Int. J. Chem. Sci.: 7(3), 2009, 1583-1590

PLASMA HOMOVANILLIC ACID IN DELUSIONAL DISORDER : IMPLICATIONS FOR DOPAMINE DYSFUNCTION

SIKTA BANDOPADHYAY, BISU SINGH, NIRMAL K. BERA[a], SUJIT K. DAS[a], CHITTA R. NAYAK[b] and T. K. CHAUDHURI[*]

Cellular Immunology Laboratory, Department of Zoology, University of North Bengal, SILIGURI – 734013 (W. B.) INDIA
[a]Department of Psychiatry, North Bengal Medical College and Hospital, SILIGURI (W. B.) INDIA
[b]Computer Centre, University of North Bengal, SILIGURI - 734013, W. B., India

ABSTRACT

Plasma concentrations of the major dopamine (DA) metabolite homovanillic acid (HVA) are useful indicators of brain DA activity[1] in clinical research[2, 3]. It is the potential index of central dopamine turnover[4] and is the most suitable instrument currently available to assess DA activity under relatively natural behavioral conditions i. e., without any pharmacological manipulation or inducing stress from study conditions[5].

In this study, We compared the plasma homovanillic acid (pHVA) concentration in the delusional disorder patients as well as in the healthy controls to investigate if the abnormalities of the central dopaminergic transmission may involve in the expression of the delusional symptoms in the patients. 30 Delusional disordered patients have been considered for this study. 30 age and sex matched healthy individuals were considered as the control. A significant increase has been found in pHVA concentration of delusion disorder patient than the healthy controls.

The finding suggests that there is a positive correlation between pHVA concentration and delusional disorder, which further provides the evidence for hyperdopaminergic activity in the brain of delusional disorder patients.

Key words : Plasma homovanillic acid, Dopamine, HPLC, Delusional disorder.

INTRODUCTION

The dopamine hypothesis continues to provide the principal conceptual gateway

[*] Author for correspondence; Tel : +91-353-2699124 (O), +91-9434377127 (M); Fax: +91-353-2699001; E-mail : dr_tkc_nbu@rediffmail.com; tapas.chaudhuri@gmail.com

into the mysteries of many psychotic disorders such as schizophrenia, attention deficit hyperactivity (ADHD), addictive disorder, alcoholism etc. Very recently, dopamine hypothesis of delusional disorder has been supported by a few studies on D2 receptor variation[6] and D4 receptor Exon 3 variation[7]. Therefore, the measurement and dynamic assessment of HVA, the major metabolite of dopamine, offer opportunities for obtaining substantial understanding of dopamine's role in delusional disorder. Plasma concentrations of the major dopamine (DA) metabolite homovanillic acid (HVA) are useful indicators of brain DA activity[1] in clinical research[2, 3]. It is the potential index of central dopamine turnover[4] and is the most suitable instrument currently available to assess DA activity under relatively natural behavioral conditions i. e., without any pharmacological manipulation or inducing stress from study conditions[5].

Recent evidence from the study of delusional misidentification syndrome indicates that delusions of very specific type may arise in association with certain well-defined brain insults. Delusional disorder is characterized by monosymptomatic paranoid symptoms. In the field of psychiatry it is the only disease where delusion is recorded as the discrete symptom. Since delusional disorder is characterized by mono-symptomatic paranoid symptoms, several investigators have suggested that delusional disorder is a naturally occurring model psychosis based on abnormalities of the dopaminergic temporolimbic system[9].

In the present investigation, We have studied the concentration of plasma homovanillic acid (pHVA) to see if the abnormalities of the central dopaminergic transmission is involved in the expression of the delusional symptoms in delusional disorder patients and if it can be used as the state marker of this disorder.

EXPERIMENTAL

Materials and methods

Subjects

30 patients with delusional disorder, classified according to DSM-IV and ICD-10 criteria, attended the psychiatric out patient department (OPD) of North Bengal Medical College and Hospital were studied. On an average of 1500 new patients with various psychiatric illness and about 4000 recurrent follow-up cases attend the OPD every year. Initially, about 50 unrelated patients were recruited with the symptoms of delusions. All subjects were screened independently by two psychiatrists using the structured clinical interview (SCID) for DSM-IV to determine the diagnosis of delusional disorder (American

Psychiatric Association, 1994). After longitudinal follow-up, 20 patients represented genuine cases of delusional disorder of various types have been considered. Most patients were clustered between the ages of 25-55 years. The male/female ratio was 1 : 2.The patients having any one of the following conditions were excluded from the study like : (i) substance abuse during the past year; (ii) any history of other general medical illness or treatment with anti-inflammatory or immunosuppressive medication; (iii) any past history of psychotic illness and (iv) any history of co morbidity. Further, the delusional patients and the family members were made to answer a questionnaire included self reported age, sex, caste, medical history, age of onset, age of severity, pedigree, disease duration among patients. Written informed consent was obtained from each subject after a complete description of the study. The study was conducted as per the norms of the "World Medical Association Declaration of Helsinki, Ethical Principles for Medical Research Involving Human Subjects". 20 age and sex matched healthy individuals with no past history of psychiatric illness belonging to the same ethnic group as the patients were used as control subjects. The subjects were mostly from middle-class urban and semirural society and belonged to a nuclear family.

Isolation of plasma

Blood samples were collected in heparinized tube from all 40 individuals. None of the participating subjects took psychotropic medication. Each sample was obtained by vein puncture. The blood was centrifuged for 10 mins at 4000 xg, within 30 mins and the plasma was collected and stored at -80°C until use. All the participants provided their written consent for giving the blood sample after the study procedure was explained.

Determination of homovanillic acid in plasma

Homovanillic acid was measured using HPLC method according to Zumarraga *et al.,* 1987[10].

Sample preparation : Plasma (0.5 mL) was mixed with 50 μmol of EDTA, 1.82mmol of heptanesulfonic acid, 0.02 mol/lit citric acid and 0.02mol/lit disodium phosphate (pH 3.1). Further, plasma proteins were precipitated by adding 20 μL of concentrated perchloric acid and vortex for several seconds. Then the samples were centrifuged for 10 mins at 42000xg. From 1.2 mL of supernatants HVA was extracted, three times with 1.5 mL of ethyl acetate. The combined organic phases were evaporated to dryness under a stream of nitrogen and the residue was dissolved in 0.4 mL of 1 mmol Na_2-EDTA. The extracts were stable for upto 3 days when frozen in liquid nitrogen and stored at -80°C.

Chromatographic system : HPLC analysis was carried out in a liquid chromatographic system consisted of a Model 6000A solvent delivery system (Waters Associates, Milford, MA); a Waters WISP 7101 automatic injector and Waters 464 pulsed ECD with glassy carbon electrode. The detector was operated at 0.6V potential between the working electrode and the Ag/AgCl reference electrode at a sensitivity of 2nA full scale deflection. The column was a 150 x 3.9 mm Novapack C_{18}, 5-μm (Waters, USA). Chromatograms were recorded with Model 023 recorder (Perkin-Emer Corp., Norwalk, CT operated at 10mV full scale. A Waters guard column containing C_{18} Corasil was used. All separations were achieved isocratically at room temperature. Flow rate 1 mL/min. and injection volume was 20 μL.

Quantification of HVA in plasma : The linearity of the detector response was estimated by injecting increasing amounts of standard HVA. Known amount of authentic HVA (Sigma-Aldrich Pvt. Ltd., USA) were added to study the linearity of the purification procedure and the analytical recovery. The standard addition calibration curve was constructed for each plasma sample. The calibration curve was extrapolated to zero concentration of added HVA. The samples were assayed on the basis of peak heights relative to those of external standards.

Statistical analysis

Statistical analysis was performed for the bivariate associations between delusional disorder patients versus normal group by employing one-way analysis of variance. Student's t-test was performed to determine the significance of differences of means among the patients and the control group.

RESULTS AND DISCUSSION

The mean value, standard deviation and coefficient of variance in both the patient group and normal controls are presented in the Table 1. In the present study, the pHVA concentrations in delusional disorder patients were found to be significantly higher (28.014 ng/mL) than in normal controls (7.437 ng/mL) ($p<0.001$) (Fig. 1).

Several studies have been done on the association between pHVA and schizophrenia but all the studies were failed to obtain consistent results [11]. This inconsistency were suggested to be due to the following reasons (i) pHVA is correlated positively with the severity of the psychotic symptoms[12,13,14]; (ii) in good responders pHVA decreases along with neuroleptic treatment[13,15,16,17,18];and (iii) pHVA is lower in

deficit-type than in non-deficit-type[14, 19, 20].

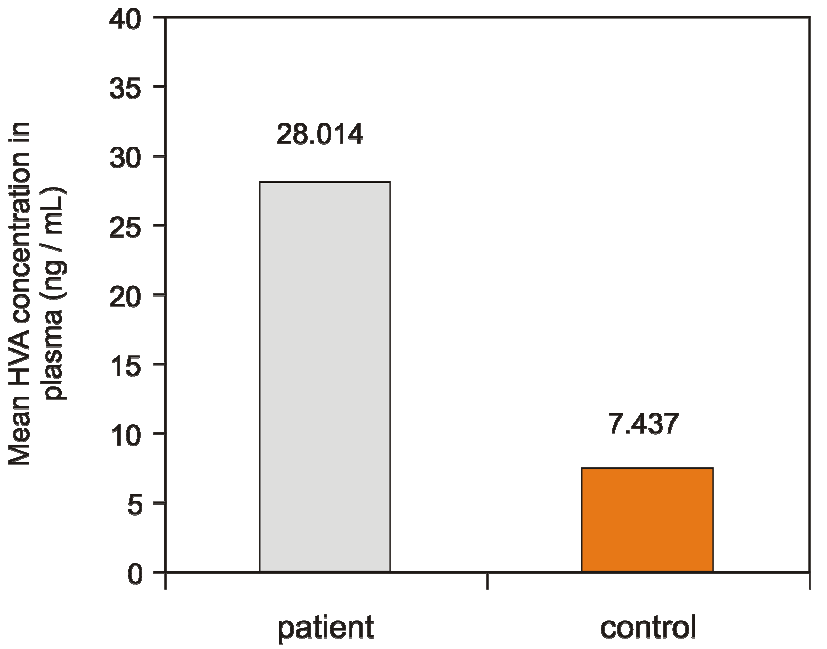

Fig. 1 : Comparison of mean HVA concentrations (ng/mL) in the plasma of both the controls and the patients

Table 1 : Plasma HVA levels (ng/mL) in both the patients and the controls

Characteristics	Patients (n = 30)	Controls (n = 30)
Range of pHVA (ng/mL)	9-95	2-20
Mean (ng/mL)	28.014	7.437
SD	19.363	5.091
Coefficient of variation	69.118%	68.448%

In contrast to these complicated results for pHVA in schizophrenia, more consistent results have been observed in delusional disorder[11]. It is suggested from earlier studies on the pHVA levels and psychosis, that consistent results has been observed in psychoses with a good prognosis, which is unrelated to their conventional diagnosis[21, 22, 23, 24]. Garver et al. (1997) have suggested that the higher pHVA psychosis is a dopamine psychosis that may have a familial origin. Our findings on pHVA in delusional disorder are

in agreement with the results of Garver et al. (1997) and Morimoto et. al (2002).

Present study had some limitations. Time of blood sampling was not strictly controlled, since OPD were included in the study. Despite the limitations of the present study the higher level of pHVA in the present finding suggests that the hyperfunction of the dopamine system, which may be at least partly responsible for the brain mechanisms underlying its delusional symptoms and could be used as state marker for the delusional disorder.

REFERENCES

1. M. D. Kohnke, G. Wiatr, W. Kolb, A. M. Kohnke, S. Schick, U. Lutz, R. Vonthein and I. Gaertner, Plasma Homovanillic Acid, A Significant Association with Alcoholism is Independent of a Functional Polymorphism of the Human Catechol-o-Methyltransferase Gene, Neuropsychopharmacol., **28**, 1004-1010 (2003).
2. F. Amin, M. Davidson and K. L. Davis, Homovanillic Acid Measurement in Clinical Research, A Review of Methodology, Schizophr. Bull., **18**, 123-148 (1992).
3. F. Amin and A. J. Friendhoff, Plasma Homovanillic Acid as a Tool to Investigate Brain Dopaminergic Activity. in Friendhoff A. J. and F. Amin (Eds), Plasma Homovanillic Acid in Schizophrenia, Implications for Presynaptic Dopamine Dysfunction, Washington, DC, American Psychiatric Press, (1997) pp. 1-16.
4. M. Davidson and K. L. Davis, A Comparison of Plasma Homovanillic Acid Concentrations in Schizophrenic Patients and Normal Controls. Arch. Gen. Psychiatry, **45**, 561-563 (1988).
5. F. Amin, A. E. Stroe, T. Kahn, P. J. Knott, R. S. Kahn and M. Davidson, Control of Renal Factors in Plasma Homovanillic Acid Measurements, Neuropsychopharmacol., **18**, 317-320 (1998).
6. A. Serretti, E. Lattuada, C. Lorenzi, R. Lilli and E. Smeraldi, Dopamine Receptor D2Ser/Cys 311 Variant is Associated with Delusion and Disorganization Symptomatology in Major Psychoses., Mol. Psychiatry, **5**, 270-4 (1999).
7. A. Serretti, R. Lilli, C. Lorenzani, E. Lattuada and E. Smeraldi, DRD4 Exon 3 Variants Associated with Delusional Symptomatology in Major Psychoses, A Study on 2, 011 Affected Subjects, Am. J. Med. Genet., **105**, 283–90 (2001).
8. D. G. Gorman and J. L. Cummings, Organic Delusional Syndrome. Seminars in Neurology, **10**, 229-38 (1990).
9. A. Munro, Delusional Disorders are Naturally Occurring 'Experimental Psychosis'. Psychopathology, **27**, 247 - 50 (1994).

10. M. Zumarraga, I. Andla, B. Barcena, M. I. Zamalloa and R. Davila, Liquid Chromatographic Method for Determination of Homovanillic Acid in Plasma, with Electrochemical Method. Clin. Chem., **33(1)**, 72-75 (1987).

11. K. Morimoto, R. Miyatake, M. Nakamura, T. Watanabe, T. Hirao and H. Suwaki, Delusional Disorder, Molecular Genetic Evidence for Dopamine Psychosis., Neuropsychopharmacol., **26**, 794-801 (2002).

12. K. L. Davis, M. Davidson, R. C. Mohs, K. S. Kendler, B. M. Davi, C. A. Johns, Y. Denigris and T. B. Horvath, Plasma Homovanillic Acid Concentration and the Severity of Schizophrenia Illness, Science, **29**, 1601-1602 (1985).

13. D. Pickar, R. Labarca, A. R. Doran, O. M. Wolkowitz, A. Roy, A. Bteier, M. Linnoila and S. M. Paul, Longitudinal Measurement of Plasma Homovanillic Acid Levels in Schizophrenic Patients. Correlation with Psychosis and Response to Neuroleptic Trearment, Arch. Gen. Psychiatry, **43**, 669-676 (1986).

14. M. Davidson and K. L. Davis, A Comparison of Plasma Homovanillic Acid Concentrations in Schizophrenic Patients and Normal Controls. Arch. Gen. Psychiatrty, **45**, 561-563 (1988).

15. D. Pickar, R. Labarca, M. Linnoila, A. Roy, D. Hommer, D. Everett and S. M. Paul, Neuroleptic-Induced Decrease in Plasma Homovanillic Acid and Antipsychotic Activity in Schizophrenic Patients, Science, **225**, 954-957 (1984).

16. W. Chang, T. Chen, C. Lee, J. Hung, W. Hu and E. Yeh, Plasma Homovanillic Acid Levels and Subtyping of Schizophrenia, Psychiatry Res., **23**, 239-244 (1988).

17. A. R. Koreen, J. Lieberman, J. Alvir, D. Mayerhoff, A. Loebel, M. Chakos, F. Amin and T. Cooper, Plasma Homovanillic Acid Levels in First Episode Schizophrenia. Psychopathology and Treatment Response, Arch. Gen. Psychiatry, **51**, 132-138 (1994).

18. S. Nagaoka, N. Iwamaoto and H. Arai, First Episode Neuroleptic-Free Schizophrenics, Concentrations of Monoamines and Their Metabolites in Plasma and Their Correlations with Clinical Responses to Haloperidol Treatment. Biol. Psychiatry, **41**, 857-864 (1997).

19. M. Ribeyre, P. Lesieur, O. Varoquaux, S. Dollfus, M. Pay and M. Petit, A Comparison of Plasma Homovanillic Acid in the Deficit and Non-Deficit Subtypes of Schizophrenia, Biol. Psychiatry, **36**, 230-236 (1994).

20. F. Thibaut, -. M. Ribeyre, N. Dourmap, J. F. Menard, S. Dollfus and M. Petit, Plasma 3-methoxy-4-hydroxyphenylglycol and Homovanillic Acid Measurements in Deficit and Nondeficit Forms of Schizophrenia, Biol. Psychiatry, **43**, 24-30 (1998).

21. M. B. Jr. Bowers, M. Swigar, P. Jatlow and N. Goicoechea, Plasma Catecholamine Metabolites and Early Response to Haloperidol, J. Clin. Psychiatrty, **45**, 248-251 (1984).

22. M. B. Jr. Bowers, Psychotic Patients with Increased Plasma HVA and MHPG or Increased Alone, Biol. Psychiatry, **34,** 584-586 (1993).

23. D. L. Garver, J. L. Steinberg, B. E. Mcdermott, J. K. Yao, J. E. Ramberg, S. Lewis and S. J. Kingsbury, Etiologic Heterogeneity of the Psychoses, is there a Dopamine Psychosis?, Neuropsychopharmacol., **14**, 191-201 (1997).

24. S. E. Ottong and D. L. Garver, A Biomodal Distribution of Plasma HVA/MHPG in the Psychoses, Psychiatry Res., **69,** 97-103 (1997).

Accepted : 23.03.2009