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The Amygdala's Neurochemical Ratios after 12 Weeks Administration of 20 mg Long-acting Methylphenidate in Children with Attention Deficit and Hyperactivity Disorder: A Pilot Study Using ^{1}H Magnetic Resonance Spectroscopy

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Objective: Recent pediatric studies have suggested a correlation between decreased amygdala volume and attention deficit and hyperactivity disorder (ADHD) symptoms, including the emotional dysregulation. To investigate the hypothesis that medication treatment of ADHD specifically improves amygdala function, we used ^{1}H magnetic resonance spectroscopy (MRS) to study the effect of 12 weeks of treatment with daily 20 mg long-acting methylphenidate on the Glu/Cr, NAA/Cr, Cho/Cr, and ml/Cr ratios in the amygdala of medication-naïve children with ADHD.

Methods: This was a prospective study, using a pre- and post-test design, on a single group of 21 children (average age 8.52 years, 17 males and 4 females) diagnosed with ADHD. Low Time Echo MRS scans sampled voxels of interest (1.5×1.5×2.0) from both the right and left amygdala.

Results: There was significant clinical improvement after 12 weeks of treatment with 20 mg long-acting methylphenidate. On ^{1}H MRS, there were no statistical significant differences of NAA/Cr ratio, Cho/Cr ratio, ml/Cr ratio before and after 12 weeks administration of 20 mg long-acting methylphenidate both in the right and left amygdala. In addition, Glu/Cr ratio decreased 14.1% in the right amygdala (p=0.029) and 11.4% in the left amygdala (p=0.008). Standardized mean effect sizes ranged from 0.14-0.32.

Conclusion: The findings are consistent with the possibility that hyperglutamatergic processes in the amygdale are related to the hyperactive-impulsive symptoms of ADHD.

KEY WORDS: Attention deficit and hyperactivity disorder; Long-acting methylhenidate; Magnetic resonance spectroscopy; Glutamatergic neurotransmission; Amygdala.

INTRODUCTION

Attention deficit and hyperactivity disorder (ADHD) is a serious mental health problem among school age children. The worldwide prevalence is around 3-10%.[1-3] The latest survey among primary school age children in Jakarta showed that 15.3% of them were diagnosed as ADHD based on clinical interview using Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV) criteria.[4] The typical symptoms of ADHD are hyperactivity, impulsivity, and inattentiveness, adversely impacting academic achievement and social interaction. In addition, several study revealed that children with ADHD show low frustration tolerance, reduced emotions and empathy, deficits in executive function, and working and spatial memory deficits.[5-8]

Prefrontal and limbic systems − in particular, the amygdala − have important roles in the pathophysiology of ADHD.[5,6,9-12] Children with ADHD showed an alteration of amygdala function compared to healthy controls, along with more hyperactivity and inattention, fear learning, negative emotions, and negative perceptions of emotional stimuli.[13-15] Other studies found that emotional lability and impulsive behavior in children correlated with dysfunction in the amygdala.[16,17]

Studies using magnetic resonance imaging (MRI) showed a volume reduction in the prefrontal cortex, which

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is connected to the limbic system. The studies suggested that disrupted connectivity between the prefrontal cortex and the hippocampus and amygdala might adversely impact attention, memory, and emotional control in children with ADHD.[10,18,19] Plessen *et al*.[13] demonstrated significant reductions in volume in the lateral and basal nuclei of the amygdala, and these reductions might be associated with poor emotional control and affective drive, related to the behavioral reward system in children with ADHD.

Methylphenidate hydrochloride is the first choice medication for ADHD. This medication is assumed to bind at the dopamine transporter, where it reduces the re-uptake of dopamine from the pre-synaptic cleft in the dorsal striatal cortex.[20,21] An animal model study showed that 20 mg methylphenidate might decrease the function of the dopamine active transporter (DAT-1) by 50% in prefrontal cortex, thereby improving dopaminergic neurotransmission at that region.[22] The efficacy of methylphenidate hydrochoride in ameliorating ADHD symptoms is estimated to be around 75-80%.[21,23-25] Wiguna *et al*.[26] demonstrated that 20 mg long-acting methylphenidate for 12 weeks reduced 79.6% of ADHD symptoms, including emotional regulation symptoms; after one month of discontinuation from the medication, measures of ADHD symptoms remained the normal range. Extrapolating from magnetic resonance spectroscopy (MRS) findings, we postulated that the clinical improvements were related to improvements in hemodynamics and neurotransmission in prefrontal and striatal cortex.

MRS is a noninvasive neuroimaging technique that can detect neurochemical concentrations of N-acetyl- aspartate (NAA), glutamate (Glu), creatine (Cr), choline (Cho), and myo-inositol (mI), which are indirect markers of neurotransmission and brain functioning.[27,28] Several previous MRS studies of children with ADHD who were drug naive or treated with methylphenidate hydrochloride showed inconsistent findings in the brain regions studied.[28-31] Therefore the aim of this pilot study was to identify, using MRS, the neurochemical ratios, normalized to Cr, in the amygdala of children with ADHD who were treated with 20 mg long-acting methylphenidate for 12 weeks.

METHODS

Study Design and Participants

This was a pre- and post-test study done without any control group. MRS was used to measure the neurochemical concentrations of NAA, Glu, Cr, Cho, and mI before and after 12 weeks of daily administration of 20 mg long-acting methylphenidate.

Participants were children with ADHD recruited from the Child and Adolescent Psychiatric Outpatient Clinic and Pediatric Outpatient Clinic of Dr. Cipto Mangunkusumo General Hospital, and the Developmental Clinic of Pantai Indah Kapuk Hospital, Jakarta. To ascertain the presence of ADHD symptoms, we adminsitered the Mini-International Neuropsychiatric Interview (MINI) for kids guideline, which had been translated into the Indonesian language by the Division of Child and Adolescent Psychiatry, Department of Psychiatry, at the University of Indonesia-Cipto Mangunkusumo General Hospital, Jakarta. Research was done between January and November 2008. Particpants were collected using the consecutive sampling technique. We used sample size tables for clinical studies to determine the needed sample size.[21] The children were between the ages of seven to 10 years and were all: newly diagnosed with ADHD, drug-naïve, without any comorbidity or chronic illness, of normal intelligence (assessed by the clinical psychologist using the Wechsler Intelligence Scale for Children), and right-handed. All parents signed the consent form which was prepared for this study. The ethics review committee of the University of Indonesia approved the procedures of this study.

Measurements

MRS procedures used in this study have been previously described,[27] and we set the internal chemical shift reference as;

1. The NAA peak peak level at 2.02 ppm
2. The Cho complex peak level at 3.22 ppm
3. The Cr peak level at 3.03 ppm
4. The mI peak level at 3.56 ppm
5. The Glu peal level at 3.65 ppm

The measurements in this study were given as the ratio between the peak-amplitude of NAA, Cho, mI, and Glu to Cr (calculated separetely, as the peak of Cr at 3.03 ppm is accpeted as an internal amplitude reference).

Also, as previously described, we periodically administered the *Skala Penilaian Perilaku Anak Hiperaktif Indonesia* (SPPAHI, the Indonesian Hyperactive Behavior Assessment Scale for Children) and the Clinical Global Impression-Severity scales (CGI-S)[32] and recorded physical parameters (weight, height, pulse, and blood pressure) and interim history (including clinical concerns and use of any other medication).[27] During the

twelve weeks of the research study, clinician contact occurred seven times.

Statistical analysis

Paired *t*-test was used to analyse neurometabolite ratio differences before and after medication administration. Repeated-measures test was used to analyse differences in SPPAHI score. We used a *p*-level of 0.05 as the criterion of statistical significance. All data was analysed using SPSS software ver. 16.0 for graduate students (SPSS Inc., Chicago, IL, USA) for graduate students. A standardized effect size was also determined using a computerized effect size calculator which can be found in the www.cem.org/evidence-based-education/effect-size-calculator.

RESULTS

During the 11 months of the recruitment and study period, 21 subjects completed the study. Fifteen subjects were diagnosed with ADHD, combined type, and six subjects were diagnosed with ADHD, predominantly inattentive type. There were 17 males and 4 females (a male-to-female ratio of 4 : 1). Their average age was 8.52 years. Other demographic characteristics of this population have been previously described.[27)]

Before and after the treatment intervention, the following significant changes in average neurochemicals ratios were observed: NAA/Cr ratio increased 2.5% in the right amygdala (p=0.541) and 4.7% in the left amygdala (p=0.295); Glu/Cr ratio decreased 14.1% in the right amygdala (p=0.029) and 11.4% in the left amygdala (p=0.008); Cho/Cr ratio decreased 6.5% in the right amygdala (p=0.296) and 8.8% in the left amygdala (p=0.091); mI/Cr ratio decreased 1% in the right amygdala (p=0.783) and 4.3% in the left amygdala (p=0.350). The effect size of the administering daily 20 mg long-acting methylphenidate ranged from low to moderate (0.14-0.32) (Table 1).

SPPAHI and CGI-S scores decreased as described in our previous study, and the medication was overall well-tolerated.[27)]

DISCUSSION

A few previous studies have demonstrated, in ADHD, changes in the amygdala, which is involved in the affective processes of fear learning, experience of negative emotion, and perception of emotional stimuli.[33,34)] Dysfunction of the amygdala and the ventromedial prefrontal cortex has been hypothesized to be involved with the unemotional traits[35)] and emotional dysregulation and impulsive behavior of ADHD. Frodl *et al*.[36)] showed that patients with higher hyperactivity lesser inattention had smaller right amygdala volumes compared to healthy controls. They also demonstrated that amygdala volume did not differ significantly in patients with versus patients without previous stimulant medication treatment.

This study showed an increase in NAA/Cr ratio after 12 weeks of long-acting methylphenidate, but this increase did not reach the *a priori* threshold of statistical significance as had been determined. The result might reflect an improvement in neuronal function (as indicated by NAA), correlated with a clinical improvement in ADHD's symptoms.[12,27)] These results seem to be consistent with previous MRS findings and current theories of ADHD pathophysiology.[28,37,38)]

Interestingly, our finding, in this pilot study, of a significant decrease in Glu/Cr ratio in both the right and left amygdala (together with a reduction in clinical hyperactivity-impulsivity) may reflect decreased transmission of glutamate, which is a neurotoxin. Glutamatergic neurotransmission in the prefrontal cortex, limbic system, and

Table 1. The mean of neurometabolite ratios in the right and left amygdala before and after administering 20 mg long-acting methylphenidate for 12 weeks

	Amygdala ratio							
	Right				Left			
	Before treatment	After treatment	*p* value	Effect size	Before treatment	After treatment	*p* value	Effect size
NAA/Cr	1.58 (0.31)	1.62 (0.26)	0.541	0.14	1.49 (0.25)	1.56 (0.19)	0.295	0.32
Glu/Cr	1.98 (0.54)	1.69 (0.50)	0.029	0.56	1.76 (0.42)	1.53 (0.43)	0.008	0.54
Cho/Cr	1.07 (0.23)	1.00 (0.16)	0.296	0.35	1.13 (0.22)	1.03 (0.15)	0.091	0.53
mI/Cr	0.98 (0.19)	0.97 (0.18)	0.350	0.19	0.93 (0.15)	0.88 (0.14)	0.350	0.34

Values are presented as mean (standard deviation).
Paired *t*-test was used in the analysis.
NAA, N-acetylaspartate; Glu, glutamate; Cho, choline; Cr, creatine; mI, myo-inositol.

hippocampus appears to be related to hyperactive and impulsive behavior and emotional dysregulation in children with ADHD. The glutamate elevation, in turn, may be the end result of the following pathophysiological pathway: increased dopamine transporter activity (specifically in children with ADHD) leads to abnormally low levels of synaptic dopamine, which ultimately leads to a failure to inhibit glutamate release.[39] In addition, the increased glutamatergic neurotransmission may reflect decreased astrocyte-mediated neuronal energy metabolism and may indicate cerebral insults.[40-42]

Several previous studies reported an elevation of glutamate spectroscopy signaling in the frontal regions, the striatum, and the anterior cingulate cortex. Younger age of ADHD onset, ADHD symptom ratings at baseline, and diminished capacity to learn and memorize were positively correlated with glutamatergic resonance. However, these findings are controversial, as not all MRS studies consistently showed significant results.[30,43]

Limitations of the current study included: no healthy or untreated control group as a comparison and a relatively small sample size (albeit comparable to many similar studies on neuroimaging in ADHD). The differences in Glu/Cr ratio might be affected by the differences of Cr peaks. In addition, the results might have been different if there was any methylphendiate dose titration during the study.

Notwithstanding these important limitations, this pilot study provided further support for current theories on the neurobiology of ADHD: specifically, the important role of the amygdala and glutamatergic neurotransmission. Our study findings should be interpreted cautiously and should be further replicated.

REFERENCES

1. Furman RA. *Attention deficit/hyperactivity disorder: an alternative viewpoint. J Int Child Adolesc Psychiatry 2002; 2:125-144*.
2. Pliszka S; AACAP Work Group on Quality Issues. *Practice parameter for the assessment and treatment of children and adolescents with attention-deficit/hyperactivity disorder. J Am Acad Child Adolesc Psychiatry 2007;46:894-921*.
3. Faraone SV, Sergeant J, Gillberg C, Biederman J. *The worldwide prevalence of ADHD: is it an American condition? World Psychiatry 2003;2:104-113*.
4. Saputro D. *Childhood hyperkinetic disorder in Jakarta-Indonesia, new screening instrument development, prevalence study, pathophysiological research, and therapy approach. Yogyakarta:Gajah Mada University;2004. [Disertation]*.
5. Herrmann MJ, Biehl SC, Jacob C, Deckert J. *Neurobiological and psychophysiological correlates of emotional dysregulation in ADHD patients. Atten Defic Hyperact Disord 2010;2:233-239*.
6. Maier SJ, Szalkowski A, Kamphausen S, Feige B, Perlov E, Kalisch R, *et al*. *Altered cingulate and amygdala response towards threat and safe cues in attention deficit hyperactivity disorder. Psychol Med 2014;44:85-98*.
7. Reimherr FW, Marchant BK, Strong RE, Hedges DW, Adler L, Spencer TJ, *et al*. *Emotional dysregulation in adult ADHD and response to atomoxetine. Biol Psychiatry 2005;58:125-131*.
8. Volkow ND, Wang GJ, Baler RD. *Reward, dopamine and the control of food intake: implications for obesity. Trends Cogn Sci 2011;15:37-46*.
9. Silvetti M, Wiersema JR, Sonuga-Barke E, Verguts T. *Deficient reinforcement learning in medial frontal cortex as a model of dopamine-related motivational deficits in ADHD. Neural Netw 2013;46:199-209*.
10. De La Fuente A, Xia S, Branch C, Li X. *A review of attention-deficit/hyperactivity disorder from the perspective of brain networks. Front Hum Neurosci 2013;7:192*.
11. Bledsoe JC, Semrud-Clikeman M, Pliszka SR. *Anterior cingulate cortex and symptom severity in attention-deficit/hyperactivity disorder. J Abnorm Psychol 2013;122:558-565*.
12. Hammerness P, Biederman J, Petty C, Henin A, Moore CM. *Brain biochemical effects of methylphenidate treatment using proton magnetic spectroscopy in youth with attention-deficit hyperactivity disorder: a controlled pilot study. CNS Neurosci Ther 2012;18:34-40*.
13. Plessen KJ, Bansal R, Zhu H, Whiteman R, Amat J, Quackenbush GA, *et al*. *Hippocampus and amygdala morphology in attention-deficit/hyperactivity disorder. Arch Gen Psychiatry 2006;63:795-807*.
14. King JA, Tenney J, Rossi V, Colamussi L, Burdick S. *Neural substrates underlying impulsivity. Ann N Y Acad Sci 2003;1008:160-169*.
15. Levy F. *Synaptic gating and ADHD: a biological theory of comorbidity of ADHD and anxiety. Neuropsychopharmacology 2004;29:1589-1596*.
16. Holland PC, Gallagher M. *Amygdala circuitry in attentional and representational processes. Trends Cogn Sci 1999;3: 65-73*.
17. Cardinal RN, Parkinson JA, Hall J, Everitt BJ. *Emotion and motivation: the role of the amygdala, ventral striatum, and prefrontal cortex. Neurosci Biobehav Rev 2002;26:321-352*.
18. An L, Cao QJ, Sui MQ, Sun L, Zou QH, Zang YF, *et al*. *Local synchronization and amplitude of the fluctuation of spontaneous brain activity in attention-deficit/hyperactivity disorder: a resting-state fMRI study. Neurosci Bull 2013;29: 603-613*.
19. Wang S, Yang Y, Xing W, Chen J, Liu C, Luo X. *Altered neural circuits related to sustained attention and executive control in children with ADHD: an event-related fMRI study. Clin Neurophysiol 2013;124:2181-2190*.
20. Hyman SE. *Methylphenidate-induced plasticity: what should we be looking for? Biol Psychiatry 2003;54:1310-1311*.
21. Volkow ND, Wang GJ, Fowler JS, Ding YS. *Imaging the effects of methylphenidate on brain dopamine: new model on its therapeutic actions for attention-deficit/hyperactivity disorder. Biol Psychiatry 2005;57:1410-1415*.
22. Russell VA, Sagvolden T, Johansen EB. *Animal models of attention-deficit hyperactivity disorder. Behav Brain Funct 2005;1:9*.
23. Swanson J, Gupta S, Guinta D, Flynn D, Agler D, Lerner M, *et al*. *Acute tolerance to methylphenidate in the treatment of attention deficit hyperactivity disorder in children. Clin Pharmacol Ther 1999;66:295-305*.

24. Biederman J, Mick E, Faraone SV. *Age-dependent decline of symptoms of attention deficit hyperactivity disorder: impact of remission definition and symptom type. Am J Psychiatry 2000;157:816-818*.
25. Steele M, Weiss M, Swanson J, Wang J, Prinzo RS, Binder CE. *A randomized, controlled effectiveness trial of OROS-methylphenidate compared to usual care with immediate-release methylphenidate in attention deficit-hyperactivity disorder. Can J Clin Pharmacol 2006;13:e50-e62*.
26. Wiguna T, Wibisono S, Sastroasmoro S. *The effect of long-acting methylphenidate 20 mg in improving clinical symptoms of ADHD. Sari Pediatri 2010;2:142-8*.
27. Wiguna T, Guerrero AP, Wibisono S, Sastroasmoro S. *Effect of 12-week administration of 20-mg long-acting methylphenidate on Glu/Cr, NAA/Cr, Cho/Cr, and mI/Cr ratios in the prefrontal cortices of school-age children in Indonesia: a study using 1H magnetic resonance spectroscopy (MRS). Clin Neuropharmacol 2012;35:81-85*.
28. Aoki Y, Inokuchi R, Suwa H, Aoki A. *Age-related change of neurochemical abnormality in attention-deficit hyperactivity disorder: a meta-analysis. Neurosci Biobehav Rev 2013;37:1692-1701*.
29. Jin Z, Zang YF, Zeng YW, Zhang L, Wang YF. *Striatal neuronal loss or dysfunction and choline rise in children with attention-deficit hyperactivity disorder: a 1H-magnetic resonance spectroscopy study. Neurosci Lett 2001;315:45-48*.
30. MacMaster FP, Carrey N, Sparkes S, Kusumakar V. *Proton spectroscopy in medication-free pediatric attention-deficit/hyperactivity disorder. Biol Psychiatry 2003;53:184-187*.
31. Courvoisie H, Hooper SR, Fine C, Kwock L, Castillo M. *Neurometabolic functioning and neuropsychological correlates in children with ADHD-H: preliminary findings. J Neuropsychiatry Clin Neurosci 2004;16:63-69*.
32. Busner J, Targum SD. *The clinical global impressions scale: applying a research tool in clinical practice. Psychiatry (Edgmont) 2007;4:28-37*.
33. Winstanley CA, Theobald DE, Cardinal RN, Robbins TW. *Contrasting roles of basolateral amygdala and orbitofrontal cortex in impulsive choice. J Neurosci 2004;24:4718-4722*.
34. Aggleton JP. *The contribution of the amygdala to normal and abnormal emotional states. Trends Neurosci 1993;16:328-333*.
35. Birbaumer N, Veit R, Lotze M, Erb M, Hermann C, Grodd W, *et al*. *Deficient fear conditioning in psychopathy: a functional magnetic resonance imaging study. Arch Gen Psychiatry 2005;62:799-805*.
36. Frodl T, Stauber J, Schaaff N, Koutsouleris N, Scheuerecker J, Ewers M, *et al*. *Amygdala reduction in patients with ADHD compared with major depression and healthy volunteers. Acta Psychiatr Scand 2010;121:111-118*.
37. Tafazoli S, O'Neill J, Bejjani A, Ly R, Salamon N, McCracken JT, *et al*. *1H MRSI of middle frontal gyrus in pediatric ADHD. J Psychiatr Res 2013;47:505-512*.
38. Arcos-Burgos M, Londoño AC, Pineda DA, Lopera F, Palacio JD, Arbelaez A, *et al*. *Analysis of brain metabolism by proton magnetic resonance spectroscopy (1H-MRS) in attention-deficit/hyperactivity disorder suggests a generalized differential ontogenic pattern from controls. Atten Defic Hyperact Disord 2012;4:205-212*.
39. Carrey NJ, MacMaster FP, Gaudet L, Schmidt MH. *Striatal creatine and glutamate/glutamine in attention-deficit/hyperactivity disorder. J Child Adolesc Psychopharmacol 2007;17:11-17*.
40. Hyder F, Patel AB, Gjedde A, Rothman DL, Behar KL, Shulman RG. *Neuronal-glial glucose oxidation and glutamatergic-GABAergic function. J Cereb Blood Flow Metab 2006;26:865-877*.
41. Struzyńska L. *A glutamatergic component of lead toxicity in adult brain: the role of astrocytic glutamate transporters. Neurochem Int 2009;55:151-156*.
42. Todd RD, Botteron KN. *Is attention-deficit/hyperactivity disorder an energy deficiency syndrome? Biol Psychiatry 2001;50:151-158*.
43. Courvoisie H, Hooper SR, Fine C, Kwock L, Castillo M. *Neurometabolic functioning and neuropsychological correlates in children with ADHD-H: preliminary findings. J Neuropsychiatry Clin Neurosci 2004;16:63-69*.