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ORIGINAL ARTICLE

Alteration of plasma alanine, glutamate, and glycine Level: A potentiate manic episode of bipolar disorder

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Abstract

Introduction: The amino acids that function as co-agonists at the N-methyl-D-aspartate (NMDA) receptor have been investigated in bipolar disorder (BD). However, studies comparing amino acid levels in the plasma of BD patients with healthy controls have yielded inconsistent results. We, therefore, conducted a study in Hospital Universiti Sains Malaysia to determine the plasma levels of glutamate, glycine, and alanine in BD patients and compared them with the healthy controls. *Materials and Methods:* An overnight fast of 10-hour plasma levels of glutamate, glycine, alanine, and tryptophan were measured in 83 bipolar patients, and were compared to a group of 82 healthy controls. *Results:* The mean (SD) age of bipolar patients was 40.9 (12.1), while the mean (SD) age for control groups was 35.6 (7.7) years. The median (25^{th}, 75^{th} percentile) of glutamate and alanine levels in bipolar patients was 111.0 (65.0,176.0) and 530.0 (446.0,629.0), respectively, while the mean (SD) of glycine level in bipolar patients was 304.0 (98.1). Significant higher glutamate, glycine, and alanine levels were found in bipolar disorder patients in the manic episode as compared to the healthy controls. *Conclusion:* Although the exact relationship between peripheral NMDA receptor co-agonist levels in the pathogenesis of BD is not well understood, these findings should be explored and may enlighten some new paths for BD therapy which could reward the patients also clinicians.

Keywords: bipolar disorder, alanine, glutamate, glycine, NMDA receptor

INTRODUCTION

Bipolar disorder (BD) is one of the most common psychiatric disorders worldwide, with a lifetime prevalence of approximately 1.3%.[1] The exact cause of BD is not yet known. A variety of biochemical, environmental, and genetic elements could be involved in both triggering and causing bipolar episodes. Currently, there is no biomarker to confirm BD, and mostly diagnosis is depending on judgment and clinical expertise.[2]

There is growing evidence that suggests that N-methyl-D-aspartate (NMDA) glutamate receptor complex abnormalities play an essential role in the pathophysiology of mood disorders.[3] Antagonism of the NMDA glutamate receptor complex induces behavioural and cognitive deficits in healthy subjects with a broad range of CNS symptoms such as psychotic phenomena, agitation, and disorientation. Whereas hypofunction of this receptor complex has an association with negative symptoms and cognitive deficits in patients with schizophrenia.[4]

In addition to the binding site for the agonist glutamate, a glycine-coupled modulatory site must be occupied by glycine to open the calcium channel of NMDA receptors.[5, 6] Glycine levels vary with brain region extracellularly and neuronal activity and are regulated by the glycine transporter-1 (GlyT1), a glycine transporter produced in astrocytes and post-synaptic neurons, in a function-dependent form.[7] Modulation of NMDA receptor responses through dynamic alterations in endogenous glycine levels was successfully utilised in schizophrenia as therapeutic interventions.[8] Some studies also

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have shown that increased glycine levels occur not only in schizophrenia but also in relapsing mania, which suggests a significant role of glycine as the coupled modulatory site for NMDA receptor with glutamate.[9,10] Besides, in vivo, a radiological study showed significantly higher glutamate and glycine levels in both the anterior and posterior cingulate cortex of patients with schizophrenia and bipolar disorder.[11] Thus it could be postulated that the dysfunction of this receptor complex also plays a vital role in the emergence of psychotic relapses.

Alanine is a non-essential amino acid, which can have several specific functions in various roles including neuron-astrocyte functional group, such as glutamate and synaptic Gamma-aminobutyric acid (GABA) entities.[12] Clinical data show that the diminished function of GABA provokes depression or manic mood states. In the brain, cerebrospinal fluid, and plasma of depressed patients, as well as in mania patients, lower levels of GABA are present.[13] In a previous study, higher plasma alanine was found in patients with Major Depressive Disorder (MDD). The alteration of this amino acid could influence the synthesis of brain neurotransmitters related to mood disorder pathophysiologic mechanism.[14] Hence, there is a possible role of alanine in BD, and this hypothesis, appropriate to a subset of mood disordered persons, is testable.

Recently, metabolomics, which quantifies and identifies metabolites in various body fluids such as serum/plasma and urine, has been used in diagnosing neuropsychiatric disorders, especially in schizophrenia and autism.[15,16] Laboratory testing is not yet commonly used to diagnose BD.[17] To the best of our knowledge, no biomarkers are available to support laboratory testing for this condition. Previous studies have also determined that dysregulation of glutamate and glycine levels occurs in the affective patients by postmortem, radiological, and cerebrospinal fluid examination.[11,18-20] However, there has been little discussion about the peripheral plasma level of glutamate, glycine, and alanine in patients with bipolar disorder. This study aims to investigate the plasma concentrations of amino acids related to the NMDA receptor, which are glutamate and glycine, and the role of the alanine in patients with bipolar disorder.

MATERIALS AND METHODS

Study Participants

A case-control study was conducted from October 2017 till June 2018 in Hospital Universiti Sains Malaysia (HUSM). Bipolar patients aged 18 to 65 years old who fulfilled the DSM-5 criteria of Bipolar I and Bipolar II were recruited from the psychiatric outpatient clinic. They were informed before the clinic visit to fast for at least 10 hours to ensure that diet intake was not affecting the amino acid level. Those with comorbid psychiatric illnesses, chronic medical diseases, pregnancy, and either had acute surgery or infarctions to the brain, which may interfere with the amino acid level were excluded from the study. These illnesses were ruled out by clinical interview by the researcher and team.

Healthy control aged from 18 to 65 were recruited from HUSM employees who volunteered to take part in the study. They were assessed to ensure that they were in good health and had no underlying medical or psychiatric illnesses. All protocols were approved and conducted in compliance with the Human Research Ethics Committee of USM (USM/JEPeM/17020091), and written informed consent was obtained from participants before the studies.

Sample size calculation and sampling method

The first objective was to evaluate the plasma levels of glutamate, glycine, and alanine in BD patients. The sample size was calculated using the formula for estimation of single mean with type 1 error (α) of 5%, a standard deviation of the parameters of interest from a previous study (σ), the precision of estimation (Δ) and anticipated dropout rates of 10%. This gave a total number of 64 BD patients that needed to be recruited.

The second objective was to compare the plasma levels of glutamate, glycine, and alanine in BD and healthy controls. The sample size was calculated using the formula for comparison of two independent means with type 1 error (α) of 5%, 1- type 2 error (power) of 80%, a standard deviation of the parameters of interest among control group from a previous study (σ), the detectable difference (Δ), a ratio between control to cases (m) is equal to 1, and anticipated dropout rates of 10% which gives a sample size of 75 bipolar patients. For this study 83 patients with bipolar disorder, and 82 healthy controls were recruited with the total numbers of study participants being 165.

For sampling methods of the cases, all eligible BD patients who came for follow up in the psychiatric outpatient clinic at HUSM within the study period were recruited. For the controls, we have obtained the healthy subjects

who volunteered to participate in the study after the advertisement placed in the HUSM clinic.

Instruments

The self-administered questionnaires included questions on sociodemographic characteristics, Beck Depression Inventory (BDI), and Saringan Status Kesihatan Mental – 20 (SSKM-20). The clinician-rated survey used in this study was the Young Mania Rating Scale (YMRS).

Young Mania Rating Scale (YMRS)
The severity of manic symptoms was assessed according to 11-items YMRS that ranges from 0 to 60, with higher scores indicate greater severity of symptoms.[21] This question tool is validated, clinician-rated in the English language, and was assessed by a trained psychiatry medical officer throughout the study duration.

Beck Depression Inventory (BDI)
BDI was used to assess the status of depression for the patient with bipolar disorder in the depressive episode. This questionnaire is a validated tool, translated to the Malay language, and had 21 questions with the multiple-choice self-report inventory. It assessed how the subjects had been feeling in the last week. The standard scores were divided into four categories from 0 to 9 indicates minimal depression, 10 to 18 mild depression, 19 to 29 moderate depression, and 30 to 63 shows severe depression. The higher the total scores indicate more severe depressive symptoms.

Saringan Status Kesihatan Mental – 20 (SSKM-20)
This tool is validated to screen for any psychiatry issues in Malaysia.[22] It contains 20 questions with *Likert scale* answer which assess the participants' feeling within a month duration. The cut-off point used was 14, and participants with scores more than 14 will be referred for assessment.

Sociodemographic porforma
Demographic characteristics of the participants in this study included basic characteristics such as age, race, gender, BMI, smoking status, diagnosis, duration of illness, and family history of mental illness.

Amino acids analysis

Samples were collected after 10 hours of fasting from subjects between 8:00 am and 9:00 am. 5mls of venous blood was withdrawn and collected in lithium heparin by a qualified officer for plasma amino acid analysis. Samples were kept in ice for transportation and were analysed using the high-performance liquid chromatography (HPLC) method.

Statistical analysis

Characteristics of participants were compared using an independent sample t-test (for age) and a chi-square test (for gender). To determine significant differences in amino acid level between BD patients and controls, we performed both independent sample t-test and the Mann Whitney test. A one-way ANOVA and Kruskal Wallis tests were performed to evaluate significant differences in the concentration level of amino acids between Bipolar I and Bipolar II and in different episodes of BD. A significance level was set at $p<0.05$.

RESULTS

Demographic data

A total of 83 bipolar patients were enrolled from HUSM psychiatric outpatient clinic, and 82 healthy control employees volunteered to participate in this study. The sociodemographic characteristics of bipolar patients and the control groups are summarized in Table 1. The mean (SD) age of bipolar patients was 40.9 (12.1), while the mean (SD) age for control groups was 35.6 (7.7) years. The participants from the bipolar group and control group differ significantly in age (p=0.001). There was no significant difference between patients and controls for gender. Among patients with bipolar disorder, ten were identified to be manic, 67 were euthymic, 4 in hypomanic, and 2 in depressive episodes at the time of assessment. Patients in the hypomanic episode and depressive episode were excluded from the comparison of amino acids in between events due to the small number of patients.

Multiple pairwise comparisons with Bonferroni correction revealed a significant difference in glutamate (p=0.011) and alanine (p <0.001) level between control vs. Bipolar I. However, there was no significant difference in glutamate between Bipolar II vs. control (p >0.95) and between Bipolar I vs. Bipolar II (p >0.95). For alanine, there was no significant difference between Bipolar II vs. control (p =0.119) and between Bipolar I vs. Bipolar II (p >0.95).

Table 1: Characteristics of Study Participants

Variables	Controls	Bipolar patients	*p* value
Number (n)	82	83	
	Mean (SD)	**Mean (SD)**	
Age (years)	35.6 (7.7)	40.9 (12.1)	**0.001** [a]
BMI	25.55 (5.17)	27.65 (5.33)	**0.011** [a]
	n (%)	**n (%)**	
Gender			0.388 [b]
Male	34 (42.0)	29 (34.9)	
Female	48 (58.0)	54 (65.1)	
Race			0.082 [b]
Malay	82 (100)	80 (96.4)	
Non-Malay		3 (3.6)	
Smoking			0.274 [b]
Yes	5 (6.10)	9 (10.8)	
No	77 (93.9)	74 (89.2)	
Family history of psychiatric illness			**<0.001** [b]
Yes	1 (1.21)	64 (77.1)	
No	81 (98.8)	19 (22.9)	
Diagnosis			
Bipolar 1		76 (91.6)	
Bipolar 2		7 (8.4)	
Episodes			
Euthymic		67 (80.7)	
Manic		10 (12.0)	
Hypomanic		4 (4.8)	
Depressive		2 (2.4)	
		Median (25^{th},75^{th} percentile)	
Duration of illness (years)		10.0 (4,21)	
	Mean (SD)	**Mean (SD)**	
YMRS		17.43 (9.87)	
BDI		23.0 (8.49)	
SSKM	8.27 (6.16)		

[a] Independent sample t-test, [b] χ^2 test

Amino acids of plasma samples from BD patients and control

The plasma amino acids examined in this study were glutamate, glycine, and alanine.

Multiple pairwise comparisons with Bonferroni correction revealed a significant difference in glutamate level between control vs. euthymic (p=0.019). However, no significant difference in glutamate level between control vs. manic (p =0.892) and between manic vs. euthymic (p>0.95). There was a significant difference in the alanine level between control vs. euthymic (p<0.001) and between manic vs. control (p=0.001). The hypomanic and depressive episode was not included in the analysis because of the very small sample, n=4 and n=2 respectively.

Post Hoc analysis with Bonferroni correction revealed the mean difference of glycine level is significant between manic and control (p=0.021). However, there was no significant difference between control vs. euthymic (p=0.212) and

Table 2: Comparison of Glutamate and Alanine Level of Bipolar Disorder Patients and Control Group

Variable	Median (25th, 75th percentile) Level concentration (μmol/L)		Z statistics	*p* value
	Control (n= 82)	Bipolar (n=83)		
Glutamate	61.5(44.3,166.0)	111.0(65.0,176.0)	-2.80	**0.005***
Alanine	430.5(370.0,477.5)	530.0(446.0,629.0)	-5.774	**<0.001***

*Mann Whitney test

Table 3: Comparison of Mean Glycine Level between Bipolar Patients and Control Group

Variable	Mean (SD) Level Concentration (μmol/L)		*t* stats (df)	*p* value
	Control (n= 82)	Bipolar (n=83)		
Glycine	273.6 (66.4)	304.0 (98.1)	-2.328 (163)	0.021*

*Independent sample t-test

Table 4: Comparison of Glutamate, Glycine, and Alanine Level Among Bipolar I, Bipolar II, and Control

Variables	Median (25th, 75th percentile) Level Concentration (μmol/L)				
	Control (n= 82)	Bipolar I (n= 76)	Bipolar II (n= 7)	Z stats (df)	*p* value
Glutamate	61.5 (44.3,166.0)	111.0 (65.3,179.0)	67.0 (51.0,166.0)	8.606 (2)	**0.014***
Alanine	430.5 (370.0,477.5)	532.0 (446.8,627.8)	516.0 (384.0,655.0)	33.395 (2)	**<0.001***
	Mean (SD)				
	Level Concentration (μmol/L)			**F stats (df)**	***p* valuea**
	Control (n= 82)	**Bipolar I (n= 76)**	**Bipolar II (n= 7)**		
Glycine	**273.6 (66.4)**	302.7 (100.1)	31.80 (77.9)	1.04 (3,79)	0.380

*Kruskal Wallis test, [a]One-way ANOVA

Table 5: The Median of Glutamate and Alanine Level among Euthymic, Manic and Control

Variables	Median (25th, 75th percentile) Level Concentration (μmol/L)				
	Control (n= 82)	Euthymic (n=67)	Manic (n=10)	Z stats (df)	*p* value
Glutamate	61.5 (44.3,166.0)	111.0 (64.0,180.0)	122.0 (61.5,167.5)	7.634 (2)	**0.022***
Alanine	430.5 (370.0,477.5)	516.0 (443.0,608.0)	571.0 (463.3,703.0)	32.91 (2)	**<0.001***

*Kruskal Wallis test

Table 6: The mean of Glycine Level among Euthymic, Manic and Control

Variable	Mean (SD) Level Concentration (μmol/L)				
	Control (n=82)	Euthymic (n=67)	Manic (n=10)	F-stats (df)	*p* value
Glycine	273.9 (66.1)	299.04 (99)	350.9 (104.6)	4.529 (2,156)	**0.012***

*One-way ANOVA

euthymic vs. manic (p=0.211). The hypomanic and depressive episode was not included in the analysis because of a very small sample, n=4 and n=2 respectively.

DISCUSSION

Previous evidence has suggested that there was an efflux of brain-to-blood amino acids.[23-25] They are commonly transported across the blood-brain barrier through a specific transporter to participate in amino acid metabolism and synthesis of neurotransmitters. Hence the level of these amino acids in plasma was directly being influenced by the level in the brain. For example, in autism patients relative to controls study showed that higher levels of glutamate are not restricted to plasma, and some studies have reported higher levels in some brain regions.[26]

The present study demonstrates the increase in plasma levels of glutamate, glycine, and alanine in different episodes of BD patients, more markedly in the manic phase as compared to the euthymic and healthy control group. These changes suggest that episodes of bipolar disease influenced the plasma levels of these amino acids.

These results are consistent with those found in the previous study, which also documented an elevated level of glutamate and glycine in bipolar patients.[9] Though, in their study, they mention that the glutamate and glycine were persistent increase until the patient in remission. Similarly, this study has found that an increased glutamate level in patients with Bipolar I disorder in the euthymic phase compared to the healthy controls.[25] In a previous study, the brain tissue also demonstrated significantly high glutamate and glycine levels both in anterior and posterior cingulate cortex in bipolar patients which may explain the elevation of both amino acids in the plasma level.[11] Nevertheless, in patients with schizophrenia, the plasma glycine levels, and the glycine/serine ratios were decreased compared to controls and patients with major depression.[27] Another research study reported a significant reduction in CSF glutamate and glycine in patients and controls in refractory affective disorder.[18]

Valproate is one of the anticonvulsants that have been widely used to treat BD patients, while lithium is the goal standard treatment.[28-30] Most medications that are commonly used to treat bipolar disorder have been postulated to interfere with the glutamate system, and it has been shown to modulate glutamatergic neurotransmission.[31-33] Few studies have recorded a significant increase in the plasma levels of glutamate and glycine relative to healthy controls, primarily due to the use of lithium and anticonvulsants.[34,35] While a study on red blood cells (RBC) showed a significant elevation of glycine levels in BD patients who were on lithium.[36] These findings indicate that lithium and valproate share common effects on the concentration of specific amino acid neurotransmitters, which may be related to their mechanism of action in bipolar disorder. Yet, later studies showed no effect on the plasma levels of glycine in treated chronic bipolar patients.[9] Previous animal studies have shown that a significant reduction in brain concentration of glutamate occurs when measured with Nuclear magnetic resonance (NMR) spectroscopy. Nonetheless, when HPLC was used, a non-significant decrease in glutamate concentrations was found with both drugs.[37] We could postulate that, according to these diverse findings, the drug-to-bipolar disorder relationship remains unresolved.

Studies showed, upon binding of glutamate and concurrent binding of a coagonist e.g glycine and alanine, the NMDA receptor is activated.[38-40] In Schizophrenia patients, plasma NMDA receptor coagonist levels have become feasible as various case-control studies have been published on this issue. However, in BD the data is still unclear and remains controversial.[41] In our study, it was demonstrated that alanine levels were higher in BD as compared to healthy controls and markedly raised in a manic episode. This optimistic result supports the theory that increasing NMDA neurotransmission via the

NMDA co-agonist site is a strategy for BD treatment. Though, the specific pathways in the pathophysiology of BD that encompass the role of alanine synthesis or metabolism remains unclear.

In summary, the study has shown that alterations in the plasma levels of glutamate, glycine, and alanine in bipolar patients, suggesting the dysfunction of NMDA receptor complexes, may play an important role in the vulnerability for the emergence of bipolar disorder. While the exact relationship between peripheral amino acids level and central functionality is not clear, the association studies with the radiological and the histological findings may be of value in understanding better mental disorder pathophysiology. Perhaps, larger sample size, a medication-related including mood stabiliser, anti-depressant and anti-psychotic effect, and a longer follow-up time would be beneficial.

This study had some limitations. First is that majority of study participants were stable bipolar patients, and only a few of the severe manic and depressed patients were admitted during the study periods, which give an imbalance of participants in each group episode for the analysis. Secondly, because of that reason, we have to exclude the BD patients who were in depressive and hypomanic episodes as a minimal number of participants that fall into these groups for comparison. Apart from that, we also cannot exclude from current research that long-term treatment with mood stabilizers, antidepressants, and antipsychotics can affect serum amino acid levels. Therefore, all of these factors need to be considering further studies.

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Authors' contribution: WNWN performed testing, analysed results and drafted the manuscript. WNWA interpretation of the data analysed results and wrote the manuscript. AAR discussed the outcomes and contributed equally to the manuscript preparation. NMY calculates the sample size and statistical analysis of the data.

Conflict of interest: The authors declare no conflict of interest in the research and publication of this paper.

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