

BRIEF ARTICLE

Prolidase activity in adult bipolar disorder patient: a case - control study

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ABSTRACT

Background: Bipolar disorder is one of the major neuropsychiatric illnesses. It is responsible for 6.8% of disability-adjusted life years among all mental disorders. Few studies have evaluated the biochemical basis of bipolar disorder. Prolidase is an enzyme that degrade extracellular matrix into proline. In stress condition, prolidase by releasing proline maintain ATP generation, redox state, apoptosis and cell proliferation. The relationship between serum prolidase with bipolar disorder was investigated in this study.

Methods: A case - control study was conducted at the Department of Laboratory Medicine in collaboration with the Department of Psychiatry of Bangabandhu Sheikh Mujib Medical University, Dhaka from March 2021 to February 2022. Thirty-five patients with bipolar disorder type I consisting of 15 euthymic and 20 manic patients were enrolled in the study according to the availability of patients as cases. Thirty-five age - and sex-matched healthy individuals were recruited from the Department of Laboratory Medicine as controls. Serum prolidase level was measured in cases and controls using venous blood.

Results: Serum prolidase level was significantly higher in cases than controls ($P=0.021$). There was no significant differences was observed between euthymic and manic patients ($P=0.629$). Significant positive correlation was found between Young Mania Rating Scale severity score and serum prolidase level ($P=0.001$).

Conclusion: Serum prolidase activity may be associated with bipolar disorder. This needs further corroboration because our sample size was small.

Keywords: bipolar disorder, prolidase level, proline, oxidative stress

INTRODUCTION

Bipolar disorder is a chronic progressive psychiatric illness characterized by an episode of mania, hypomania and depression interspersed with euthymia.¹ Around the world, 46 million people had bipolar disorder in 2017.² In South Asia, the prevalence of bipolar disorder is 0.6%,³ which is similar to the prevalence (0.5%) in Bangladesh.⁴ Research findings suggest that men and women have equal frequencies of bipolar disorder around the world.⁵ The diagnosis of bipolar disorder is based upon clinical interview. Due to disease heterogeneity, accurate diagnosis of bipolar disorder is difficult. It may take 10 years on average for a person to be correctly diagnosed with bipolar disorder.⁶ There is a

need to find out a reliable peripheral blood biomarker. A recent study showed that serum prolidase activity may have a role in bipolar disorder pathogenesis.⁷

Measuring the level of serum prolidase may be helpful to predict the disease. A review of literature identified 20 protein analytes with excellent predictive performance for bipolar disorder.⁷ The panel had a good predictive performance to identify misdiagnosed bipolar disorder and a fair to good predictive performance to differentiate between bipolar patients and controls.⁷ Three analytes of this panel belong to structurally related family of protease which are matrix-metalloproteinase that play important role in extracellular matrix degradation.⁷ Prolidase is a

HIGHLIGHTS

1. Prolidase level is highly significant in adult patients with bipolar disorder.
2. Prolidase level is significantly correlated with bipolar disorder severity.
3. Measurement of serum prolidase may be helpful in early detection of bipolar disorders among adults.

member of the matrix metalloproteinase family that degrades the extracellular matrix into proline and hydroxyproline.⁸ An increased prolidase level is associated with increased proline peptides.⁹ Disorder of proline metabolism was found to be associated with adult bipolar disorder.¹⁰

The link has been also found between hyperprolinemia and the development of bipolar disorder.¹¹ In bipolar disorder, brain energy generation and basal metabolic rate is increased in case of mania.¹² Normal physiological processes also happen in excess in bipolar disorder such as excitotoxicity, excessive apoptotic activity and cellular senescence.¹³ Proline plays a key role in maintaining cellular homeostasis and excess physiological processes such as apoptosis and autophagy in stressful condition.¹⁴ In stressful condition, PRODH/POX enzyme is involved in generating more reactive oxygen species during oxidation of proline in mitochondrial proline cycle.¹⁴ As a result, anti-oxidant capacity of the brain is limited. Impairment of signal transduction, neuronal plasticity and cell flexibility occurs due to lipid peroxidation of membranes by oxidative stress which is responsible for neural injury.¹⁵ A study from Turkey suggested prolidase as a reliable diagnostic marker for adult bipolar disorder.¹⁶ Another study also found significant higher prolidase level in bipolar disorder patients.¹⁷ However, the relationship of prolidase with Young Mania Rating Scale (YMRS) has not been examined previously. The objective of this study was to determine association between prolidase activity with bipolar disorders. Its correlation with YMRS was also examined.

METHODS***Study design***

This case-control study was conducted at the Department of Laboratory Medicine in collaboration with the Department of Psychiatry of Bangabandhu Sheikh Mujib Medical University, Dhaka from March 2021 to February 2022.

Subjects

Study sample consisted of bipolar disorder type-I patients and healthy controls. Thirty-five known cases of bipolar disorder type-I with manic and euthymic episode who attended in- and out-patient Department of Psychiatry were enrolled in this study. Diagnosis was made according to DSM-V criteria by a psychiatrist and symptoms severity was evaluated using Bangla version of YMRS¹⁸. YMRS ≤ 12 regarded as euthymia and YMRS > 12 regarded as mania. Patients with history of illicit drug use, depression, acute systemic disease, chronic systemic disease (diabetes mellitus, hypertension, ischemic stroke, malignancy), severe head injury or seizure were excluded from this study. Age and sex matched 35 healthy individuals from the Department of Laboratory Medicine were included as controls. Participants' demographic data and clinical features were recorded. All the participants were informed about the aim of the study and written informed consent was obtained.

Specimen collection

Random venous blood (3 ml) samples were collected in a biochemistry tube from the antecubital vein. The biochemistry tube was centrifuged at 3000 rpm for 5 min after an incubation period of 30 min. The separated serum specimen was kept in eppendorf tube and stored at -20°C until analysis were performed. Investigation was done at the Department of Laboratory Medicine. All sample were tested on four successive occasions within one month of sample collection.

Measurement of prolidase

Serum prolidase level was measured by human prolidase (peptidase D) ELISA (enzyme linked immunosorbent assay method) kit using the principle of

Sandwich-ELISA. The micro-ELISA plate was pre-coated with an antibody specific to human prolidase. Sample and standard were added to the micro-ELISA plate wells. The plate was covered and incubated for 45 min at 37°C. Each well was aspirated and washed with wash buffer four times. Then horseradish peroxidase (HRP) conjugate detection antibody specific for human prolidase was added to each micro plate well and incubated at 37°C. The washing process was repeated five times. Chromogenic solution A and chromogenic solution B were added to each well. The well containing human Peptidase D Protein (PEPD) detection antibody with HRP conjugate appeared blue in color. It was gently mixed and incubated. Then stop solution was added to each well and color turned yellow. The optical density was measured with spectrophotometer. The optical density value was proportional to the concentration of human prolidase. The concentration of human PEPD in the samples was calculated by comparing the optical density of the sample to the standard curve.

Statistical analysis

Data analysis was performed using IBM SPSS version 26. Categorical variables were expressed as frequency (percentage), and numerical variables as mean (standard deviations) or median (interquartile range) depending on the distribution of data. In case of normally distributed variables, the significant differences between the groups were estimated by t-

tests. Wilcoxon test was done for skewed data. Correlation of serum prolidase with YMRS score was determined by Spearman rank correlation co-efficient test. A probability value of less than 0.05 was considered statistically significant.

RESULTS

TABLE 1 shows the sociodemographic variables of the participants. Mean age of the participants was 32.9 years for cases and 36.1 years for controls, with a predominance of males(66%). The mean duration of the

TABLE 1 Demographic characteristics of the study subjects (n=70)

	Bipolar disorder type-I n=35	Healthy Control n=35
Age in years, mean (±SD)	32.9 (12.0)	36.1 (9.4)
Gender, male/female (n)	23/12	18/17
Disease duration in years, mean (±SD)*		-
Euthymic (n=15)	5±4.4	
Manic (n=20)	7±4.2	
Mean YMRS score†		-
Euthymic	3±0.7	
Manic	23±9.3	
Treatment, yes/no (n)	15/20	-

*SD indicates standard deviation

†Young Mania Rating Scale

disease was 5.0 years.

FIGURE 1(A) shows comparison of serum prolidase levels between cases and controls. Median (IQR) prolidase level of cases was 510.0 U/L and controls was 440.0 U/L. The difference was statistically significance ($P=0.021$). **FIGURE 1(B)** shows the comparison of serum prolidase level between euthymic

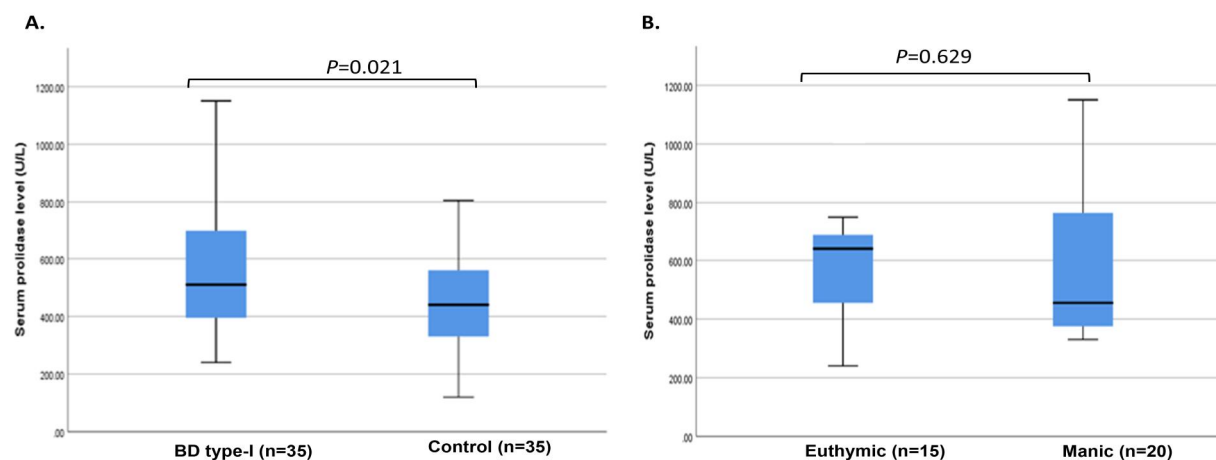


FIGURE 1 (A) Serum prolidase levels in bipolar disorder patients and healthy controls; **(B)** serum prolidase levels between euthymic and manic participants with bipolar disorder.

and manic participants among bipolar disorder patients. Median prolidase in euthymic BD patient was 640.0 and in manic episode of BD was 455.0 U/L. The difference was not statistically significant ($P=0.629$). However, there was a significant positive correlation ($r=0.87$) between serum prolidase level and YMRS severity score (FIGURE 2).

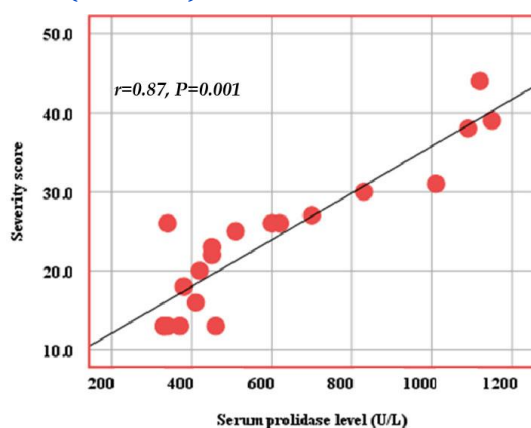


FIGURE 2 Correlation between serum prolidase level and severity score in bipolar disorder (n=35).

DISCUSSION

We report here that a higher level of serum prolidase level in adult bipolar disorder patients, compared to healthy controls. The age and sex distribution of series may not be directly comparable to many other studies¹⁵⁻¹⁹ because of differences in study settings, subject selection and sample sizes.

Selek et al. had determined higher prolidase levels in bipolar disorder patients compared to their control group which was statistically significant ($P<0.001$) and consistent with current study.¹⁶ Increased serum prolidase level in bipolar disorder was also reported by Ceylan et al.¹⁷, which is consistent with the findings of the present study.

Statistically significant difference of serum prolidase level was not found between euthymic and manic groups in this study ($P=0.629$). Selek et al.¹⁶ in their study also did not find any significant difference of serum prolidase level among sub-group of bipolar disorder patients which is consistent with findings of the present study.

The present study found significant positive correlation of serum prolidase level with YMRS symptom score in

manic patients of bipolar disorder ($r=0.87$). It seems that with increase disease process, prolidase level increases. As per our knowledge, no such correlation was observed between serum prolidase level and YMRS score previously. Although prolidase correlate with YMRS score in manic patients of bipolar disorder, the correlation in euthymic and manic was not statistically significant (data not shown). A small sample size might be responsible for this. Lifestyle and dietary patterns may also affect the prolidase activity.

Measuring serum prolidase of patients in different episodes and comparison of prolidase of patients with and without treatment is needed to be performed to identify the relation of prolidase with the characteristic property of the disorder.

Limitations

The study was conducted with a small sample size. Lack of measurement of proline level alongside prolidase was also a limitation.

Conclusion

Our finding indicates that serum prolidase activity is associated with bipolar disorder. It is correlated with the YMRS symptom scores also. Prolidase activity may have a significant role with the disease activity.

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Author Contributions

Conception and design: JDJ, DP, MSA. Acquisition, analysis, and interpretation of data: RMR, SY. Manuscript drafting and revising it critically: JDJ, DP, MSA, MMAB, RMR, SF, MSI, SY. Approval of the final version of the manuscript: JDJ, DP, MSA, MMAB, RMR, SF, MSI, SY. Guarantor accuracy and integrity of the work: DP.

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This study did not receive any external funding.

Conflict of Interest

Authors declare no conflict of interest.

Ethical Approval

The study was conducted following the Declaration of Helsinki as the cornerstone document on human research ethics. Before commencing the study, approval of this project was taken from the Institutional Review Board of Bangabandhu Sheikh Mujib Medical University (memo no BSMMU/2021/6952).

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REFERENCES

- Sagar R, Pattanayak RD. Potential biomarkers for bipolar disorder: Where do we stand? The Indian journal of medical research. 2017; 145(1): 7. DOI: https://doi.org/10.4103/ijmr.IJMR_1386_16.
- Dattani S, Ritchie H, Roser M. Mental Health. Published online at OurWorldInData.org. 2021; Available at: <https://ourworldindata.org/mental-health> [Online Resource].
- Naveed S, Waqas A, Chaudhary AMD, Kumar S, Abbas N, Amin R, Jamil N, Saleem S. Prevalence of common mental disorders in South Asia: a systematic review and meta-regression analysis. Frontiers in psychiatry. 2020; 11, 899. DOI: <https://doi.org/10.3389/fpsy.2020.573150>.
- World Health Organization, Bangladesh WHO Special Initiative for Mental Health Situational Assessment. WHO, 2020.
- Harrison P, Cowen PT, Burns, Faze M. Shorter Oxford Textbook of Psychiatry. Seventh Edition, New York, Oxford University Press. 2018.
- Hirschfeld RM, Vornik LA, Perceptions and impact of bipolar disorder: how far have we really come? Results of the national depressive and manic-depressive association 2000 survey of individuals with bipolar disorder. The Journal of clinical psychiatry. 2003; 64(2): 14089.
- Haenisch F, Cooper JD, Reif A, Kittel-Schneider S, Steiner J, Leweke FM, Rothermundt M, van Bevern NJ, Crespo-Facorro B, Niebuhr DW, Cowan DN. Towards a blood-based diagnostic panel for bipolar disorder. Brain, behavior, and immunity. 2016; 52: 49-57. DOI: <https://doi.org/10.1016/j.bbi.2015.10.001>.
- Surazynski A, Milyk W, Palka J, Phang JM. Prolidase-dependent regulation of collagen biosynthesis. Amino acids. 2008; 35(4): 731-38. DOI: <https://doi.org/10.1007/s00726-008-0051-8>.
- Verma AK, Raj J, Sharma V, Singh TB, Srivastava S, Srivastava R. Plasma prolidase activity and oxidative stress in patients with Parkinson's disease. Parkinson's Disease. 2015. DOI: <https://doi.org/10.1155/2015/598028>.
- Güneş M, Bulut M, Demir S, İbiloğlu AO, Kaya MC, Atlı A, Kaplan İ, Camkurt MA, Sir A. Diagnostic performance of increased prolidase activity in schizophrenia. Neuroscience letters. 2016; 613: 36-40. DOI: <https://doi.org/10.1016/j.neulet.2015.12.036>.
- Duarte M, Afonso J, Moreira A, Antunes D, Ferreira C, Correia H, Marques M, Sequeira S. Hyperprolinemia as a clue in the diagnosis of a patient with psychiatric manifestations. Brain Dev. 2017 Jun;39(6):539-541. DOI: <https://doi.org/10.1016/j.braindev.2017.01.008>.
- Caliyurt O, Altıay G. Resting energy expenditure in manic episode. Bipolar disorders. 2009; 11(1): 102-06. DOI: <https://doi.org/10.1111/j.1399-5618.2008.00649.x>.
- Berk M, Kapczinski F, Andreazza AC, Dean OM, Giorlando F, Maes M, Malhi GS. Pathways underlying neuroprogression in Bipolar disorder: Focus on inflammation, oxidative stress and neurotrophic factors. Neuroscience & Biobehavioral Reviews. 2011; 35(3): 804-17. DOI: <https://doi.org/10.1016/j.neubiorev.2010.10.001>.
- Huynh TYL, Zareba I, Baszanowska W, Lewoniewska S, Palka J. Understanding the role of key amino acids in regulation of proline dehydrogenase/proline oxidase (prodh/pox)-dependent apoptosis/autophagy as an approach to targeted cancer therapy. Molecular and cellular biochemistry. 2020; 466(1): 35-44. DOI: <https://doi.org/10.1007/s11010-020-03685-y>.
- Akarsu S, Bolu A, Aydemir E, Zincir SB, Kurt YG, Zincir S, Erdem M, Uzun Ö. The relationship between the number of manic episodes and oxidative stress indicators in Bipolar disorder. Psychiatry investigation. 2018; 15(5): 514. DOI: <https://doi.org/10.30773/pi.2016.12.31>.
- Selek S, Altındag A, Saracoglu G, Celik H, Aksoy N. Prolidase activity and its diagnostic performance in bipolar disorder. Journal of affective disorders. 2011; 129(1-3): 84-86. DOI: <https://doi.org/10.1016/j.jad.2010.09.003>.
- Ceylan MF, Hesapcioglu ST, Kasak M, Senat A, Erel O. Increased prolidase activity and high blood monocyte counts in pediatric Bipolar disorder. Psychiatry research. 2019; 271: 360-64. DOI: <https://doi.org/10.1016/j.psychres.2018.11.066>.
- Ara H, Nahar JS, Rahman W, Ahmed S, Arafat SY. Psychometric Properties of Bangla Young Mania Rating Scale. Malaysian Journal of Psychiatry. 2019; 28(2): 44-52. Available at: https://journals.lww.com/mjp/Abstract/2019/28020/Psychometric_Properties_of_Bangla_Young_Mania.6.asp
- Chowdhury MI, Hasan M, Islam MS, Sarwar MS, Amin MN, Uddin SN, Rahaman MZ, Banik S, Hussain MS, Yokota K, Hasnat A. Elevated serum MDA and depleted non-enzymatic antioxidants, macro-minerals and trace elements are associated with bipolar disorder. Journal of Trace Elements in Medicine and Biology. 2017 Jan 1;39: 162-8. DOI: <https://doi.org/10.106/j.jtemb.2016.09.012>.