Original Research Article

Association of Major Depression with Serum Prolidase activity and Oxidative Stress

Abstract

Aim: Stress is a major causative factor for the progression of major depressive disorder (MDD). The present study aimed to know the association of serum prolidase activity (SPA) and oxidative stress with the progression of MDD.

Place and Duration of Study: The study was carried out at the Department of Biochemistry, Sir Sunder Lal Hospital, Institute of Medical Sciences (IMS), Banaras Hindu University (BHU), Varanasi, Uttar Pradesh, India. The duration of study was September-2011 to August-2016.

Methodology: 80 patients with MDD and 80 healthy controls of matched age and genders were selected. Serums SPA, total oxidant status (TOS), oxidative stress index (OSI) and total antioxidant status (TAS) were measured spectrophotometrically.

Results: Increased SPA, TOS, and OSI were observed in patients with MDD than healthy controls (all $P < 0.001$). However, TAS was significantly decreased ($P < 0.001$). SPA, TOS and OSI were also increased in patients with > 1 years of MDD than patients with ≤ 1 years of MDD. Positive, linear and significant correlations were observed between duration of MDD and SPA, and TOS, and OSI (all $P < 0.001$). However, negative, linear and significant correlation was observed between duration of MDD and TAS ($P < 0.001$).

Conclusions: The study concluded that SPA and oxidative stress have been significantly increased in the patients with MDD than healthy individuals. Increased SPA and oxidative stress have been significantly correlated to progression of MDD and may be responsible for its pathogenesis.

Key Words: Major Depressive Disorder, Oxidative Stress, Serum Prolidase Activity, Total Oxidant Status, Total Antioxidant Status, Oxidative Stress Index

1. Introduction

Major Depressive disorder (MDD) is a chronic most commonly as well as frequently occurring serious disorder that negatively affects the quality of life. It can alter the morbidity as well as mortality [1]. Including suicide, it is associated with an overall 50% increase in the risk of morbidity [2, 3]. The interaction of both genetic and environmental factor can play a role in the development of depression [4]. Gender, age, socioeconomic status, stressful life events, childhood adversity, and co-morbidities or medical childbirth are counted as risk factor for depression [5].

Prolidase is a cytosolic manganese-dependent exopeptidase which cleaves dipeptides with proline or hydroxy-proline at carboxy- terminal end [6]. Proline or hydroxy-proline is an end product of prolidase that participates in collagen metabolism, cell growth and protein synthesis. It involves in deactivations of neuropeptides and can influence the biological as well as conformational properties of neuropeptides [6-8].

Oxidative stress is a condition of imbalances between oxidants and antioxidants [9]. Numbers of evidences are supporting the involvement of oxidative and nitrosative stress in the pathophysiology of MDD [10]. Altered status of both oxidants and antioxidants [(which includes reactive oxygen species (peroxide), reactive nitrogen species (NO), glutathione, vitamin E, zinc, coenzyme Q10, manganese superoxide dismutase and catalase)] have reported in the patients with MDD [10, 11]. Several of our previous studies are suggesting that altered status of prolidase activity have correlated to altered status of oxidative stress in different diseases such as non-ulcer dyspepsia, diabetes, diabetic nephropathy, end stage renal disease and Parkinson's disease [6, 8 and 12]. In our previous study, we have been observed altered status of malondialdehyde, nitrite, ceruloplasmin, ascorbic acid and superoxide dismutase in patients with MDD [11]. Thus in the continuation of our previous study, present

study aimed with an explorative study on the association of serum prolidase activity, TOS, TAS, and OSI in the patients with MDD.

2. Materials and Methods

The study was conducted in the Department of Biochemistry in the association of Department of Psychiatry, Sir Sunder Lal Hospital, Institute of Medical Sciences (IMS), Banaras Hindu University (BHU), Varanasi, India from the period of September-2011 to August-2016. The study was approved by the ethical committee of the IMS, BHU. Written and signed consent form was taken from every studied subject.

A total 80 drug naive and fresh cases of major depressive disorder were included in the study. All the cases were selected for the study from the out-patient department (OPD) of Psychiatry, Sir Sunder Lal hospital, IMS, BHU, Varanasi. All of them belonged to the population of Uttar Pradesh and Bihar state of North India. Cases were diagnosed as per the DSM IV by the consultant psychiatrist. The Diagnostic and Statistical Manual of Mental Disorders, fourth edition, (DSM-IV) standardized by American Psychiatric Association (APA) in 2000, is the reference used for diagnosis of major depression [13]. All the selected cases were gone through a structured interview (questionnaire) as per DSM-IV criteria of major depression. It required the presence of a chronic and continual depressed mood for at least two weeks. A minimum five out of nine possible depressive symptoms were required that occurred throughout the period of trouble, and one of the five symptoms must be anhedonia or depressed mood [13].

A total 80 healthy subjects of matched age and gender were taken from the general population and considered as control group. Cases and controls, addicted to tobacco, alcohol or any other substances or aged greater than 60 years were excluded from the study. The patients with discontinuous depressed mood were also excluded. All the control subjects

included for the study were healthy, not addicted to tobacco or alcohol, non-diabetics, normotensive and showed no evidence of any chronic and/or acute infection. The subjects failing to above inclusion criteria were excluded from the study. The subjects who did not agreed to sign informed consent form were also excluded from the study.

2.1 Specimen Collection

From every studied subjects, 5mL of blood was withdrawn by venipuncture (from peripheral vein) method in clean dry glass tube. Serum was separated by centrifugation at 3000 r.p.m. for 10 minutes. All serum samples were stored at -80^0C. Care was taken to avoid samples hemolysis. Repetition of thaw of serum samples was also avoided.

2.2 Estimation of serum prolidase activity (SPA)

Reagents such as diluting solution, standard proline solution, enzyme substrate (94 mmol/L glycyl-l- proline) and Chinard's reagent were prepared. Serum prolidase activity was measured with the use of our previous standardized method [8, 14]. The enzyme activity was expressed in millimolar per minute per liter (mmol Min^{-1} L^{-1}).

2.3 Estimation of Serum Total Anti-oxidant Status (TAS)

TAS of serum was estimated with using a method developed by Erel (2004) [15]. Following reagents and procedures were used for the estimation of TAS-

Reagent-1: 75mM Clark and Lubs solution (pH 1.8) was prepared as; [5.591 gram of potassium chloride (KCl) was diluted in 1000 mL of deionized water. 6.41 mL of 36.5% hydrochloric acid (HCl) was dissolved in 1000 mL of deionized water. Then finally, 800 mL of above prepared KCl solution was mixed with 200 mL of above prepared HCl solution (pH maintained at 1.8)]. After this, 3.17 gram of orthodianisidine dihydrochloride (final 10 mM)

was mixed in this Clark and Lubs solution. Mixing was followed by addition of 0.01764 gram of Fe $(NH_4)_2$ $(SO_4)_2.6H_2O$ (final 45 µM). At 4^OC, it is stable for six months. (Note; orthodianisidine is a carcinogenic and very toxic substance. Thus, gloves and face mask were used during the handling of this substance)

Reagent-2: 7.5 mM of H_2O_2 solution was prepared (0.641mL of 35% H_2O_2 (Sigma Aldrich) was maintained to 1000 mL volume with the Clark and Lubs solution). At 4^OC, it is stable for one month.

Procedure: 2000 µL of reagent-1 was mixed with 50 µL of serum sample and 100 µL of reagent-2. Absorbance was taken at 444 nm. The first absorbance was taken before mixing of reagent-1 and reagent-2 (this OD was deducted from respective test as sample blank). The last reading of test was taken after 3- 4 minutes of mixing of reagent-1 and reagent-2. (Final OD = OD of regaent1 plus sample plus reagent 2 - OD of reagent1 plus sample).

Finally, TAS was estimated by preparation of standard linear calibration graph of Trolox (a Vitamin-E analogue). For this, 2.0 mM of Trolox was serially diluted as 0.2 mM, 0.4 mM, 0.6 mM, 0.8 mM, 1.0 mM, 1.2 mM, 1.4 mM, 1.6 mM, 1.8 mM. Results of TAS were expressed in millimolar Trolox equivalent per liter (mmol Trolox Eq./L).

2.4 Estimation of Serum Total Oxidant Status (TOS)

TOS of serum was estimated with using a method developed by Erel (2005) [16]. Following reagents and procedures were used for the measurement of TOS-

Reagent-1: 150 µM xylenol orange (114 mg) and 140 mM NaCl (8.18 gram) were dissolved in 900 mL of 25 mM-H_2SO_4 solution. Then, 100 mL of glycerol was added to this solution (final concentration of glycerol, 1.35 M). The pH of solution was maintained at 1.75. At $4\ ^OC$ it is stable for six months.

Reagent-2: 1.96 gram of ferrous ammonium sulfate (5 mM) was mixed with 3.17 gram of o-dianisidine dihydrochloride (10 mM) in 1000 mL of 25 mM- H_2SO_4 solution. At 4 ^{O}C, reagent is stable for six month.

Procedure: 2250 µL of reagent 1 was added to 350 µL of serum sample and 110 µL of reagent 2. The bichromatic absorbance was taken (main wavelength 560 nm, secondary wavelength 800 nm).The first absorbance was taken before mixing of reagent-1 and reagent-2 (this OD was deducted from respective test as sample blank). The last reading of test was taken after 3-4 minutes of mixing of reagent-1 and reagent-2. For final absorbance, absorbance of a test at 800 nm was deducted from the absorbance at 560 nm of respective test (Final Absorbance = Absorbance at 560 nm – Absorbance at 800 nm).

The assay was calibrated with H_2O_2 standard solution. 100 µmol of standard H_2O_2 solution was serially diluted with deionized water and results were drawn with the help of linear calibrated graph. Thus, results of test was expressed in terms of micromolar hydrogen peroxide equivalent per liter (µmol H_2O_2 Eq./L).

2.5 Calculation of Oxidative Stress Index (OSI)

Values of OSI were estimated with the help of following formula [8, 12 and 14]–

$$\text{OSI (Arbitrary Unit)} = \frac{\text{TOS (mmol } H_2O_2 \text{ Eq. / L)}}{\text{TAS (µmol Trolox Eq. / L)}}$$

2.6 Statistical Analysis

Standard statistical methods were used for the data interpretation. Microsoft office excels worksheet and SPSS (16) software was used for the calculation. Data, which follow normal distributions, were expressed as mean ± SD (standard deviation). A p-value < 0.05 was

considered as significant; student's t-test was used. Pearson's correlation was calculated for the correlative observations.

3. Results

In present study, Non-significant differences of mean age of the cases and controls were observed 39.11 ± 10.64 and 39.70 ± 9.89 years respectively (Table-1).

3.1 Observed status of SPA, TOS, TAS and OSI

The observed SPA, TOS and OSI were significantly increased in the cases than controls ($P < 0.001$, Table-1). However, TAS was significantly decreased in the cases than controls ($P < 0.001$, Table-1).

3.2 Status of Serum SPA, TOS, TAS and OSI with respect to progression of MDD

Total 80 cases of patients with MDD were categorized on the basis of duration of disease (Group-1; Duration of disease ≤ 1 years, range 0.42 – 1 year, n = 50, and group-2; Duration of disease > 1 years, range 1.5 – 6 years, n = 30). In this regards, significantly increased SPA, TOS and OSI were observed among the group of patients with disease duration > 1 year than the patients with disease duration ≤ 1 year ($P < 0.001$, Table-2). However, serum TAS was significantly decreased in the group-2 than group-1 ($P < 0.001$, Table-2).

3.3 Correlation of SPA and Serum Oxidative Stress with progression of MDD

On correlative observations, it was observed that positive, linear and significant correlation was observed between duration of disease (MDD), and SPA, and TOS, and OSI ($r = 0.879$, 0.646 and 0.695 respectively, all $P < 0.001$) (Figure-1A, 1B and 1D). However, negative linear and significant correlation was observed between duration of disease (MDD) and serum TAS ($r = - 0.619$, $P < 0.001$) (Figure-1C).

4. Discussion

In our previous study we have observed that increased oxidative stress in term of different individual oxidative stress markers in the patients with MDD [11]. Thus in present study we planned to observe oxidative stress in term of TOS, TAS and OSI in the patients with MDD. Along with this, serum prolidase activity (SPA) was also assessed. Present explorative study included 80 subjects of both cases of MDD and healthy individuals of matched age and gender. All the studied subjects have age below 60 years old.

Prolidase is an enzyme which cleaves the glycyl-l-proline and provides proline as end product [6]. Proline is usually circulated in the central nervous system (CNS), and may be act as neuromodulator in synaptic transmission [17, 18]. It is believed that glutamate is involved in the etiology of depression [19]. Several present literatures represented that proline and glutamate receptor interacts with each other [20]. It has been reported in literature that proline inhibited the glutamate release in cerebrospinal fluid (CSF), which induced the glutamatergic signaling in the hippocampus [21, 22]. It has also reported in literature that increased proline may be neurotoxic and damage brain by the decrease in glutamate uptake [23]. In present study, it is observed that serum prolidase activity has been increased in the patients with major depressive disorder than the healthy individuals (Table 1). Same results are reported by Kokacya et al., 2014, in Turkey population [24]. In addition to this, we also evaluated the serum prolidase activity with respect to progression of major depression in term of duration of depression and correlation. It is observed that serum prolidase activity has been significantly increased in the patients with > 1 years of MDD than the patients with ≤ 1 years of MDD (Table 2). Increased serum prolidase activity has been also significantly, positively and linearly correlated to duration of MDD ($r = 0.879$, $P < 0.001$) (Figure 1A). Recently, it is reported that elevated proline in peripheral circulation is associated and development of psychiatric disorders included schizophrenia [25]. Thus, it seems that increased prolidase

activity can leads to increase in proline concentration in circulation. Increased proline concentration may interfere with glutamate signaling of depressive patients and may be responsible for the progression and pathogenesis of major depressive disorder. But further explorative study is needed for better clarification.

An imbalance in the control of oxidants and antioxidants in human system leads to oxidative stress. Previously, we have reported that the status of different oxidants (malondialdehyde and nitrite) and antioxidants (superoxide dismutase, ascorbic acid and ceruloplasmin) are not in balance manner in the patients with MDD. Its observed value altered as compared to healthy individuals [11]. Delwing et al., 2003, reported that proline itself is able to increase the oxidative stress in the brain [26]. In present study, we have been observed significantly increased TOS and OSI in the patients with MDD than healthy individuals. However, significantly decreased TAS has been observed (all $P < 0.001$, Table 1). Same observations are done by Kokacya et al., 2014, in Turkey population [24]. Additionally, we have been also observed the association of oxidative stress with the progression of MDD. TOS and OSI have been significantly increased in the patients with > 1 years of MDD than the patients with ≤ 1 years of MDD ($P < 0.001$, Table 2). While, TAS has been significantly decreased ($P < 0.001$, Table 2).

Serotonin, 5-hydroxytryptamine, is a neurotransmitter involved in number of physiological functions such as regulation of body temperature, sleep, hormonal regulation, anxiety, depression and schizophrenia [27]. It is well documented that decreased status of serotonin is associated with the pathogenesis of major depression [28]. Evidences support that reactive oxygen species (ROS) can leads to oxidation of serotonin. Additionally, oxidation of serotonin as well as dopamine (a neurotransmitter of central nervous system) can also lead to increase in reactive radical load, and finally the status of serotonin status has dropped in depression [29, 30]. On the other hand, along with serotonin, cortisol a glucocorticoid

hormone is also associated with the chronic stress and depression. Chronic stress can lead to increase in cortisol status along with increased oxidative stress [31, 32]. Thus, it is clear that the neurotransmitters such as serotonin (decreased level) and cortisol (increased level) is associated with increased oxidative stress in depression. In the present study increased oxidative stress has been observed. Thus, it seems that increased oxidative stress may be associated with the pathogenesis of major depression via the alteration in serotonin and cortisol mediated signalling.

On correlative observations, it has been observed that duration of MDD significantly, positively and linearly correlated to TOS, and OSI ($r = 0.646$, $P < 0.001$ and $r = 0.695$, $P < 0.001$ respectively) (Figure 1B and 1D). While, significant and negative correlation has been observed between duration of MDD and TAS ($r = - 0.619$, $P < 0.001$) (Figure 1C). Thus, it seems that altered status of different oxidative stress markers as well as increased proline (as product of prolidase) may lead to increase in oxidative stress in the patients with MDD. This increase in oxidative stress may be responsible for the pathogenesis and progression of MDD.

5. Conclusions

The study concluded that serum prolidase activity, total oxidant status and oxidative stress index have been significantly increased in the patients with major depressive disorder than healthy controls. However total antioxidant status has been significantly decreased. It is also concluded that serum prolidase activity and oxidative stress have been progressively increased with the progression of major depressive disorder and may be associated with its pathogenesis. It seems that increased prolidase activity may lead to elevation of proline in circulation. This elevated circulatory proline may contribute to the increase in oxidative stress and/or it may alter the glutamate, serotonin and cortisol mediated signaling in the progression and pathogenesis of major depressive disorder.

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Table-1: Status of serum prolidase activity (SPA) and oxidative stress (TOS, TAS, and OSI) in cases and controls.

Variables	Cases (n= 80)	Controls (n= 80)	P- value
Age (Years)	39.11 ± 10.64	39.70 ± 9.89	NS
Gender	M- 48, F- 32	M- 45, F- 35	NS
SPA (mmol Min^{-1} L^{-1})	112.92 ± 24.19	91.19 ± 20.91	< 0.001
TOS (µmol H_2O_2 Eq./ L)	16.86 ± 2.59	13.17 ± 2.78	< 0.001
TAS (mmol Trolox Eq./L)	0.95 ± 0.33	1.56 ± 0.54	< 0.001
OSI (Arbitrary Unit)	21.22 ± 10.88	10.01 ± 5.16	< 0.001

SPA – Serum Prolidase Activity, TOS – Total Oxidant Status, TAS – Total Antioxidant Status, OSI – Oxidative Stress Index, n – Numbers of Subjects, NS – Non Significant, M- Male, F-Female

Table-2: Status of serum prolidase activity (SPA) and serum oxidative stress (TOS, TAS and OSI) with respect to disease duration (duration of major depression).

Variables	Duration of disease ≤ 1 years Range 0.42 – 1 year, n = 50	Duration of disease > 1 years Range 1.5 – 6 years, n = 30	P- value
SPA (mmol Min^{-1} L^{-1})	99.50 ± 14.98	135.31 ± 19.67	< 0.001
TOS (µmol H_2O_2 Eq./ L)	15.70 ± 2.43	18.78 ± 1.47	< 0.001
TAS (mmol Trolox Eq./L)	1.10 ± 0.28	0.69 ± 0.23	< 0.001
OSI (Arbitrary Unit)	15.85 ± 6.90	30.18 ± 10.45	< 0.001

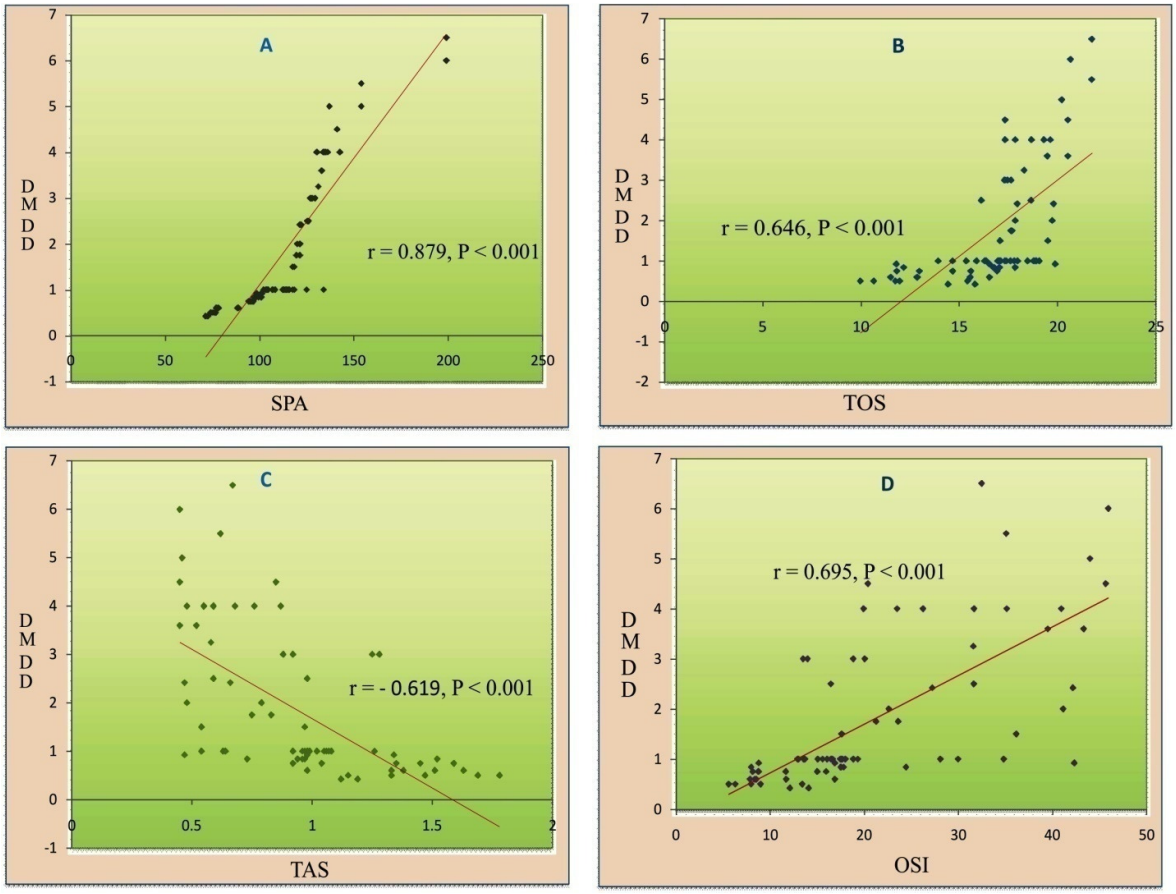

Figure-1: Scattered diagram showing Pearson's correlation between duration of depression and SPA (Fig A), and TOS (Fig B), and TAS (Fig C), and OSI (Fig D). r = Pearson's correlation coefficient, D-MDD = Duration of major depressive disorder in years.