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Article *in* Journal of the Pakistan Medical Association · February 2015

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ORIGINAL ARTICLE

Prolidase activity and oxidative stress in patients with schizophrenia: A preliminary study

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Abstract

Objective: To determine whether serum prolidase levels are associated with the etiopathogenesis of schizophrenia.

Methods: The study was conducted at the psychiatry outpatient clinics of the University Hospitals of Recep Tayyip Erdogan and Afyonkocatepe in spring 2013. It comprised patients with schizophrenia who were consecutively recruited from the Psychiatry outpatient clinics of the hospital. An equal number of healthy individuals were recruited from the community. Each patient underwent a detailed diagnostic evaluation by psychiatry residents by using the Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders-IV. Serum prolidase activity and oxidative parameters were measured in patient and control groups. The severity of psychotic symptoms was assessed using the positive and negative syndrome scale. SPSS 16 was used for statitistical analysis.

Results: There were 30 subjects in each group, with 18(60%)females among the patients and 21(70%) among the controls. Serum prolidase level was significantly higher in schizophrenia patients compared to the controls ($p<0.001$). Total Oxidative Stress and Oxidative Stress Index parameters were found to be significantly different between the patients and the controls ($p=0.024$ and $p<o.001$). Serum prolidase level did not show any correlation with markers of oxidative stress in the patients.

Conclusion: Prolidase activity, glutamate transmission and oxidative stress may be inter-related in the etiopathogenesis of schizophrenia.

Keywords: Prolidase activity, Schizophrenia, Proline, Glutamat. (JPMA 65: 131; 2015)

Introduction

Schizophrenia is a severe mental disorder, which is characterised by thought disturbance, abnormal perception, impaired cognition and bizarre behaviour.[1] Though genetic and environmental factors have been known to contribute to the clinical phenotype, the exact aetiology and pathophysiology of schizophrenia have not been fully elucidated.[2]

Prolidase is an essential cytosolic enzyme that specifically splits imidodipeptides with C-terminal proline or hydroxyproline.[3] The enzyme is widely distributed in the body, including the plasma, brain, thymus, uterus and heart.[4] It is an important enzyme in proline nutrition and in the recycling of proline for protein synthesis.[5] Its main physiological activity is related to collagen synthesis and cell growth.[6] In addition, prolidase is thought to be involved in the regulation of various hormone-releasing factors and neurotransmitters in the brain.[7] Disruptions in proline metabolism were found to have an association with behavioural difficulties, mental retardation, autism spectrum disorder and schizophrenia.[8,9] Prolidase activity has been found to be distributed throughout regional and subcellular spheres of brain.[10] It was shown that maintenance of proline levels are regulated by prolidase in brain.[7]

Experimental studies indicate that there is interaction between proline and the N-methyl-D-aspartate (NMDA) receptor.[11,12] Proline may contribute to mental retardation and seizure by activating the NMDA receptors.[13] A study[14] suggested that extracellular proline regulates the basal function of some glutamate synapses. It has been known that glutamate is a major excitatory neurotransmitter, which is thought to be connected to schizophrenia.[15,16] Most studies reported that the dysfunction of glutamatergic neurotransmission may play an important role in the pathogenesis of schizophrenia. In particular, the hypofunction of NMDA receoptors is considered to be a key factor in schizophrenia.[17,18] Recently, increased serum levels of prolidase have been demonstrated to be associated with bipolar affective disorder[19] and Alzheimer's disorder,[20] which are also related to glutamatergic transmission.[21,22] Considerable evidence has been accumulated to indicate a correlation between

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oxidative stress (OS) and prolidase activity in clinical studies.[23,24] Therefore, we hypothesised that the prolidase activity may be impaired in patients with schizophrenia and there may be an association between prolidase activity and OS in these patients. We evaluated serum prolidase levels and oxidative parameters in patients with schizophrenia and healthy controls in order to determine whether the serum prolidase levels are associated with the etiopathogenesis of schizophrenia, and whether there is a relationship between prolidase activity and oxidative parameters in these patients.

Patients and Methods

The case-control study was conducted in spring 2013 after approval from the institutional ethics committee. The study comprised patients with schizophrenia who were consecutively recruited from the Psychiatry outpatient clinics of the hospital. An equal number of healthy individuals were recruited from the community. Informed written consent was obtained from all the subjects. All patients underwent a detailed diagnostic evaluation by psychiatry residents in accordance with the Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders (DSM)-IV (SCID-I) criteria.[25] The exlusion criteria included patients who had any other comorbid psychiatric disorder and those with a history of inadequate cardiac function, renal dysfunction, diabetes, liver disease and any cancer. The severity of psychotic symptoms was assessed using the positive and negative syndrome scale (PANSS), validity and reliability studies of whose Turkish version have already been conducted.[26]

The controls were matched for age and gender. The controls had no clinical psychiatric disorder. They had not taken any psychotropic drugs for at least two months prior to the study. Their psychiatric conditions were evaluated by the same psychiatrists in accordance with SCID Axis-I, and they were all free of Axis-I disorders. They had no past neurological, endocrinological, hepatic and renal diseases.

Serum prolidase activity was measured in the two groups. Venous blood samples were immediately centrifuged and stored at -20°C for analysis. Serum Xaa-pro dipeptidase/prolidase (PEPD) was measured using an enxyme-linked immunoabsorbent assay (ELISA) test kit (Human Xaa-Pro Dipeptidase/Prolidase (PEPD) ELISA Kit, Cusabio biotech)according to the manufacturer's procedure. This assay employed the quantitative sandwich enzyme immunoassay technique. Absorbance (OD) of each well was determined at 450nm by a microtiter plate reader (Multiskan GO, Thermo Scientific) within 5 minutes. Standard curves were fitted using Titri ELISA software. The fitted curve was then used to convert sample OD readings to PEPD concentrations.

Serum total anti-oxidant capacity (TAC) was measured using a novel automated measurement method developed by Erel.[27] This method involves the production of a potent biological hydroxyl radical. In the assay, ferrous ion solution (present in Reagent 1) is mixed with hydrogen peroxide (present in Reagent 2). Thus, it is possible to measure the anti-oxidative effect of the sample against the potent free radical reactions initiated by the production of the hydroxyl radical. The assay is characterised by excellent precision values of less than three percent. The results were expressed as mmolTroloxEq/L.

Total oxidant status (TOS) of serum was determined using a novel automated measurement method, developed by Erel.[28] Oxidants present in the sample oxidise the ferrous ion-o-dianisidine complex to ferric ion. The oxidation reaction is enhanced by glycerol molecules, which are abundantly present in the reaction medium. The ferric ion makes a coloured complex with xylenol orange in an acidic medium. The colour intensity, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules present in the sample. The assay was calibrated with hydrogen peroxide and the results were expressed in terms of micromolar hydrogen peroxide equivalent per liter(μmol H_2O_2Equiv/L).

Percent ratio of TOS level to TAC level was accepted as Oxidative stress index (OSI). For its calculation, the resulting unit of TAC was changed to mmol/L, and the OSI value was calculated according to the following formula: OSI (arbitrary unit)= TOS (mmol H_20_2 Equiv./L)/ TAC (mmolTroloxEq/L).[29]

Statistical analysis was performed using SPSS 16. Mean ages were compared by student's t test, Pearson's chi-square or linear by linear association tests was used to compare the gender, education, marital status and work status differences between the two groups. Normality assumptions of continuous variables were checked via Shapiro-Wilk's test. Normality was not assigned in TAS, TOS and OSI, therefore non-parametric Mann Whitney U test was applied. Student's t test was employed to compare prolidase values between schizophrenia patients and controls. Correlation analysis was performed by spearman test. Results were considered significant at $p<0.05$.

Results

There were 30 subjects in each group, with 18(60%) females among the patients and 21(70%) among the

Table-1: Demographic and clinical characteristics.

Parameters		Patients (n=30)	Controls (n=30)	p
Age (mean ± Standard Deviation)		27.3±5.4	28.8±5.4	0.220a
Gender	Female	18	21	0.417b
	Male	12	9	
Marital Status	Single	21	13	*0.018b
	Married	7	17	
Education	Primary school	13	10	0.095c
	High School	17	15	
	University degree	0	5	
Work Status	Employed	9	14	0.184b
	Unemployed	21	16	
PANSS positive score (mean±SD)		25.3±4.4	N/A	
PANSS negative scores (mean±SD)		25.7±4.3	N/A	
Duration of illness		5.7±2.7	N/A	

*p<0.05, a: Student t-Test, b: pearson chi-square, c: linear by linear association
N/A:Not applicable
PANSS: Positive and Negative Syndrome Scale.

Table-2: Serum TAS and TOS levels.

	Patients (n=30)	Controls (n=30)	p[a]
TAS (mmolTroloxEq/L)	0.8±0.2	1.1±0.3	*0.002
TOS (Immol H_2O_2 Eq./L)	26.8±5.5	22.9±3.8	*0.024
OSI	34.8±17.3	28.3±19.7	<0.001

*p<0.05, a: Mann Whitney U test
TAS: total antioxidant status
TOS: total oxidant status
OSI: Oxidative stress index

Table-3: Correlation analyses between serum prolidase levels and other parameters in the patient with schizophrenia.

	Prolidase levelr	p[a]
TAS	-0.22	0.240
TOS	-0.73	0.703
OSI	0.18	0.324

[a]:Spearman correlation test
TAS: Total antioxidant status
TOS: Total oxidant status
OSI: Oxidative stress index

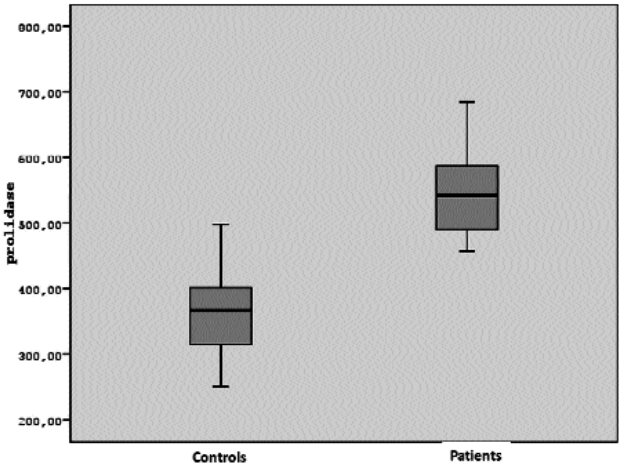

Figure: Prolidase activity in patients and controls (p<0.001) (Student's t-test).

controls. Likewise, there were 21(70%) unmarried/single individuals among the patients and 13(43.3%) among the controls. Demographic variables did not show any statistically significant difference between the groups except for the marital status (p=0.018) (Table-1). The mean serum prolidase activity levels in patients with schizophrenia and controls were 555.68±81.34 U/L and 364.08±58.2 U/L, respectively. Mean serum prolidase activity difference between the two groups was statistically significant (p<0.001) (Figure-1). TOS levels and OSI were significantly higher in patients with schizophrenia compared to controls (P=0.024 and <0.001, respectively). TAS was significantly lower in the patients group (p=0.002) (Table-2). The mean serum prolidase level did not show any correlation with TAS, TOS levels and OSI in patients (Table-3).

Discussion

As the results show, the study found that the levels of serum prolidase, TOS and OSI in patients were significantly higher than those in the controls. TAS levels were significantly decreased in the patients, and, finally, that serum prolidase levels were not correlated with TAS

and TOS levels and OSI.

There is evidence that proline plays a role as a neuromodulator in synaptic transmission.[30] A pioneering study performed in rats[31] observed that proline is normally present in cerebrospinal fluid (CSF) and inhibit glutamate release. Another study[32] found that elevated proline levels induce glutamatergic signalling in the hippocampus and increased glutamatergic tone causes release of dopamine in the prefrontal cortex. Chronic administration of proline in rats leads to a significant impairment of learning/memory.[33]

One study[34] observed that plasma proline level was negatively correlated with intelligence quotient (IQ) in patients with mental disorders. It has been recently hypothesised that prolidase deficiency may lead to mental retardation by the high amount of proline residues.[35] Studies[36,37] suggest that hyperprolinaemia may be associated with schizophrenia and schizoaffective disorders. Another[38] suggests that elevated proline is risk factor for schizophrenia. Our findings together with the earlier ones support the notion that disruptions in the proline pathway might have an association with schizophrenia.

Our study found that oxidative imbalance is present in patients with schizophrenia. This finding is consistent with previous studies.[39-41] Most studies showed that there is a relationship between OS and increased prolidase activity in some diseases such as ovarian cancer, mitral stenosis and helicobacter pylori infection.[42-44]

The assessment of prolidase activity in neuropsychiatric disorders has been so far limited to a few studies. One study[19] found high serum prolidase activities in patients with bipolar affective disorder compared with controls, and suggested that it may be associated with OS. Another one[20] found increased serum prolidase activities and lower total antioxidant levels in patients with Alzheimer's disease compared to healthy controls. Based on these results, researchers noted that OS may be the reason behind elevated prolidase levels.

Our study found that there was no correlation between the oxidative parameters and prolidase activity. This could mean that there is no direct association between OS and prolidase activity in schizophrenia. Taking these findings into account, increased prolidase activity in patients with schizophrenia may be due to the relevance between OS, proline metabolism and glutamate transmission. In schizophrenia, it was demonstrated that there was glutamate exitotoxicity-induced OS.[45,46] A study[8] reported that proline may also decrease glutamate uptake in presynaptic neurons, causing exitotoxic cell death by overstimulation of NMDA. A study[47] suggested that the induction of OS may occurr secondary to NMDA receptor stimulation by proline in the brain. It seems that more research is needed to clarify the detail of the mechanism.

In terms of limitations, our sample size was small. Besides, we assessed only one time point for the measurement of prolidase levels. Replication with large samples and longitudinal follow-up will be needed to overcome the limitation. Finally, we did not study plasma proline concentrations.

Conclusion

Serum prolidase level was significantly higher in schizophrenia patients compared to the healthy subjects in our study. This finding, together with those of previous studies, implies that prolidase activity, glutamate transmission and OS may be inter-related in the etiopathogenesis of schizophrenia.

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