タイトル	Analyses of N-Methyl-D-Aspartate Receptor-Related Metabolites in the Serum of Antipsychotic-Naïve Individuals with at-Risk Mental State
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公開者	The Medical Society of Toho University
発行日	2020.06.01
ISSN	21891990
掲載情報	Toho Journal of Medicine. 6(2). p.97-97.
資料種別	学術雑誌論文
内容記述	Original Article
著者版フラグ	publisher
JaLCDOI	info:doi/10.14994/tohojmed.2019-026
メタデータのＵＲＬ	https://mylibrary.toho-u.ac.jp/webopac/TD39126483

Original Article

Analyses of *N*-Methyl-D-Aspartate Receptor-Related Metabolites in the Serum of Antipsychotic-Naïve Individuals with at-Risk Mental State

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ABSTRACT

Introduction: The criteria for "at-risk mental state" have been advocated as a preventive approach to psychosis treatment although their pathophysiological mechanisms remain unclear. Reliable biomarkers to predict transitions from at-risk mental state to psychosis are urgently needed. Since abnormalities in *N*-methyl-D-aspartate receptor co-agonists have been reported in the serum of patients with schizophrenia, several of these metabolite levels in individuals with at-risk mental state were investigated and these levels with clinical symptoms were correlated.

Methods: Serum levels of glutamate, cysteine, glycine, γ-glutamylcysteine, glutathione, D-serine, and L-serine were investigated in antipsychotic-naïve individuals with attenuated psychotic symptoms (n = 28) and compared with those in antipsychotic-naïve individuals with first-episode psychosis (n = 13) and those in healthy controls (n = 41). The serum metabolite levels were measured using high-performance liquid chromatography with fluorescence detection or liquid chromatography with tandem mass spectrometry. Correlations between clinical symptoms and serum metabolite levels in individuals with at-risk mental state were also examined.

Results: The glutathione and D-serine levels were significantly lower, whereas glutamate levels were significantly higher in individuals with attenuated psychotic symptoms than in healthy controls. Additionally, glutathione levels were significantly decreased in individuals with first-episode psychosis compared with those in healthy controls. In individuals with attenuated psychotic symptoms, clinical scores were not correlated with serum levels of metabolites related to the *N*-methyl-D-aspartate receptor.

Conclusions: The results of this study suggest that abnormally altered levels of metabolites related to the *N*-methyl-D-aspartate receptor can already occur in individuals with attenuated psychotic symptoms.

Toho J Med 6 (2): 90–97, 2020

KEYWORDS: *N*-methyl-D-aspartate receptor, at-risk mental state, attenuated psychotic symptom, schizophrenia, serum biomarker

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DOI: 10.14994/tohojmed.2019-026

Received Oct. 29, 2019: Accepted Nov. 30, 2019
Toho Journal of Medicine 6 (2), June 1, 2020.
ISSN 2189–1990, CODEN: TJMOA2

Introduction

Schizophrenia is one of the severe and chronic mental disorders and can have a deteriorating course. To improve its prognosis, it is important to minimize the duration of untreated psychosis, which is significantly associated with quality of life, social functioning, and cognitive function.[1] Furthermore, early detection of high-risk state for psychosis for subsequent intervention prevents the transition to psychosis and improves the prognosis after psychosis onset. The at-risk mental state (ARMS) has been advocated as the preventive approach to psychosis treatment.[2] Although a meta-analysis indicates that ARMS is associated with higher psychosis transition rates,[3] not all of the affected individuals necessarily convert to psychosis. To date, it is difficult to predict individuals with ARMS who will transition to psychosis relying only on symptom features. Therefore, establishing reliable biomarkers in individuals with ARMS is urgently needed. To identify reliable biomarkers, it is necessary to examine molecules based on the pathophysiology of schizophrenia.

Although the etiology of schizophrenia has not been fully elucidated yet, some convincing hypotheses have been suggested. Among others, *N*-methyl-D-aspartate (NMDA) dysfunction, also termed the "glutamate hypothesis," has been proposed because NMDA hypofunction causes negative symptoms and cognitive impairment, as well as positive symptoms, in animal and human experiments.[4] Ketamine and phencyclidine, which are NMDA antagonists, induce schizophrenic-like symptoms in healthy humans.[4] The NMDA receptor is an ionotropic glutamate receptor, which is activated by binding its agonist glutamic acid (Glu), whereas glycine (Gly) or D-serine can bind as co-agonists to the regulatory site of the submit.[5–7] In the early 1990s, additional modulatory effects on NMDA receptors by the tripeptide glutathione (GSH) have been described.[8] Besides, GSH is regarded as one of the endogenous antioxidants and is implicated in the "oxidative stress hypothesis" that has been advocated as the etiology of schizophrenia.[9] GSH is synthesized from Glu, cysteine (Cys), and Gly.[10] First, γ-glutamylcysteine (γ-GluCys) is synthesized from glutamate and cysteine. In a second step, glycine is added to γ-GluCys to synthesize GSH. Glutamate metabolites are also agonist or co-agonist of the NMDA receptor and intimately related to the NMDA receptor.

Several studies indicated that serum levels of γ-GluCys, GSH, and D-serine are lower and those of Glu are higher in patients with schizophrenia compared with healthy controls,[11, 12] whereas another study detected decreased Gly levels in patients with schizophrenia.[13] Previous studies examining the relationships between blood levels of metabolites related to the NMDA receptor in patients with schizophrenia and their symptoms reported that GSH serum levels were negatively correlated with the total Positive and Negative Syndrome Scale (PANSS)[14, 15] score of these patients[16] and plasma D-serine levels were correlated with the improvement of their positive symptoms.[17] However, an analysis of the metabolites related to the NMDA receptor in the serum of individuals with ARMS has not been reported yet. To investigate alterations of biological metabolites in these individuals, we determined serum levels of metabolites related to the NMDA receptor including Glu, Cys, Gly, γ-GluCys, GSH, D-serine, and L-serine in antipsychotic-naïve individuals with ARMS and compared these levels with those in antipsychotic-naïve individuals with first-episode psychosis (FEP) and in healthy controls. Furthermore, we also correlated the serum levels of these NMDA receptor-related metabolites with the clinical symptoms in individuals with ARMS.

Materials and Methods

Participants

This is a cross-sectional observational study of individuals with attenuated psychotic symptoms (APS, n = 28, those with FEP, n = 13), and healthy controls (n = 41). All participants were enrolled between June 1, 2014, and December 31, 2018, and individuals with APS or FEP were recruited from the Toho University Omori Medical Center in Tokyo. To minimize the heterogeneity present in ARMS, which could affect the data, we extracted individuals with APS among the ARMS population of this center. All individuals with APS and FEP were help-seeking patients who had already been affected by psychotic symptoms. Individuals with APS satisfied the following criteria: A) participants fulfilled the APS criteria of the Structured Interview for Psychosis-Risk Syndromes/Scale of Psychosis-Risk Symptoms (SIPS/SOPS).[18] We used the Japanese version of SIPS/SOPS, which we previously demonstrated to have excellent interrater reliability.[19] The SIPS/SOPS were administered by trained psychiatrists; B) they were 15-40 years old; C) they were native Japanese speakers; and D) they were able to obtain written informed consent. The exclusion criteria for individu-

als with APS were as follows: E) they took antipsychotic medication during the study period or had taken antipsychotics in the past 6 months; and they had F) severe learning disorder, G) organic brain disorder, H) medical history of psychosis, I) severe suicidal thoughts, J) alcohol and drug abuse, K) IQ below 70, and L) comorbidity or medical history of hepatic, renal, or endocrine diseases including diabetes.

Individuals with FEP who experienced the first onset of psychotic symptoms were identified by trained psychiatrists using the Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders (SCID-I).[20, 21] The enrolled participants satisfied the criteria B) to D) and all exclusion criteria E) to L).

Additionally, we recruited healthy participants. Healthy controls satisfied the following inclusion criteria: A) they had no history of any mental disorder according to the SCID-I Non-Patient Edition[21, 22] with B) to D) as described above. The exclusion criteria for healthy controls were from E) to L) as mentioned above.

We assessed the clinical symptoms and social functions of study participants with APS. The clinical symptoms were assessed using the PANSS, Beck Depression Inventory second edition (BDI-II),[23] Clinical Global Impression (CGI),[24] and the modified Global Assessment of Functioning (GAF) scale.[25, 26] Social functions were assessed by the World Health Organization Quality of Life 26 (WHO-QOL 26),[27, 28] and the Schizophrenia Cognition Rating Scale (SCoRS).[29] We used the SCoRS Interviewer's Global Rating as a parameter.[30] Moreover, we determined the duration of untreated illness, which was defined as the interval between the onset of the prodrome and the administration of the first treatment.[31]

The study protocol was approved by the Ethical Research Committee of the Toho University School of Medicine (A17039-26012). All participants provided written informed consent, or if participants were under 20 years of age, their parents provided written informed consent with the participants giving written assent.

Blood sampling and metabolite analysis

Blood samples (10 mL) were collected from an antecubital vein between 8:00 AM and 9:00 AM before breakfast after fasting from 9:00 PM on the previous day to avoid the effects of food intake. The serum was separated by centrifugation and stored at −80°C. The serum levels of Cys, Gly, γ-GluCys, GSH, Glu, D-serine, and L-serine were measured using high-performance liquid chromatography with fluorescence detection[32] or a liquid chromatography with tandem mass spectrometry-based method that we reported before.[33]

Statistical analysis

A one-way analysis of variance (ANOVA) or chi-squared test was employed to analyze group differences in demographic variables. The ANOVA was utilized for the statistical analysis of metabolite concentrations with $p < 0.05$ considered statistically significant. We used Turkey's HSD test as a post hoc test for multiple comparisons. The Spearman rank correlation test was performed to determine statistical correlations between variables. Statistical analyses were carried out using the Statistical Package for Social Science version 23.0 (IBM, Armonk, NY).

Results

Demographics and characteristics of the study participants

The demographics of the participants are presented in Table 1. There were significant group differences in age and sex, whereas the body mass index did not significantly differ among the three study groups. The clinical characteristics of the APS and FEP groups are also shown in Table 1.

Serum levels of metabolites related to the NMDA receptor in the three study groups

The serum levels of metabolites related to the NMDA receptor are listed in Table 2. The levels of GSH (APS group 4.3 ± 1.8 μM, healthy controls 6.4 ± 2.4 μM; $p = 0.001$) and D-serine (APS group 0.9 ± 0.2 μM, healthy controls 1.1 ± 0.3 μM; $p = 0.010$) were in the APS group significantly lower than in healthy controls. By contrast, the Glu levels (APS group 184.3 ± 102.2 μM, healthy controls 131.2 ± 67.4 μM; $p = 0.022$) were significantly elevated in the APS group compared with the control group. Moreover, the serum levels of GSH (FEP group 3.8 ± 2.4 μM, healthy controls 6.4 ± 2.4 μM; $p = 0.002$) were significantly decreased in the FEP group compared with the healthy controls. The serum levels of the three metabolites that were significantly different among the three groups are shown in Fig. 1.

Relationships between relative metabolite concentrations and clinical symptoms

Since the serum concentrations of Glu, GSH, and D-serine were significantly different between the APS and control groups, we additionally examined their relationships with demographic characteristics like age, with the clinical

Table 1 Demographic characteristics

Characteristic	Control N = 41	APS N = 28	FEP N = 13	P-value
Age mean (SD), yr	21.6 (2.4)	18.9 (4.9)	28.3 (10.3)	**0.000** * *
Gender				
Female: Male	30:11	10:18	8:5	**0.008** * *
Body Mass Index, mean (SD)	21.7 (4.7)	21.0 (3.0)	20.8 (5.0)	0.69
PANSS		66.9 (15.1)	93.2 (20.8)	-
Positive, mean (SD)	-	15.8 (3.9)	24.4 (4.8)	-
Negative, mean (SD)	-	17.0 (5.4)	24.5 (7.5)	-
General, mean (SD)	-	34.1 (9.1)	45.0 (10.8)	-
BDI-II	-	24.5 (12.3)	19.6 (9.9)	-
CGI, mean (SD)	-	4.6 (0.7)	5.5 (0.8)	-
GAF, mean (SD)	-	46.0 (8.5)	33.9 (12.9)	-
WHOQOL-26, mean (SD)	-	2.7 (0.6)	2.9 (0.4)	-
SCoRS Interviewer's Global Rating (SD)	-	3.9 (1.8)	4.8 (2.2)	-
DUI (SD), M	-	29.2 (61.3)	31.3 (41.8)	-

*: $p<0.05$, * *: $p<0.01$

Significant values are highlighted by bold font.

APS, attenuated psychotic symptoms; PANSS, Positive and Negative Syndrome Scale; BDI-II, Beck Depression Inventory-II; CGI, Clinical Global Impression; GAF, Global Assessment of Functioning; WHOQOL-26, World Health Organization Quality of Life-26 Scale; SCoRS, Schizophrenia Cognition Rating Scale; DUI, duration of untreated.

Table 2 Comparison of serum levels of metabolites related to the NMDA receptor among the three groups

	Control N = 41	APS N = 28	FEP N = 13	ANOVA	Post-hoc		
	A	B	C		A vs B	A vs C	B vs C
Glu (SD), μM	131.2 (67.4)	184.4 (102.2)	160.4 (59.2)	**0.028** *	**0.022** *	0.488	0.647
Cys (SD), μM	225.4 (34.8)	237.8 (40.8)	242.3 (34.5)	0.226	0.358	0.929	0.323
γ-GluCys (SD), μM	4.3 (1.1)	3.7 (1.2)	3.9 (1.1)	0.168	0.161	0.553	0.931
Gly (SD), μM	264.7 (57.7)	237.0 (50.0)	243.1 (64.9)	0.119	0.119	0.553	0.945
GSH (SD), μM	6.4 (2.4)	4.3 (1.8)	3.8 (2.4)	**0.000** * *	**0.001** * *	**0.002** * *	0.783
D-serine (SD), μM	1.1 (0.3)	0.9 (0.2)	1.0 (0.4)	**0.012** *	**0.010** *	0.325	0.687
L-serine (SD), μM	128.7 (20.0)	124.0 (23.4)	130.2 (48.8)	0.719	0.778	0.764	0.984

*: $p<0.05$, * *: $p<0.01$

Significant values are highlighted by bold font.

APS, attenuated psychotic symptoms; FEP, first episode psychosis; Glu, glutamic acid; Cys, cysteine; γ-GluCys, γ-glutamylcysteine; GSH, glutashione; Gly, glycine.

symptoms evaluated by scores in SOPS and its subscales, BDI-II, GAF, CGIC, WHO-QOL26, and SCoRS Interviewer's Global Rating, and with the duration of untreated illness (Table 3). There was no correlation between the metabolite concentrations and the age of the participant. Similarly, we did not detect any relationship between the concentrations of the three examined metabolites Glu, GSH, and D-serine and the clinical symptoms and characteristics.

Discussion

Regarding the serum analysis of endogenous compounds, we recently reported a significant decrease in serum L-lactate levels in individuals with ARMS.[34] However, to the best of our knowledge, this is the first investigation that reports serum levels of metabolites related to the NMDA receptor in individuals with ARMS.

The serum levels of GSH in individuals with APS and those with FEP were significantly lower than those in

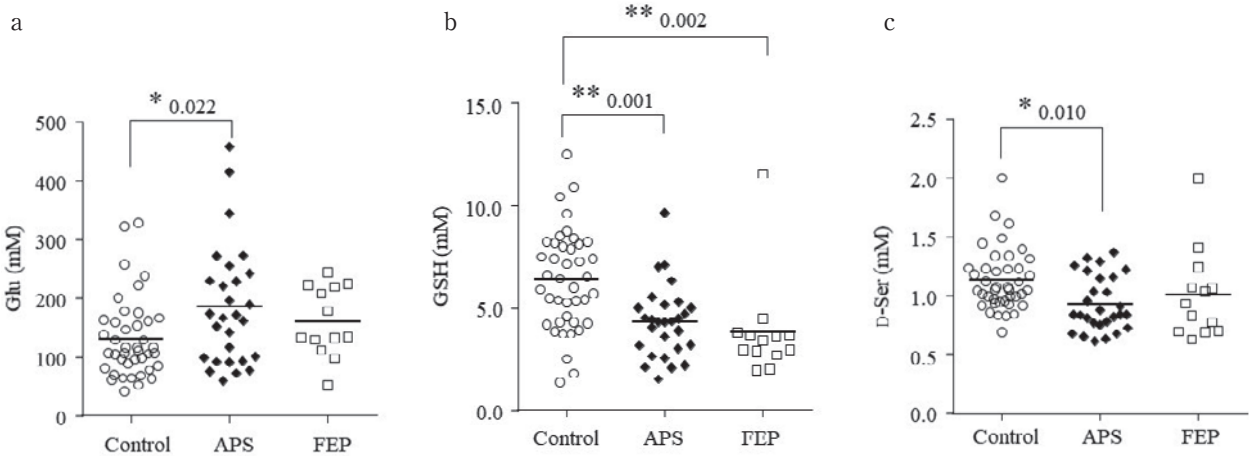

Fig. 1 Comparison of serum levels of metabolites related to the NMDA receptor among the three study groups.
(a) The level of glutamic acid (Glu) in the APS group is significantly higher than that in healthy controls. (b) The glutathione (GSH) levels in the APS and FEP groups are significantly decreased compared with healthy controls. (c) The level of D-serine in the APS group is significantly lower than that in healthy controls.
*: $p<0.05$, **: $p<0.01$
Quantitative variables were compared using the one-way analysis of variance (ANOVA).
The y-axis represents the metabolite concentration. Bars indicate mean values.

Table 3 Correlations of serum levels of metabolites related to the NMDA receptor and clinical symptoms in individuals with APS

	Glu	GSH	D-ser
Age	0.034	0.002	−0.04
SOPS total	−0.17	−0.14	−0.19
Positive subscale	0.12	−0.09	−0.01
Negative subscale	−0.20	−0.09	−0.26
Disorganaized subscale	−0.22	−0.17	−0.29
General subscale	−0.06	−0.17	−0.16
BDI-II	0.35	−0.32	0.19
GAF	−0.06	−0.04	0.27
CGIC	0.07	−0.16	−0.09
WHO-QOL26	−0.21	0.20	−0.24
SCoRS Interviewer's Global Rating	−0.36	−0.05	−0.26
DUI	−0.11	−0.08	−0.07

There was no correlation between clinical scores and serum levels of metabolites related to the NMDA-receptor in individuals with APS.
SOPS, the Scale of Psychosis-Risk Symptoms Positive and Negative Syndrome Scale; BDI-II, Beck Depression Inventory II; CGI, Clinical Global Impression; GAF, Global Assessment of Functioning; WHO-QOL26, World Health Organization Quality of Life 26; SCoRS, Schizophrenia Cognition Rating Scale; DUI, duration of untreated illness.

healthy controls. Decreased GSH levels in the serum of individuals with schizophrenia have already been reported by several groups.[11,35] Moreover, it has been demonstrated that GSH can bind to and stimulate NMDA receptors.[9] In the view of the glutamate hypothesis, decreased GSH levels may, thus, be related to the NMDA hypofunction. Therefore, our results suggest that NMDA hypofunction could occur in APS as well as FEP. Although the Glu levels in individuals with APS were increased, there were no significant differences in Glu levels between control and individuals with FEP. We propose that Glu levels in individuals with APS could be increased to compensate for their

NMDA hypofunction in contrast to participants with FEP. This suggests that increased Glu levels may be a crucial factor to distinguish between APS and FEP individuals. However, further longitudinal studies are needed to elucidate the mechanisms of how this alteration in APS causes the transition to schizophrenia.

The D-serine levels were significantly lower in individuals with APS compared with those in healthy controls. Although decreased D-serine levels in the serum or plasma of individuals with schizophrenia have been reported previously by Calcia et al.[36] and our group,[11] we detected similarly decreased D-serine levels in individuals with APS. *In vivo*, D-serine is biosynthesized by serine racemase (EC.5.1.1.18) and degraded by D-amino acid oxidase (DAO; EC.1.4.3.3).[37] Previously, Madeira et al.[38] reported increased DAO activity in the brain of individuals with schizophrenia. The results of the present study suggest that individuals with APS may exert increased DAO activity.

According to our results in individuals with APS, there was no correlation between their clinical scores and their serum levels of metabolites associated with NMDA receptor activation. We suggest that the reason for the lack of such relationships may be the relative attenuation of symptoms and cognitive dysfunction in APS compared with those in schizophrenia.

In the present study, however, several limitations should be mentioned. First, our sample size was comparatively small, and larger sample sizes are required to confirm the results of the present study. However, these data are valuable. It is also difficult to collect a large number of data from antipsychotic-naïve individuals with APS and FEP. Second, this study examined only serum levels of metabolites. It is possible that the metabolite levels in the peripheral blood samples may not have sufficiently reflected the status of these metabolites in the brain tissue of the study participants. However, some studies reported that the serum levels of some metabolites are correlated with their corresponding cerebral levels.[39] Third, other potentially confounding factors, such as the menstrual cycle phase, daily food consumption, and drinking, which could have affected the metabolite levels, were not taken into consideration in our analysis because of the lack of this information. Fourth, we should evaluate disease control to confirm specificity. In the next study, we will recruit other mental disorder groups such as depression. Last, there were significant differences in sex among the three study groups. A previous study found no sex differences in metabolite concentrations.[40] However, the sample size of this study was comparatively small, and larger studies are required to clarify the influence of sex differences on peripheral metabolite concentrations.

In conclusion, the present study indicated that abnormal levels of metabolites related to the NMDA receptor occurred in individuals with APS. Future longitudinal studies should investigate the relationship between the identified candidate metabolites and the clinical course of individuals with APS who transition to schizophrenia, and multicenter studies are needed to confirm our biomarker results.

Disclaimer: Masafumi Mizuno is one of the Editors of Toho Journal of Medicine. He was not involved in the editorial evaluation or decision to accept this article for publication at all.

Acknowledgements: The authors thank all participants for providing the blood samples for this study. The authors thank T. Funatogawa, T. Yamaguchi, N. Katagiri for their help to design this study. The authors thank I. Kimura for her analysis of metabolites. The authors thank T. Sakamoto, R. Onuma, S. Furukawa, K. Kobata, A. Uta, and Y. Morita for their technical assistance in the HPLC measurements.

This work was financially supported by the Toho University Joint Research Fund (2015-2016), Inokashira Hospital Grants for Psychiatry Research (2017), and a Japan Society for the Promotion of Science (JSPS) Grant-in-Aid for Scientific Research (C) (grant number 16K 08944).

Conflicts of interest: None declared.

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