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## Glutamate concentration in the serum of patients with schizophrenia

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### Abstract

Glutamate is the major neurotransmitter with multiple functions in the central nervous system. Glutamate-mediated excitotoxicity is involved in the pathophysiological processes in schizophrenia. The purpose of this study was to determine the concentration of glutamate in the serum of patients with paranoid schizophrenia compared with healthy individuals, and depending on the duration of the schizophrenic process and leading clinical symptoms. We investigated the level of glutamate in the serum of 158 patients with paranoid schizophrenia and 94 healthy persons. Higher concentrations of glutamate in schizophrenic patients compared with healthy persons have been found. The maximum concentrations of glutamate were detected in patients with disease duration of more than ten years. Glutamate level in the serum does not depend on the prevailing negative or positive clinical symptoms. The increased concentration of glutamate can hypothetically contribute to dopaminergic and glutamatergic imbalance, leading to the development of psychotic symptoms and cognitive dysfunction.

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## 1. Introduction

Schizophrenia is a chronic mental illness characterized by psychotic or positive symptoms (hallucinations and delusions), and negative and cognitive symptoms (apathy, social withdrawal, decreased attention, decreased executive function, abnormal psychomotor speed of processing and impairment of verbal memory)<sup>1</sup>. Despite treatment advances, schizophrenia remains as a seriously disabling, lifelong illness that is among the world's top ten causes of long-term disability<sup>2</sup>.

Difficulties in designing new and effective methods of diagnosis, treatment and prevention of mental illness are associated with a lack of understanding of the molecular mechanisms of processes involved in the development of the mental component of these disorders. According to the literature review most studies for the past decades have been conducted within the various individual hypotheses of schizophrenia pathogenesis. The neurotransmitter dopamine hypothesis of schizophrenia is of most current interest<sup>3,4,5</sup>. The dopamine hypothesis is based on the discovery that drugs that inhibit dopaminergic transmission eliminate symptoms of acute psychosis, agitation, anxiety and hallucinations. Dopamine was first discovered neurotransmitter involved in the mechanism of the disease. So far, primarily the dopamine D2-receptors, which are the most widespread dopamine receptors in the brain and are synthesized in a larger amount than other receptors were supposed to be involved in the pathogenesis of schizophrenia<sup>5</sup>.

Although the dopamine hypothesis has been a useful model in our understanding and study of the psychotic state, it does not explain the deteriorating course in terms of cognition and function seen in the first few years of schizophrenia. Glutamate antagonists are well known to induce positive and negative psychotic symptoms more akin to schizophrenia than the positive symptoms induced by dopamine agonists alone<sup>6,7</sup>. It has been proposed that this deterioration course may be partially explained by cortical neuronal toxicity secondary to enhanced glutamate exposure, which in turn is thought to reflect a compensatory increase in cortical glutamatergic activity due to hypofunction of the N-methyl-D-aspartate (NMDA) receptor<sup>8</sup>.

Glutamate is the most widely distributed excitatory neurotransmitter in the brain and also acts as an intermediate in cerebral energy metabolism<sup>9</sup>. The glutamatergic hypothesis of schizophrenia is currently well recognized, claiming a crucial role of the glutamate system in the genesis of schizophrenia<sup>8</sup>, although presently the underlying abnormalities are not fully understood. The glutamatergic abnormalities have been linked to a possible excitatory/inhibitory imbalance related to N-methyl-d-aspartate (NMDA) and metabotropic receptors<sup>9,10</sup>.

Recent studies confirm the important role of glutamate in the pathophysiology and treatment of schizophrenia<sup>7,11,12</sup>. However, the published data on the serum concentration of this amino acid in schizophrenia are ambiguous and controversial<sup>8,13,14</sup>. Inconsistency of results is due to the lack of comprehensive analysis of complexity of this multifactorial disease. An important component of this analysis is estimation of the influence of the disease duration, leading negative and positive symptoms, pharmacotherapy on the concentration of glutamate. So, the objective of this study was investigation the glutamate concentration in schizophrenic patients, depending on the duration of the diseases and leading negative and positive symptoms.

## 2. Materials and Methods

### 2.1. Clinical Samples

Informed consent was obtained from each subject after explanation of the study after approval of the study protocol by the institutional bioethics committee. Subjects were included from two psychiatric departments (for permanent and temporal hospitalization) of the Mental Health Research Institute in Tomsk and Kemerovo Region Psychiatric Hospital.

We included subjects with informed consent and clinical diagnosis of schizophrenia (ICD-10: F20), and excluded subjects with non-Caucasian physical appearance (e.g., Mongoloid, Buryats, or Khakassians), subjects with clinically-relevant withdrawal symptoms, and those with organic disorders. Clinical and demographic data were extracted from patients' medical files. In total, 158 Russian Caucasian patients met the inclusion criteria aged from 18 to 60 years (average age  $37.26 \pm 12.64$  years). Duration of disease is  $13.37 \pm 11.08$  years.

### 2.2. Medication

On the day of assessment and taking blood samples, a complete documentation of the medications used was compiled by the raters. As a psychotropic drug therapy, patients received medications as a monotherapy or combinations thereof: traditional antipsychotics (haloperidol, chlorprothixene, etc.), or atypical antipsychotics. The dose of the antipsychotic medication was converted into chlorpromazine equivalents (CPZEQ), according to the literature<sup>15,16</sup>.

### 2.3. Laboratory examination

The control group consisted of 94 physically and mentally healthy persons corresponding to the examined patients in terms of sex and age.

Blood was taken from the cubital vein of the examined fasted persons in the morning. Glutamate concentration in serum was measured spectrophotometrically on the instrument Epoch (USA) using reagent kits Glutamate Assay Kitt (BioVision, USA). Discoloration of the solution is recorded by a spectrophotometer at  $\lambda = 450$  nm. Colouring is proportional to the amount of glutamate in the sample. Concentration of amino acids in the examined samples is calculated on the basis of the calibration curve after measuring the optical density of the solution in the wells (nmol /  $\mu$ l).

### 2.4. Statistical Analysis

Statistical Analysis has been done using statistical software SPSS 17.0 for Windows. Samples were tested for normality by the Shapiro-Wilk test. The significance of differences has been determined by Student t-test for independent samples with normal distribution; the mean and standard deviation have been calculated. When the distribution of independent samples was different from the normal, the significance of differences has been determined by U-Mann-Whitney test; the median and quartiles have been calculated.

## 3. Results and Discussion

All of the subjects, except 2, were using antipsychotics and 35 subjects (24%) were utilizing depot antipsychotics on the day of assessment. Table 1 presents the main demographic and clinical features of the studied population.

Table 1. Basic demographic data presented as sample mean  $\pm$  standard deviation or as the number (*n*) and the percentage (%).

Characteristics	Schizophrenic patients n=158	Male n=111	Female n=47
Age, years	37.26 $\pm$ 12.64	34.48 $\pm$ 11.44	43.79 $\pm$ 13.05*
Age of the beginning of illness, years	23.87 $\pm$ 9.66	23.59 $\pm$ 8.14	25.15 $\pm$ 11.22
Duration of illness, years	13.37 $\pm$ 11.08	11.44 $\pm$ 8.11	18.64 $\pm$ 14.86*
Daily dose of antipsychotics (chlorpromazine equivalents)	971.8 $\pm$ 848.6	962.8 $\pm$ 827.0	986.6 $\pm$ 894.3
Subjects using atypical antipsychotics, n (%)	51(34.9)	34(37.4)	17(30.9)
Leading positive symptoms, n (%)	92 (58.3)	64 (57.7)	28 (59.6)
Leading negative symptoms, n (%)	66 (41.7)	47 (42.3)	19 (40.4)

\*  $p < 0.0001$  – significant difference between male and female.

Serum glutamate in schizophrenic patients was significantly higher ( $21.35 \pm 5.5$  nmol/ $\mu$ l) compared to the healthy control group ( $13.69 \pm 5.25$  nmol/ $\mu$ l;  $p < 0.001$ ) (Fig. 1). Analysis of association of glutamate levels in the serum of schizophrenic patients with the leading positive or negative clinical symptoms showed no significant differences ( $21.28 \pm 5.34$  nmol/ $\mu$ l;  $22.55 \pm 4.67$  nmol/ $\mu$ l; respectively). The maximum concentrations of glutamate were detected in patients with disease duration of more than ten years (Fig. 2).

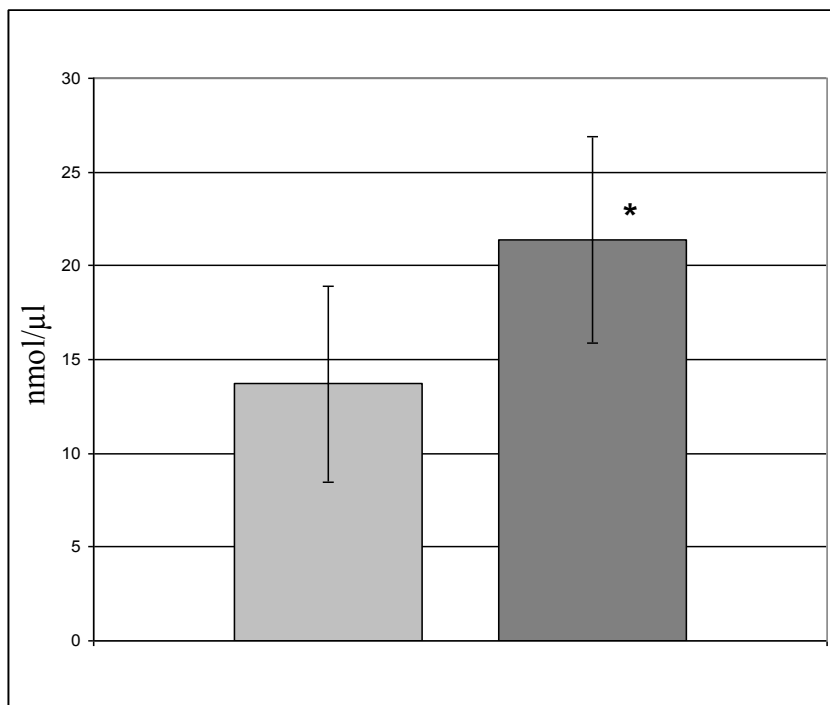


Fig. 1. Glutamate concentration in the serum of healthy controls and patients with schizophrenia (\*  $p < 0.0001$  – significant difference between schizophrenic patients and healthy controls)

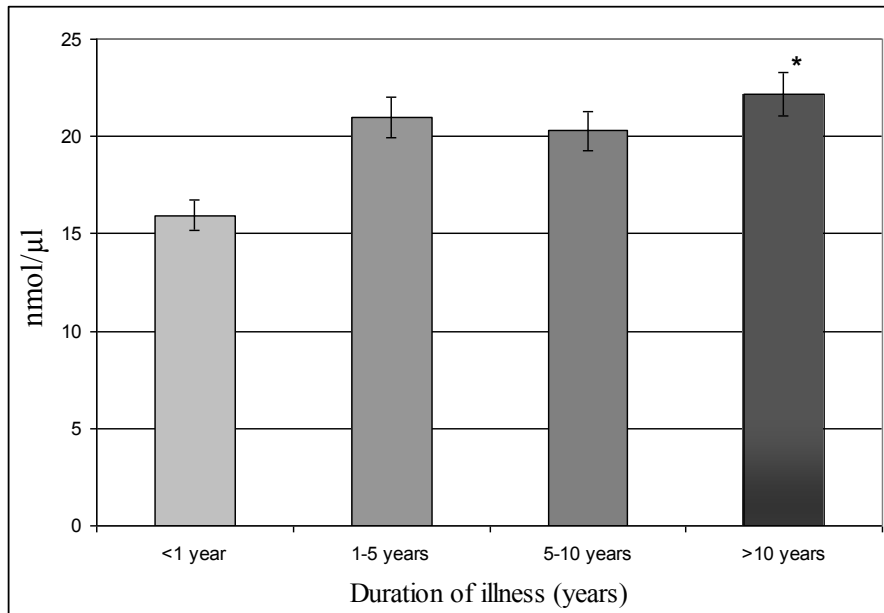


Fig. 2. Glutamate concentration in the serum of patients with schizophrenia depending on the duration of the schizophrenic process (\*  $p < 0.05$  - significant difference between schizophrenic patients with disease duration of more than 10 years and less than one year)

The interaction between glutamate and dopamine is widely documented<sup>3</sup>. In schizophrenia, dopaminergic dysregulation is thought to be the final common pathway resulting from an altered glutamatergic neurotransmission. Disruption of the cortical glutamatergic afferents induce decreased tonic dopamine release with a subsequent disinhibition of phasic dopamine release, causing abnormal responses to insignificant stimuli<sup>6</sup>.

Glutamate concentration changes in schizophrenia could be caused by various factors, in particular, by deranged kinase signaling pathways. In light of the evidence of association between the mutation (N251S)-PIP5K2A and schizophrenia, we carried out comprehensive studies of functional regulation of neuronal PIP5K2A kinase and its mutants (N251S)-PIP5K2A, associated with schizophrenia, neuronal KCNQ potassium channels responsible for the stability of the resting potential of neurons and EAAT3 glutamate transporters preventing neurotoxic effect<sup>17</sup>. The new fundamental data demonstrated in *Xenopus laevis* oocyte expression system and in cultured human HEK293 cells that PIP5K2A is a signaling element in the regulation of neuronal glutamate EAAT3 transporters have been obtained. Comparative study of functional regulation PIP5K2A and its mutant form (N251S)-PIP5K2A, associated with schizophrenia revealed a stimulating effect of PIP5K2A kinase on the neuronal EAAT3 glutamate transporters work in *Xenopus laevis* oocyte expression system and impairment of this function in mutant form (N251S)-PIP5K2A. The modulation of protein expression of neuronal glutamate transporters EAAT3 by PIP5K2A kinase and its mutant form (N251S)-PIP5K2A, associated with schizophrenia, on *Xenopus laevis* oocytes membrane and in cultured human HEK293 cells has been detected for the first time. It was shown that diphosphorylated phosphoinositide PI(4,5)P<sub>2</sub> stimulates electrogenic glutamate transport in EAAT3 expressing *Xenopus laevis* oocytes. Effects of PIP<sub>2</sub> and PIP5K2A-coexpression EAAT3 activity are not cumulative.

Significant functional dysregulation of neuronal KCNQ potassium channels and glutamate EAAT3 transporters by mutant associated with schizophrenia (N251S)-PIP5K2A kinase, greatly explains the increase in mesencephalic dopamine action potential in schizophrenic patients (the carriers of this mutation), as well as a metabolic disorder of glutamate in the brain of patients, that leads to the development of psychotic symptoms. We confirmed the association of polymorphic gene variant (N251S)-PIP5K2A with schizophrenia which was shown earlier in several independent international studies<sup>18</sup>.

Imbalance in the functioning of the dopaminergic and glutamatergic systems is associated with the failure of protective neurosteroid and neurotrophic factors shown in schizophrenia and in the development of neuroleptic induced side effects<sup>19</sup>. Low concentrations of DHEAS found in patients with schizophrenia and especially during prolonged schizophrenic process, contribute to hypofunction of NMDA receptors and the development of cognitive dysfunction<sup>20</sup>.

Limiting factors of our study are the following. Firstly, according to the literature, the increased glutamatergic excitotoxicity has been directly detected in brain structures<sup>6</sup>. We determined the glutamate concentration in the serum of patients. Although the blood serum glutamate level does not correlate with the cerebral glutamate level, its concentration in serum can be a biomarker of number of processes<sup>13</sup>. Based on the results of longstanding detailed research of plasma glutamatergic amino acid levels Ohnuma and Arai<sup>11</sup> (the Juntendo University Schizophrenia Projects (JUSP), concluded that these amino acid levels may be diagnostic, therapeutic, or symptomatic biological markers. Peripheral blood levels of endogenous amino acids may reflect the degree/change in symptoms in schizophrenia and the status of cognitive functions in schizophrenia.

The next limiting factor is the fact of receiving prolonged antipsychotic therapy by patients. Antipsychotic therapy can significantly influence the level of peripheral glutamate. The paper of van der Heijden et al.<sup>14</sup> showed that glutamate at baseline was significantly higher in patients as compared to controls. During treatment, a significant further increase of glutamate, not related to response, was observed. Glutamate levels correlated significantly with negative symptom scores at baseline and weeks 3, 6 and 14. Tortorella et al.<sup>21</sup> demonstrated that 12 weeks of clozapine administration significantly reduced serum levels of glutamate but did not restore the values observed in normal controls, nor did it affect other amino acid concentrations. These data show changes in serum amino acids that may influence central serotonergic, dopaminergic and glutamatergic transmission in neuroleptic-resistant schizophrenics.

#### 4. Conclusion

Thus, our results are consistent with literature data and confirm the possibility of the application of glutamate

levels in the blood serum as a biomarker for schizophrenia. Increasing the concentration of glutamate hypothetically contributes to the deepening of the dopaminergic and glutamatergic imbalance, leading to the development of psychotic symptoms, the severity of which, in turn, is associated with cognitive dysfunction.

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