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PROLIDASE ACTIVITY AND OXIDATIVE STRESS IN PATIENTS WITH MAJOR DEPRESSIVE DISORDER

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SUMMARY

Background: *The aim of the current study was to determine whether the serum prolidase levels are associated with the etiopathogenesis of depression.*

Subjects and methods: *This study included 29 patients with major depressive disorder (MDD), who were consecutively recruited from the psychiatric outpatient clinic, and 30 healthy individuals recruited from the general community. Each patient underwent a detailed diagnostic evaluation by two psychiatrists using the Structured Clinical Interview for DSM-IV (SCID-I). Serum prolidase activity and oxidative parameters were measured in the patient and control groups. The severity of depressive symptoms was assessed using the Hamilton Depression Rating Scale.*

Results: *Serum prolidase level was significantly higher in patients with MDD compared to healthy subjects ($p<0.001$). Total Oxidant Status (TOS) levels and Oxidative Stress Index (OSI) were also significantly higher in patients with MDD ($p<0.001$), whereas no significant difference was observed between the groups in the TAS levels ($p=0.297$). Serum prolidase level did not show any correlation with markers of oxidative stress in patients with MDD.*

Conclusion: *Increased serum prolidase levels in patients with MDD may be interpreted as the interaction of prolidase activity, glutamate transmission and oxidative stress. It is suggested that prolidase activity is involved in the etiopathogenesis of depressive disorder.*

Key words: *prolidase activity - oxidative stress - major depressive disorder - MDD - proline*

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INTRODUCTION

Prolidase or prolinedipeptidase is one of the unique enzymes capable of degrading dipeptides in which a proline or hydroxyproline reduce is located at the C-terminal position (Endo & Matsuda 1991). Prolidase has been described in various tissues, including the plasma, hearth, thymus, brain and uterus (Zanaboni et al. 1994). It was previously regarded as the collagen synthesis and cell growth (Wu et al. 2011), but now it has been recognized for various hormone-releasing factors and neurotransmitters in the brain (Chi et al. 2009). Proline is abundant in the brain (Yu et al. 2009). Disruption in proline metabolism was found to be associated with attention deficit, mental retardation, autism spectrum disorder and schizophrenia (Mitsubuchi et al. 2008, Wyse & Netto 2011). Prolidase has also been detected in brain (Hui & Lajtha 1978) and it has been shown that the maintenance of proline levels is regulated by prolidase (Sayre et al. 2001). Prolidase deficiency is related to different degrees of mental retardation (Lupi et al. 2008).

Biological factors undoubtedly play a role in the etiology of major depressive disorder (MDD) (Maric & Adzic 2013, Muller 2013). Glutamatergic transmissions have been implicated in the pathophysiology of depression (Gibbon et al. 2012). An interaction between proline and glutamate has been demonstrated in animal models (Henzi et al. 1992, Ortiz et al. 1992) and it has been suggested that proline-induced glutamate excitotoxicity may be of clinical significance in psychiatric disorders (Wyse & Netto 2011).

Elevated serum levels of prolidase have been associated with oxidative stress in some organic diseases such as mitral stenosis, helicobacter pylori infection and ovarian cancer (Rabus et al. 2008, Aslan et al. 2007, Camuzoglu et al. 2009). We hypothesize that the prolidase activity may be impaired in patients with Major Depressive Disorder (MDD) and there may be an association between prolidase activity and oxidative stress in depression. To the best of our knowledge, this is the first research to investigate the relationship between prolidase activity and depression. The aim of this study was to determine whether the serum prolidase levels are associated with the etiopathogenesis of depression, and whether there is a relationship between prolidase activity and oxidative parameters in patients with MDD.

SUBJECTS AND METHODS

Patients

Twenty-nine (29) patients with (MDD), who were consecutively recruited from psychiatry outpatient clinics at the university hospital, were included in this study. Diagnoses of MDD were made by using the Structured Clinical Interview for DSM-IV (SCID-I). The severity of depressive symptoms was assessed using the Hamilton Depression Rating Scale, whose validity and reliability were demonstrated by Akdemir et al. (2001). Inclusion criteria were age between 18 and 65 years and being medication-free for at least 30 days prior to blood sampling. The exclusion criteria included patients who had any other comorbid psychiatric disorder according to DSM-IV and those with a history of inadequate cardiac function, renal dysfunction, diabetes, liver disease, and cancer.

Controls

The control group consisted of 30 healthy individuals recruited from the community. They were matched in terms of age and gender with the patients. They had no clinical psychiatric disorder and had not taken any drug for at least one month prior to the study. Their psychiatric conditions were evaluated by the same psychiatrists using the SCID-I. All volunteers were free of Axis-I disorders. They had no past neurological, endocrinological, hepatic and renal diseases. All participating subjects gave their informed consent. The study was approved by the local ethics committee.

Prolidase Assay

Serum prolidase activity was measured in patient and control groups. Venous blood samples were immediately centrifuged and stored at -20°C for further analysis. Serum Xaa-prodipeptidase/prolidase (PEPD) was measured using ELISA (enzyme-linked immunosorbent assay) test kit (Human Xaa-Pro Dipeptidase/ Prolidase (PEPD) ELISA Kit, Cusabio biotech) according to the manufacturer's protocol. This assay employs the quantitative sandwich enzyme immunoassay technique. Absorbance (OD) of each sample well determined at 450 nm with a microliter 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 absorbance readings to PEPD concentrations.

Measurement of total antioxidant capacity (TAC)

Serum TAC was measured using a novel automated measurement method developed by Erel (2004). 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 characterized by excellent precision values of less than three percent. The results are expressed as mmol Trolox Eq/L.

Measurement of total oxidant status (TOS)

TOS of serum was determined using a novel automated measurement method, developed by Erel (Erel 2005). Oxidants present in the sample oxidize 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 colored complex with xylene orange in an acidic medium. The color intensity, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules present in the sample. The assay is calibrated with hydrogen peroxide and the results are expressed in terms of micromolar hydrogen peroxide equivalent per liter (μmol H_2O_2 Equiv/L).

Oxidative stress index (OSI)

Percent ratio of TOS level to TAC level was accepted as 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_2O_2 Equiv./L)/ TAC (mmol Trolox Eq/L) (Harma et al. 2006).

Statistical analysis

SPSS® for Windows 15.0 software was used for the statistical analysis of the data. The significance of differences between the groups was estimated by the t-test. Chi-square test was used to compare the proportions. Correlation analysis was performed using the Pearson correlation coefficient. Results were considered significant at $p<0.05$.

RESULTS

Mean prolidaseactivity of MDD was 596.4093± 129.934 U/L in the patients and 371.4461±52.792 U/L in the controls (Table 1). Prolidase activity was significantly higher in patients ($p<0.001$). Socio-demographic variables did not show any statistically significant difference between the two groups. TOS levels and OSI levels were significantly higher in patients with MDD compared to controls ($p<0.001$). TAS levels showed no significant difference between the groups ($p=0.297$) (Table 2). The mean serum prolidase level did not show any correlation with TAS, TOS levels and OSI among the patients with MDD (Table 3).

Table 1. Demographic and clinical characteristics of patients with MDD and control subjects

Parameters		Patients (n=30)	Controls (n=30)	p
Age (mean±SD)		30.9±6.3	29.03±5.7	0.290
Gender	Female	20	16	0.417
	Male	10	14	
Marital Status	Single	15	9	0.114
	Married	15	21	
Education	Primary school	3	7	0.063
	High School	19	16	
	University degree	7	7	
HAM-D score (mean±SD)		16.4±2.6	N/A	
Duration of illness		1.6±1.0	N/A	

*p<0.05; N/A: Not applicable; HAM-D: Hamilton Depression Rating Scale

Table2. Serum total antioxidant status (TAS), total oxidant status (TOS) levels and OSI levels in patients with MDD and healthy control group

	Patients (n=30)	Controls (n=30)	p
TAS (mmolTroloxEq/L)	1.2±0.2	1.2±0.1	0.305
TOS (lmmol H_2O_2 Eq/L)	13.8±7.4	9.9±4.7	<0.001
OSI	11.8±7.4	7.8±3.6	<0.001

TAS: total antioxidant status; TOS: total oxidant status; OSI: Oxidative stress index

Table3. Correlation analysis between the serum prolidase levels and other parameters in patients with MDD

	Prolidase level r	p
TAS	0.228	0.226
TOS	-0.043	0.820
OSI	-0.131	0.490

TAS: total antioxidant status, TOS: total oxidant status, OSI: Oxidative stress index

DISCUSSION

The main finding of our study was the higher serum prolidase level in patients with MDD than healthy control subjects. Proline is widely distributed in the central nervous system (CNS) (Hauptmann et al. 1983), suggesting that proline may serve as a neuromodulator in synaptic transmission (Crump et al. 1999). Glutamate is believed to be involved in the etiology of depression (Palucha et al. 2005). Experimental studies indicate that there is an interaction between proline and glutamate receptors (Ortiz et al. 1992). Proline was shown to inhibit glutamate release in the cerebrospinal fluid and to induce glutamatergic signaling in the hippocampus (Cohen & Nadler 1997a,b). Delwing et al. (2007) suggest that high proline may be neurotoxic and predispose to brain damage by the decrease glutamate uptake. It was reported that prolidase deficiency may lead to mental retardation by the high amount of proline residues (Lupi et al. 2008).

Oxidative stress has been postulated to have an important role in the pathogenesis of depression (Michel et al. 2012). Depressed patients in this study had a significant increase of OSI level compared to control subjects. The index shows the oxidative imbalance in depressive patients. This finding is consistent with previous results (Yanik et al. 2004, Cumurcu et al. 2009) reporting that oxidative metabolism is impaired in patients with MDD. However, oxidative stress in patients was not correlated with the prolidase activity. In some previous studies, it was found that there is an association between oxidative stress and prolidase activity in other diseases such as helicobacter pylori infection and Alzheimer's disease (Aslan et al. 2007, Arikanoglu et al. 2013).

The assessment of prolidase activity in neuropsychiatric disorders has so far been limited to only two studies. Selek et al. (2011) investigated serum prolidase levels in patients with bipolar affective disorder and found that serum prolidase level was significantly higher in patients than controls, suggesting that increased prolidase activity may result from oxidative stress. Our findings support the idea that prolidase activity may play a role in psychiatric disorders. In a study conducted on patients with Alzheimer's disease, increased serum prolidase activities were reported and the authors suggested that oxidative stress may be the main reason for this finding (Arikanoglu et al. 2013). Increase in the serum prolidase level could be explained in two different ways. First, possible reason of elevated prolidase levels may be the altered activity of glutamate-proline pathway in depression. Second, oxidative stress may be an indirect effect of the level of prolidase activity. Indeed, it was reported that proline can induce oxidative stress in the brain (Delwing et al. 2013). Further studies are required to clarify the role of prolidase in the pathophysiology of depression.

There were several limitations in our study. First, our sample size was rather small. Second, 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 these limitations.

CONCLUSION

In this study it was shown that serum prolidase levels were significantly higher in patients with major depression compared to control subjects. These levels were not associated with oxidative stress parameters, which may be interpreted as an interaction of prolidase activity, glutamate transmission, and oxidative stress. It is suggested that prolidase activity has a significant role in the etiopathogenesis of depression.

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Conflict of interest: None to declare.

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