ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES Knowledge to Innovation

Vol 8, Issue 5, 2015

ISSN - 0974-2441

Research Article

CHANGES IN URINARY MONOAMINE METABOLITES WITH ANTIPSYCHOTIC TREATMENT IN SCHIZOPHRENIA

MANU ARORA[1], DAYA RAM[2], SAMIR KUMAR PRAHARAJ[3]*

[1]Department of Psychiatry, Psychiatric Diseases Hospital, Government Medical College, Jammu, Jammu & Kashmir, India. [2]Department of Psychiatry, Central Institute of Psychiatry, Ranchi, Jharkhand, India. [3]Department of Psychiatry, Kasturba Medical College, Manipal - 576 104, Karnataka, India.
Email: samirpsyche@yahoo.co.in

Received: 18 May 2015, Revised and Accepted: 11 June 2015

ABSTRACT

Objective: Monoamine neurotransmitters have been considered important mediators of schizophrenia pathology and antipsychotic drug action. This study examines the level of monoamine metabolites, homovanillic acid (HVA), 5-hydroxy indole acetic acid (5-HIAA), and vanillylmandelic acid (VMA), monoamine metabolites of major neurotransmitters dopamine, serotonin and norepinephrine, respectively in urine of patients with schizophrenia as compared to normal controls and the change in monoamine metabolites with antipsychotic treatment.

Methods: Thirty-four drug-free patients with schizophrenia diagnosed with Diagnostic and Statistical Manual of Mental Disorders Fourth Edition (DSM-IV) criteria and 15 normal controls were taken for the study. Patients were assessed for psychopathology using positive and negative syndrome scale (PANSS) scale at baseline and 4 weeks after the treatment. Urinary monoamine metabolites (HVA, 5-HIAA, and VMA) were measured before and after 4 weeks of treatment using high-performance liquid chromatography.

Results: There was a trend toward higher levels of HVA and VMA in the patients as compared to controls. There was a trend toward reduction in 5-HIAA levels with treatment in patients with schizophrenia. No correlation was found between the levels of monoamine metabolites and psychopathology. Significant positive correlation was found between 5-HIAA with VMA and HVA.

Conclusion: The present study indicates that noninvasive measurement of monoamine metabolites in urine may be of value in differentiating patients with schizophrenia from controls.

Keywords: Monoamine, Homovanillic acid, 5-Hydroxy indole acetic acid, Vanillyl mandelic acid, Schizophrenia.

INTRODUCTION

Biochemical psychiatry has emerged over the past decades as an important conceptual and experimental approach to the understanding mental illness. All biochemical theories of schizophrenia are predicated on the belief that a qualitatively or quantitatively abnormal substance may play a role in the production of schizophrenic symptoms. Monoamine neurotransmitters have been considered important mediators of schizophrenia pathology and antipsychotic drug action. The dopamine hypothesis appears to be well founded in pharmacological studies and is the working hypothesis of schizophrenia [1]. The norepinephrine hypothesis is also a major working hypothesis in schizophrenia [2]. The serotonin hypothesis dates back to the early days of hallucinogenic models of psychosis in general and schizophrenia in particular [3].

Evidence for the "dopamine hypothesis" of schizophrenia stems primarily from observations on the similarity of amphetamine psychosis to acute paranoid schizophrenia, as well as from the observations that dopamine agonists exacerbate schizophrenic symptoms, whereas, dopamine antagonist effects of neuroleptics reduce the symptoms [1]. This theory regards schizophrenia as a state of excessive activity of dopaminergic neurons in certain areas of the brain. As compared to healthy subjects, a higher daily excretion and higher urinary variations of homovanillic acid (HVA) was found for chronic patients with paranoid schizophrenia. Inversely, a lower HVA excretion and probably an inversion of the circadian rhythm of urinary HVA were found for chronic undifferentiated schizophrenic patients [4]. In a study by Markianos *et al*., 42 schizophrenia patients under chronic neuroleptic treatment (11-24 years) were studied, 20 without and 22 with tardive dyskinesia [5]. Urinary HVA values were higher in both with or without tardive dyskinesia, compared with normals. The study by Kemali *et al*. found increased norepinephrine levels in the urine of schizophrenia subjects, using a radioenzymatic technique [6].

The search for the abnormalities in these neurotransmitters, or their metabolites had lead to the understanding of the pathophysiology of schizophrenia. The studies have shown inconsistent results, and Indian literature in this area is sparse. Therefore, the current study was carried out with the objective to examine the level of monoamine metabolites, HVA, 5-hydroxy indole acetic acid (5-HIAA) and vanillyl mandelic acid (VMA) in urine of patients with schizophrenia as compared to normal controls and to see the change in monoamine metabolites with antipsychotic treatment.

METHODS

Participants

The study was a hospital-based prospective study conducted at the Central Institute of Psychiatry, Ranchi, and a tertiary center in eastern India. The study was approved by Institutional ethics committee. Patients fulfilling the Diagnostic and Statistical Manual of Mental Disorders Fourth Edition (DSM-IV) criteria for schizophrenia, in the age group 18-65 years and drug free for at least 4 weeks prior to the assessment (8 weeks, if receiving long-acting depot antipsychotic) were included. Written informed consent was obtained from all the participants. Patients with presence of comorbid psychiatric disorder and history of organicity, systemic illness were excluded. Age and sex matched subjects and free of any diagnosable psychopathology was included as normal controls. Fig. 1 shows the flow of participants in the study.

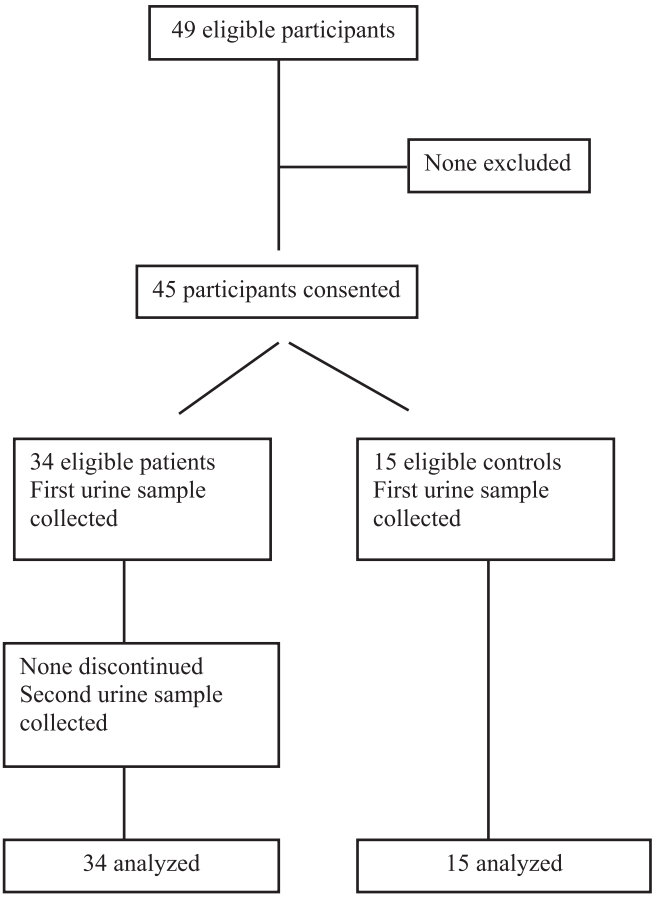

Fig. 1: The flow of participants through each stage of the study

Tools

A semi-structured pro-forma was used for recording socio-demographic and clinical details. Psychopathology was assessed using positive and negative syndrome scale (PANSS) [7]. Seven subscale scores including positive syndrome, negative syndrome, general psychopathology, anergia, thought disorder, paranoid belligerence, and depression were calculated. High-performance liquid chromatography (HPLC) (Waters® 1500 Series) was used for assessing levels of HVA, 5-HIAA, and VMA, the major metabolites of dopamine, serotonin and norepinephrine, respectively.

Procedure

Eligible patients (N=34) and normal controls (N=15) were assessed using semi-structured pro-forma. In patients, psychopathology was assessed using PANSS. All subjects were kept nil orally after midnight and they remained supine. First morning urine sample was collected from all the subjects at baseline and the samples were stored at −40°C within half an hour. Patients were started on treatment with antipsychotics as decided by the treating team. After 4 weeks, the patients were again assessed with PANSS scale and second urine samples were collected. The estimation of levels of HVA, 5-HIAA, and VMA in the urine was done by HPLC using Waters® 1500 series HPLC pump. Electrochemical detection was done by "Waters" 464-pulsed electrochemical detector. The working potential was kept at 750 mv and flow rate of 1 ml/minute. Iso-VMA was used as an internal standard. Breeze Software (Waters 2000) was used to calculate the results.

Statistical analysis

Data obtained were analyzed with SPSS version 16. Group differences between patients and controls were analyzed using independent *t*-test and Chi-Square test for continuous and categorical variables, respectively. Paired *t*-test was used to examine changes in psychopathology over time. Univariate ANCOVA was used to analyze group differences in monoamine metabolite levels between patients and controls at baseline after controlling for covariates. Repeated measures ANOVA were used to study the changes in monoamine metabolite levels over time in patients. Effect sizes were reported as eta-squared (η^2). The level of significance was kept at $p<0.05$ (2-tailed).

RESULTS

Sample characteristics are summarized in Table 1. There was a significant difference in terms of education, marital status, domicile, and occupation between the groups. Futhermore, the mean height and weight was lower in patients with schizophrenia compared to controls. Table 2 shows the change in psychopathology in patients with schizophrenia. There was the significant reduction in PANSS subscale scores after 4 weeks of treatment in all domains ($p<0.05$).

Table 3 shows baseline differences in monoamine metabolic levels (HVA, 5-HIAA, and VMA) between patients and controls after controlling for height and weight. There was a trend toward higher levels of HVA and VMA in patients with schizophrenia compared to controls, but with small effect size. Table 4 shows repeated measures ANOVA for change in monoamine metabolic levels (HVA, 5-HIAA, and VMA) after 4 weeks of treatment from baseline levels. There was a trend toward reduction in 5-HIAA levels (Pillai's Trace $F=3.28$, $p=0.079$), with small effect size. There was the significant positive correlation between the levels of 5-HIAA with HVA ($r=0.43$, $p<0.05$) and VMA ($r=0.69$, $p<0.001$). There was no correlation between PANSS subscale scores with monoamine metabolite levels at baseline or at 4 weeks.

DISCUSSION

The literature review supports the monoamine hypotheses of schizophrenia, although there are several inconsistencies; i.e. there are both positive and negative findings for monoamine metabolites. Our

Table 1: Socio-demographic and clinical characteristics

Variables	Patients (N=34)	Controls (N=15)	χ^2/t	p
Age, mean (SD)	31.76 (6.92)	31.80 (3.86)	0.01	0.985
Height (cm), mean (SD)	160.88 (8.74)	168.83 (4.99)	3.28**	0.002
Weight (kg), mean (SD)	49.65 (7.99)	64.60 (10.87)	5.39***	<0.001
Age of onset of illness, mean (SD)	26.09 (5.83)	-	-	-
Duration of illness (mths), mean (SD)	68.06 (61.12)	-	-	-
Drug free period (mths), mean (SD)	22.18 (40.57)	-	-	-
Antipsychotic dose (chloropromazine equivalent, mg), mean (SD)	485.35 (270.57)	-	-	-
Gender, n (%)				
Male	30 (88.2)	14 (93.3)	0.29	0.587
Female	4 (11.8)	1 (6.7)		
Education, n (%)				
Below matric	21 (61.8)	0	16.21***	<0.001
Above matric	13 (38.2)	15 (100)		
Marital status, n (%)				
Single	8 (23. 5)	8 (53. 3)	4.20*	0.040
Married	26 (76.5)	7 (46.7)		
Domicile, n (%)				
Urban	9 (26. 5)	13 (86.7)	16.64***	<0.001
Rural	18 (52.9)	0 (0)		
Semi urban	7 (20.6)	2 (13.3)		
Religion, n (%)				
Hindu	29 (85.3)	15 (100)	2.45	0.117
Muslim	5 (14.7)	0 (0)		
Occupation, n (%)				
Skilled	8 (23.5)	13 (86.7)	16. 94***	<0.001
Unskilled	26 (76.5)	2 (13. 3)		
Subtype, n (%)				
Paranoid	18 (52.9)	-	-	-
Non-paranoid	16 (47.1)	-		
Family mental illness, n (%)				
Present	13 (38.2)	-	-	-
Absent	21 (61.8)	-		
Suicide attempt, n (%)				
Present	5 (14.7)	-	-	-
Absent	29 (85.3)	-		

SD: Standard deviation. *p<0.05, **p<0.01, ***p<0.001 (2-tailed)

Table 2: Change in PANSS subscale scores from baseline (N=34)

PANSS subscales	Mean (SD)		Paired t	p
	Baseline	After 4 weeks		
Positive syndrome	21.85 (7.46)	13.46 (5.62)	6.93***	<0.001
Negative syndrome	26.57 (8.09)	17.13 (8.05)	6.44***	<0.001
General psychopathology	45.13 (7.12)	30.13 (8.62)	9.75***	<0.001
Anergia	14.37 (5.00)	9.33 (4.61)	6.19***	<0.001
Thought disorder	9.80 (3.58)	6.67 (3.16)	7.05***	<0.001
Activation	6.73 (1.50)	4.00 (1.39)	7.42***	<0.001
Paranoid belligerence	11.03 (2.83)	6.63 (2.57)	9.69***	<0.001
Depression	7.33 (2.70)	6.23 (2.72)	2.35*	0.026

PANSS: Positive and negative syndrome scale, SD: Standard deviation. *p<0.05, **p<0.01, ***p<0.001 (2-tailed)

Table 3: ANCOVA showing group differences in urinary metabolites between patients and normal controls at baseline (after adjusting for height and weight)

Variables	Mean (SD)		F (df=1,45)	p	Effect size (η^2)
	Patients (N=34)	Controls (N=15)			
HVA	0.024 (0.041)	0.005 (0.005)	3.29	0.076	0.068
5-HIAA	0.058 (0.085)	0.044 (0.137)	2.04	0.161	0.043
VMA	0.045 (0.056)	0.016 (0.020)	3.51	0.067	0.073

HVA: Homovanillic acid, 5-HIAA: 5-Hydroxy indole acetic acid, VMA: Vanillyl mandelic acid, SD: Standard deviation. *p<0.05, **p<0.01, ***p<0.001 (2-tailed)

study not only examined the relationship of monoamine metabolites with psychopathology but also studied the changes in their levels with antipsychotic treatment. The result of the study revealed that the patients had significantly higher concentration of HVA, as compared to controls. HVA is the final metabolite of dopamine and is excreted mostly in a free unconjugated form in the urine. The result is in accordance with the dopamine hypothesis, which suggests elevated concentration of HVA in cerebrospinal fluid (CSF), plasma or urine. Studies measuring HVA in plasma have found increase levels in schizophrenic patients as compared to controls [8-10].

Table 4: Repeated measures ANOVA showing change in monoamine metabolite levels (HVA, 5-HIAA and VMA) at baseline and after 4 weeks of treatment (N=34)

Monoamine metabolite	Mean (SD)		Pillai's trace F (df=1,33)	p	Effect size (η^2)
	Baseline	After 4 weeks			
HVA	0.024 (0.041)	0.017 (0.034)	1.02	0.319	0.030
5-HIAA	0.058 (0.085)	0.027 (0.055)	3.28	0.079	0.091
VMA	0.045 (0.056)	0.044 (0.068)	0.01	0.942	0

HVA: Homovanillic acid, 5-HIAA: 5-Hydroxy indole acetic acid, VMA: Vanillyl mandelic acid, SD: Standard deviation. *p<0.05, **p<0.01, ***p<0.001 (2-tailed)

The patients also had significantly higher concentration of VMA, metabolite of norepinephrine as compared to healthy controls. Studies of plasma norepinephrine and its metabolite also give clue to norepinephrine involvement in schizophrenia. The result of this study is in accordance with reported studies of norepinephrine or its metabolites in urine, such as Gershan *et al*. observed that 24 hrs urinary MHPG was elevated in schizophrenia population [11], and Barbeito *et al*. found elevated urinary norepinephrine levels in sample of 21 schizophrenic patients [12].

There was no difference in the amount of 5-HIAA in the urine between the patients and the controls. A meta-analysis of 12 studies were performed by Tuckwell and Koziol found that there is no evidence to suggest that 5-HIAA is altered in schizophrenia, whether in the acute phase or chronic phase [13].

There was no relationship of urinary HVA and VMA levels with PANSS subscale scores; this is similar to previous studies which did not find such association [14,15]. In contrast, some studies report weak association of plasma HVA levels with specific subscale scores [16]. The possibility that HVA and VMA which were increased as compared to the control population, did not show any change with clinical improvement, suggests that they may be a trait marker for the psychosis rather than state marker. A similar lack of correlation of dopamine and norepinephrine metabolites was observed in previous studies [17]. However, in Akiyama *et al*. study, there was an increase in the plasma HVA levels in the first week with antipsychotic treatment, which reached baseline after 4 weeks [18]. This rise in HVA levels correlated significantly with positive symptom scores at week 5. Furthermore, higher pretreatment plasma HVA levels may predict a better clinical response to antipsychotics [19].

The result of the study indicates that the monoamine metabolites, 5-HIAA, HVA, and VMA in urine significant correlated with each other at baseline measurements and after treatment. The present observation of correlation between these metabolites is compatible with the idea that the balance between dopamine and serotonin function in the CNS is of physiologic importance in the human; and suggest that these are coupled. Similar findings of cross-sectional correlation between CSF concentration of 5-hydroxy indole acetic and HVA have been demonstrated in previous studies [20,21]. Furthermore that the peripheral noradrenegic system contributes to about 70% of the HVA. This correlation is likely to be reflected in urine and plasma, as in our study.

CONCLUSION

There was a trend toward higher urinary levels of HVA and VMA in the patients as compared to controls, but no difference was seen for 5-HIAA. For HVA and VMA, there was no change found with antipsychotic treatment, whereas there was a trend toward reduction in 5-HIAA levels. The main advantages of measuring urinary monoamine metabolites are that it is both noninvasive and painless. Because of the possible cumulative effect, urinary monoamine metabolites over several hours have been suggested to be more sensitive in detecting minor changes in production rates of these metabolites than a single plasma measurement [22]. Repeated estimations of these metabolites should be done during the study period to follow them more accurately. Considering intra-individual variability, repeated sampling should be done. The conclusions drawn from these studies have led to various hypotheses, and none is proven beyond doubt. The major limitation in our study was small sample size, which reduced study power. Furthermore, females were underrepresented in our study, which limits generalization across gender.

REFERENCES

1. Howes OD, Kapur S. The dopamine hypothesis of schizophrenia: Version III – the final common pathway. Schizophr Bull 2009;35(3):549-62.
2. van Kammen DP, Peters J, Yao J, van Kammen WB, Neylan T, Shaw D, *et al*. Norepinephrine in acute exacerbations of chronic schizophrenia. Negative symptoms revisited. Arch Gen Psychiatry 1990;47(2):161-8.
3. Vollenweider FX. Advances and pathophysiological models of hallucinogenic drug actions in humans: A preamble to schizophrenia research. Pharmacopsychiatry 1998;31 Suppl 2:92-103.
4. Mathieu P, Lemoine P, Szestak M, Greffe J, Gros N, Echassoux C. Homovanillic acid (HVA) urinary excretion and day/night rhythm of chronic schizophrenic patients. Preliminary observations. Encephale 1985;11(5):199-202.
5. Markianos M, Tripodianakis J, Garelis E. Neurochemical studies on tardive dyskinesia. II. Urinary methoxyhydroxyphenylglycol and plasma dopamine-beta-hydroxylase. Biol Psychiatry 1983;18(3):347-54.
6. Kemali D, Del Vecchio M, Maj M. Increased noradrenaline levels in CSF and plasma of schizophrenic patients. Biol Psychiatry 1982;17(6):711-7.
7. Kay SR, Fiszbein A, Opler LA. The positive and negative syndrome scale (PANSS) for schizophrenia. Schizophr Bull 1987;13(2):261-76.
8. Davis KL, Davidson M, Mohs RC, Kendler KS, Davis BM, Johns CA, *et al*. Plasma homovanillic acid concentration and the severity of schizophrenic illness. Science 1985;227(4694):1601-2.
9. Chang WH, Chen TY, Lee CF, Hung JC, Hu WH, Yeh EK. Plasma homovanillic acid levels and subtyping of schizophrenia. Psychiatry Res 1988;23(3):239-44.
10. Baeza I, Castro-Fornieles J, Deulofeu R, de la Serna E, Goti J, Salvà J, *et al*. Plasma homovanillic acid differences in clinical subgroups of first episode schizophrenic patients. Psychiatry Res 2009;168(2):110-8.
11. Gershan S, Shopsin B, Wilk S. Urinary MHPG in schizophrenic population. Neuropsychobiology 1976;2:145-60.
12. Barbeito L, Lista A, Silveira R, Dajas F. Resting urinary catecholamine excretion in schizophrenics: Methodology and interpretation of results. Biol Psychiatry 1984;19(10):1419-25.
13. Tuckwell HC, Koziol JA. On the concentration of 5-hydroxyindoleacetic acid in schizophrenia: A meta-analysis. Psychiatry Res 1996;59(3):239-44.
14. Crowley TJ, Hoehn MM, Rutledge CO, Stallings MA, Heaton RK, Sundell S, *et al*. Dopamine excretion and vulnerability to drug-induced Parkinsonism. Schizophrenic patients. Arch Gen Psychiatry 1978;35(1):97-104.
15. Koreen AR, Lieberman J, Alvir J, Mayerhoff D, Loebel A, Chakos M, *et al*. Plasma homovanillic acid levels in first-episode schizophrenia. Psychopathology and treatment response. Arch Gen Psychiatry 1994;51(2):132-8.
16. Zhang ZJ, Peet M, Ramchand CN, Shah S, Reynolds GP. Plasma homovanillic acid in untreated schizophrenia – relationship with symptomatology and sex. J Psychiatr Res 2001;35(1):23-8.
17. Javaid JI, Sharma RP, Janicak PG, Davis JM. Plasma HVA in psychiatric patients: Longitudinal studies. Psychopharmacol Bull 1990;26(3):361-5.
18. Akiyama K, Tsuchida K, Kanzaki A, Ujike H, Hamamura T, Kondo K, *et al*. Plasma homovanillic acid levels and therapeutic

outcome in schizophrenics: Comparisons of neuroleptic-naive first-episode patients and patients with disease exacerbation due to neuroleptic discontinuance. Biol Psychiatry 1995;38(10):639-48.
19. Chang WH, Hwu HG, Chen TY, Lin SK, Lung FW, Chen H, *et al.* Plasma homovanillic acid and treatment response in a large group of schizophrenic patients. Schizophr Res 1993;10(3):259-65.
20. Agren H, Mefford IN, Rudorfer MV, Linnoila M, Potter WZ. Interacting neurotransmitter systems. A non-experimental approach to the 5HIAA-HVA correlation in human CSF. J Psychiatr Res 1986;20(3):175-93.
21. Lindström LH. Low HVA and normal 5HIAA CSF levels in drug-free schizophrenic patients compared to healthy volunteers: Correlations to symptomatology and family history. Psychiatry Res 1985;14(4):265-73.
22. Contreras SA, Maas JW, Seleshi E, Bowden CL. Increase in human urine homovanillic acid concentration after neuroleptic treatment is the same with or without debrisoquin administration. Arch Gen Psychiatry 1987;44(12):1109-10.