1	63.7 ± 75.1	0.840
SNRIs[a]	3	8	0.597
SSRIs[a]	10	14	0.281
TCAs[a]	2	8	0.301
TeCAs[a]	1	4	0.441
Benzodiazepines[a]	14	33	0.072
Mood stabilizers[a]	7	17	0.502
Antipsychotics, 1st generation[a]	6	9	0.559
Antipsychotics, 2nd generation[a]	4	5	0.466
Number of hospitalization[*]	1.7 ± 1.1	3.0 ± 3.2	0.111
5-HTTLPR frequency *ss/ls/ll*[a]	1/13/4	10/21/4	0.121
5-HTTLPR *ss/ls+ll*[a]	1/17	10/25	0.048

[*] Values presented as means ± SDs;
[a] Values presented as number of patients; BDI: Beck Depression Inventory; HARS: Hamilton Anxiety Rating Scale; HDRS: Hamilton Depression Rating Scale; MDD: Major Depressive Disorder; SNRIs: serotonin-norepinephrine reuptake inhibitors; SSRIs: selective serotonin reuptake inhibitors; TCAs: tricyclic antidepressants; TeCAs: tetracyclic antidepressants; S′/S′ (S) vs L′ carriers (L) for the *serotonin transporter* gene

2.2. Clinical assessment

Detailed interview on socio-demographic and clinical data, including treatment course and family history of mental disorders, was obtained from all patients and HC, as well as family members when necessary. Participants fulfilled the self-reporting Beck Depression Inventory (BDI) (Beck et al., 1961), and interviewers filled in the Hamilton Depression Rating Scale (HDRS) (Hamilton et al., 1960) and the Hamilton Anxiety Rating Scale (HARS) (Hamilton et al., 1959). Blood samples were taken for genetic studies.

2.3. Genetic testing

DNA was extracted from blood using standard protocols. The 5-HTTLPR deletion (L/S) polymorphism was assayed using gel electrophoresis of PCR products as previously reported (Alexander et al., 2009). Additionally, all samples containing the L allele were genotyped for SNP rs25531 (A/G) by restriction fragment length polymorphism analysis with *Hpa*II restriction enzyme. We analyzed two length polymorphisms of 5-HTTLPR: a short (*s*) and a long (*l*) allele, but due to a limited number of patients we divided patients into two genetically-driven groups: (a) *s* allele homozygotes, and (b) *l* allele carriers (*ll+ls*) (Table 1).

2.4. Transcranial sonography

For TCS we used a color-coded phased-array ultrasound system, equipped with a 2.5 MHz transducer (ProSound Alpha 10, Aloka, Japan). The ultrasound parameters chosen were penetration depth of 14–16 cm and a dynamic range of 45–50 dB. Image brightness, contrast and time-gain compensation were adjusted to get the best image. The examination was performed through a preauricular acoustic bone window scanning supra and infratentorial brain areas in axial planes by tilting the probe. Echogenicity of the pontomesencephalic brainstem raphe (BR) was evaluated by bilateral TCS investigation (the side with better visible structure was used for further analyses). Using the red nucleus (RN) or basal cisterns echogenicity as a reference, the BR echogenicity was semiquantitatively classified as normal (equal signal intensity with the RN and/or basal cisterns) or abnormal if (a) the raphe signals were missing (not visible), or (b) were of reduced echogenicity (the echogenic line of the BR was interrupted or appears abnormally slight and thin) on both sides of insonation (Walter et al., 2002; Krogias et al., 2011a). All TCS assessments were performed by two experienced examiners who were blinded to the clinical data. In case of discrepant assessments, a consensus was accomplished between the examiners.

2.5. Statistics

Differences of demographic and clinical characteristics between groups with or without BR abnormalities were assessed using analysis of variance for continuous and non-parametric stististics (Chi square test and Mann-Whitney test) for categorical data. A discriminant forward step wise analysis was used to develop a model for predicting presence/absence of the BR abnormalities, with gender, presence of GAD, social phobia, panic attacks, the HARS item 5 and HTLPR frequency as predictors. Predictors were ranked by the probability of the Wald chi-square test. Results were considered statistically significant at $p \leq 0.05$. Interrater reliability for the BR TCS was assessed by Cohen's kappa. Statistical analysis was performed with SPSS (v.16.0 for Windows; SPSS Inc., Chicago, IL).

3. Results

The inadequate temporal acoustic bone windows were found in 5 patients (8.6%). Therefore, 53 MDD patients and 54 HC were included