

Folic acid, vitamin B₁₂ and homocysteine levels following olanzapine administration in schizophrenia patients

Fahmida Hasnat, Zesmin F. Dewan, Mir Misbahuddin, Nargis Akhter, Jhunu Shamsunnahar and Habiba Akhter Bhuiyan

Article Info

Department of Pharmacology, Faculty of Basic Science and Paraclinical Science, Bangabandhu Sheikh Mujib Medical University, Shahbag, Dhaka, Bangladesh (FH, ZFD, MM, NA, HAB); Department of Psychiatry, Faculty of Medicine, Bangabandhu Sheikh Mujib Medical University, Shahbag, Dhaka, Bangladesh (JS)

For Correspondence:

Fahmida Hasnat
dr.fahmidahasnat@gmail.com

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Abstract

The purpose of this study was to assess the levels of folic acid, vitamin B₁₂ and homocysteine in the serum of schizophrenia patients (n=20) and to evaluate the effect of olanzapine on these biomarkers. The blood was also collected from the 10 healthy volunteers as the control. Compared to control, the serum folic acid (p=0.005) and vitamin B₁₂ levels (p=0.211) were higher in the schizophrenia patients, whereas no difference was evident in the serum homocysteine level. But significantly higher levels of serum folic acid (p=0.005), vitamin B₁₂ (p=0.047) and significantly lower level of serum homocysteine (p=0.000) were observed after 10 weeks of olanzapine administration. BPRS score was reduced significantly after the intervention. The Pearson correlation coefficient test showed a statistically not significant negative relationship between the serum folate, vitamin B₁₂ and homocysteine levels. In conclusion, olanzapine can significantly elevate the serum folic acid and vitamin B₁₂ levels whereas it can lower the serum homocysteine level which may contribute to the improvement of symptoms of schizophrenia.

Introduction

Mental illness has reached an alarming proportion over the globe and is increasing day by day. Among them, schizophrenia is one of the commonest psychotic illnesses. Schizophrenia is a disabling mental disorder that causes disruptions in thought processes, perceptions, emotions, and cognitive function, resulting in a clinical phenotype with symptoms such as delusions, hallucinations, negativism, or intellectual and social deterioration. It has a lifetime prevalence of approximately 1% of the world's population.¹ Schizophrenia presumably includes a complex pathophysiology and multifunctioning genes might be involved in its pathophysiology. Non-genetic factors such as ethnicity, drug or alcohol abuse, lifestyle, use of medications, prenatal and neonatal infections, maternal malnutrition, and complications during birth may play an important role in the development of schizophrenia.²

Several biological studies have suggested that the alteration in the normal metabolic process leading to alteration of the levels of folic acid and vitamin B₁₂, and subsequently increased levels of homocysteine might play an important role in the pathophysiology of schizophrenia.³⁻⁶ Folic acid and vitamin B₁₂ are important cofactors for the synthesis of purine and thymidine precursors of nucleic acid.⁷ The metabolism of some amino acids are e.g., interconver-

sion of serine to glycine and conversion of homocysteine to methionine or cysteine⁸ and the synthesis of s-adenosylmethionine (SAM). SAM is the major methyl group donor for the various reactions of methylation, by promoting the conversion of homocysteine into methionine.⁸

Homocysteine, a sulfur-containing amino acid, is metabolized by remethylation using folate and vitamin B₁₂ as cofactors. Recent several studies have reported that increased plasma homocysteine levels were observed in schizophrenia. It has also been estimated that 5 µmol/L increases in the plasma homocysteine level may lead to an increased risk of schizophrenia by 70%.⁹ Several studies have shown a positive correlation between the homocysteine levels and the severity of negative symptoms of schizophrenia.¹⁰⁻¹¹ Several drugs may interfere the metabolic pathways of folic acid and vitamin B₁₂, leading to an alteration of the plasma homocysteine levels. However, still, there are some controversial results and a lack of evidence regarding these association.

In this study, patients with schizophrenia were studied to determine any alteration in homocysteine, folate, and vitamin B₁₂ levels and their correlation with the second generation antipsychotic drug olanzapine which is commonly used to treat the schizophrenic patients. Assessment of the brief psychiatric rating scale (BPRS) score also acts as an aid to the fulfillment of the



aim of the present study. This study was carried out to evaluate the association of olanzapine between the serum folate, vitamin B₁₂, and homocysteine levels in patients with schizophrenia, so that the development and progression of schizophrenia can be prevented by taking the interventions that could reduce the level of homocysteine.

Materials and Methods

This study was conducted from March 2016 to July 2017. A total of 41 schizophrenic patients fulfilling the inclusion criteria were enrolled in this study but only 20 patients ultimately completed this study. Detailed history and clinical examination were done.

Inclusion criteria were patients with schizophrenia from 18 years to 45 years of either sex diagnosed by a psychiatrist and prescribed olanzapine as part of their treatment schedule. Patients were diagnosed according to the DSM-5 criteria. Patients taking olanzapine were advised to report after 10 weeks. Healthy age-matched persons of both sexes were enrolled from the Department of Pharmacology were included as the control for comparison. They were adult persons without suffering from any systemic/serious disease.

Exclusion criteria were a) patients with schizophrenia with age range of <18 years and >45 years; b) patients receiving antipsychotic drugs within the last 30 days; c) patients having serious systemic infection, systemic diseases or malignancy; d) pregnancy and lactation during the last year.

Five milliliter of blood was collected before and after 10 weeks of olanzapine administration. BPRS scoring was also done by the researcher under the guidance of a psychiatrist.

Blood sample collection, processing and preservation

With all aseptic precaution, the blood samples of schizophrenic patients were collected from a convenient vein with a disposable plastic syringe and collected into a clot activator test tube. It was then kept in standing position till the clot formation. Then the serum was separated from the cells by centrifugation (3,500 rpm for 10 min). Finally stored at -20°C in a refrigerator until the estimation of folic acid, vitamin B₁₂ and homocysteine.

Chemicals and reagents

Folic acid was purchased from the Sigma-Aldrich (USA). The organic solvents methanol purchased from (HPLC grade) the Merck Millipore Corporation, Germany and acetonitrile from the Daejung, Korea. Dimethyl sulfoxide and orthophosphoric acid (85% pure) were purchased from the Merck,

India. Potassium dihydrogen orthophosphate was purchased from the Loba Chemie, India. Freshly prepared distilled water was used daily.

Estimation of folic acid

Estimation of serum folic acid was done using the HPLC.^{12,13} The HPLC system consisted of a Thermo Fisher Scientific Ultimate 3000 series, USA. All solutions were filtered and sonicated for 2 min and prepared fresh daily. The standard solution of folic acid was prepared by weighing 1 mg of folic acid into a test tube and dissolved it in 1 mL of dimethyl sulfoxide (1 mg/mL). Then made serial dilution into 100, 10, 1 µg/mL. The solution A of the mobile phase was prepared by adding phosphoric acid (150 µL) and potassium dihydrogen orthophosphate (408 mg) into the 300 mL of distilled water. The pH of this solution was adjusted to 2.3. The solution B was the methanol (about 120 mL). The solution A of 280 mL was mixed with 120 mL of solution B. This solution was sonicated for 2 min and prepared fresh daily. All the solutions of folic acid were wrapped with aluminum foil to prevent degradation from the light.

Acetonitrile (800 µL) was added to 400 µL of fresh serum in a test tube. Then, vortex the sample for 30 sec and wait for 10 min. Again centrifuge the sample at 3,500 rpm for 10 min. The supernatant was collected and placed it into the HPLC. The pH of the entire mixture was 2.3 and the flow rate was 0.8 mL/min. Separation was achieved by using column C-18, maintaining at 35°C temperature in a column oven and the detection was conducted at its maximum wavelength of 290 nm.

Estimation of vitamin B₁₂

Estimation of serum vitamin B₁₂ was done using the HPLC.^{12,13} The standard solution of vitamin B₁₂ was prepared by weighing 1 mg of vitamin B₁₂ into a test tube and dissolved it in 1 mL of solution A (1 mg/mL). Then the serial dilutions into 100, 10, 1 and 0.1 µg/mL were made. The mobile phase was prepared by adding trifluoroacetic acid (100 µL) into 400 mL of distilled water (solution A). The pH of this solution was adjusted to 2.9. Solution B was the methanol (about vitamin B₁₂). The solution A (350 mL) was mixed with 150 mL of solution B. This 500 mL of solution was sonicated for 2 min and prepared fresh daily. All the solutions of vitamin B₁₂ were wrapped with aluminum foil to prevent degradation from light.

Dichloromethane (800 µL) was added to 400 µL of fresh serum in a test tube for the precipitation of protein. Vortex and centrifuge the sample at 3,500 rpm for 10 min to separate the precipitate from the supernatant. The supernatant was separated to which 400 µL of methanol was added. Wait for 10 min. Again vortex and centrifuge the sample to observe the two clear layers of the solution.

Separate the upper layer and evaporate the methanol using nitrogen/argon gas. 200 µL of solution A was added to the test tube to dissolve vitamin B₁₂. Pour the sample into HPLC vial. The pH of the entire mixture was adjusted to 2.9 and the flow rate was 0.5 mL/min. The separation was achieved using column C-18, maintaining at 30°C temperature in a column oven and the detection was conducted at its maximum wavelength of 230 nm.

Estimation of serum homocysteine

Serum homocysteine level was measured by auto-analyzer (Architect, Abbott Plus).

Statistical analysis

Data were processed and analyzed using computer software SPSS (Statistical Package for Social Science) version 17 and online calculator (Social

Science Statistics). The results have expressed as the mean ± SD for the continuous variables and the Wilcoxon Signed-Rank test and Mann-Whitney U test were done in case of non-parametric data. Chi-square (χ²) test was employed to compare the categorical data between groups, while unpaired and paired sample t-test were used to compare the continuous data between and within groups respectively. The correlation was done using Pearson correlation statistics to observe the relationship between clinical and biochemical parameters. The level of significance was set at 5% and p<0.05 was considered significant.

Results

The mean age of the patients with schizophrenia was 25.7 ± 6.1 years and that of the control group was 34.0 ± 2.8 years (Table I). Eighty percent of the study group were married and from the rural area compared to the control group.

Table II shows that serum folic acid level was significantly higher in the study group than the control group (p=0.005) and the percentage of difference was 182.5. Serum vitamin B₁₂ level was also higher but statistically not significant (p=0.211) in between the control and the study group. Lack of significant difference was found in the total homocysteine level of the control and the study group (p=0.810).

Following intervention for 10 weeks, the mean ± SD of serum folic acid level was increased to 27.7 ± 26.6 ng/mL, which was statistically significant (p=0.005). Consequently, statistically significant (p=0.047) higher level of vitamin B₁₂ was observed after 10 weeks of olanzapine administration. Highly significant changes were seen in the serum homocysteine levels after intervention (p=0.000). In study group after 10 weeks of olanzapine administration, BPRS score was reduced significantly (p=0.000) than the baseline level.

There was a negative correlation between BPRS score and serum folic acid, vitamin B₁₂ and homocysteine level after 10 weeks of olanzapine treatment which were not statistically significant.

Discussion

This is the first study in Bangladesh to investigate the serum folate, vitamin B₁₂ and homocysteine concentrations in drug naïve or acute schizophrenic patients before and after 10 weeks of olanzapine administration. Most of the studies done in recent years included either only male schizophrenic patients or with different onsets of diseases treated with different generations of antipsychotic drugs.¹⁴ Our data provide evidence that altered levels of

Table I

Characteristics of patient and control

	Group	
	Patient (n=20)	Control (n=10)
Age (years)	25.7 ± 6.1	34 ± 2.8
Sex		
Male	1	4
Female	9	6
Marital status		
Married	16	9
Unmarried	4	1
Habitat		
Rural	16	1
Urban	4	9

Figures in the parentheses indicate corresponding%; ^aChi-squared Test was done to analyze the data; Data were analyzed using ^bUnpaired t-test and were presented as mean ± SD; n = number of participants in each group

Table II

Serum folic acid, vitamin B₁₂ and homocysteine levels in study group

Parameters	Baseline control (n=10)	Olanzapine administration (n=20)	
		Before	After
Serum folic acid (ng/mL)	6.7 (2.9)	18.9 (15.4)	27.7 (26.6)
Serum vitamin B ₁₂ (pg/mL)	698.7 (270.6)	1081 (703)	1408 (658)
Serum homocysteine (µmol/L)	14.3 (5.6)	14.6 (6.1)	11.8 (6.3)
Brief psychiatric rating scale (BPRS)		61.1 (4.7)	36.6 (2.7)

Data were presented as mean (SD)

serum folic acid, vitamin B₁₂ and homocysteine levels exist in the schizophrenia patients and olanzapine had the significantly higher effect on these above three levels.

The present study shows that the serum folic acid remained at a significantly higher level ($p=0.005$) and vitamin B₁₂ level was not significantly higher in schizophrenia patients prior to olanzapine administration as compared to the control group. These findings do not correlate with some other studies where serum folate and vitamin B₁₂ levels were reduced in patients with schizophrenia.^{5,15} Result of the present study is contradictory with those studies might be due to drug naïve or acutely diagnosed schizophrenic patients before administration of antipsychotics were enrolled in this study. But in the above studies, chronic or refractory schizophrenic patients who were already received antipsychotic drugs were recruited.

Antipsychotics hamper the folate transport mechanism systems which might be one of the causes of folate deficiency. Genetical mutations were observed and assessed in schizophrenic patients of the above studies. However, genetical mutations were not assessed in the present study. Moreover, dietary habit, socio-economic status and lifestyles appear different in this country from the European countries. These factors might influence the serum folic acid and vitamin B₁₂ levels. Moreover, most of the participants in the present study were rural people and from the low socio-economic condition and consumed a vegetarian cereal pulse diet, which is a rich source of folic acid and vitamin B₁₂. Like this study, some authors reported in schizophrenic patients where the mean serum vitamin B₁₂ levels were significantly higher than those in the control group.¹⁶ The present study did not obtain significantly different levels of serum homocysteine compared to control and schizophrenia group before the intervention. In agreement with this findings, similar results were obtained in some studies.^{9,17} Also, some authors reported that elevated homocysteine levels were observed in various sub-groups of schizophrenia patients^{9,18} including both drug naïve first episode psychosis patients. As no folate deficiency was observed in the present study between the control and schizophrenia patients before olanzapine administration and there remain an inverse relationship of serum homocysteine levels with those of the folates. This might be possible that the serum homocysteine levels were not increased due to lack of being converted to methionine and resulting accumulation of homocysteine.

The present study has observed serum folic acid ($p=0.005$) and vitamin B₁₂ ($p=0.047$) levels in schizophrenia patient were increased significantly after 10 weeks of olanzapine treatment. Also highly significant lower level of serum homocysteine ($p=0.000$)

was obtained in schizophrenia patients after olanzapine treatment. Only a few studies have been studied discretely and separately on these topics with contradictory results and therefore the mechanism, cause and consequences of these three levels in schizophrenia patients remain unclear. The results of one of the recent studies showed that no differences were found regarding serum vitamin B₁₂ and folic acid after atypical antipsychotic treatment.¹⁹ An observational study of drug-naïve first episode schizophrenia patients showing lack of significant differences in homocysteine levels in the course of antipsychotics therapy.²⁰ In the cross-sectional study, which revealed significantly decreased serum folate level, but not homocysteine or vitamin B₁₂ level on chronic schizophrenia patient, who received high doses of typical antipsychotics, e.g. chlorpromazine >400 mg.¹⁴ The findings of above two studies does not conform to the present study as because in the present study the patients were not folic acid, vitamin B₁₂ deficient, especially folic acid levels were significantly higher in schizophrenia patients before and after olanzapine treatment compared to those in the controls. Another cross-sectional study by Wysokinski and Kioszewska (2013)²¹ shows that no significant differences were found in serum homocysteine levels between healthy controls and schizophrenia patients who received clozapine monotherapy. So, we can say that differences in lifestyle between the study groups may account for differences in folate and vitamin B₁₂ levels and also the different methodologies of homocysteine, folate and vitamin B₁₂ assessment and genotypic differences in the samples studied could also be possible factors for the inconsistencies in results.

The scores of BPRS of pretreated and post-treated schizophrenia patients were assessed. BPRS scores of schizophrenia patients at baseline was reduced significantly ($p=0.000$) after 10 weeks of drug treatment. In the present study, the statistically insignificant negative trend of association between BPRS score and serum folic acid, vitamin B₁₂ and homocysteine levels have been observed.

It was evident in the present study that, while the lowered serum folate and vitamin B₁₂ levels were elevated after 10 weeks of olanzapine administration in schizophrenia patients, the serum homocysteine levels were decreased. This supported the existing inverse relationship between folate, vitamin B₁₂ with homocysteine. Along with them, the decrease in the scores of BPRS has suggested to us that olanzapine, an antipsychotic drug of the atypical group, was able to reverse observed changes in levels of serum folate, vitamin B₁₂ and homocysteine in schizophrenia patients. So, nutrient supplementation especially the folate and vitamin B₁₂ are not necessary to reduce the homocysteine level in

preventing the development and progression of schizophrenia.

Conclusion

Olanzapine significantly elevated the serum folic acid, vitamin B₁₂ levels and significantly lowered the serum homocysteine level in schizophrenia patient. The brief psychiatric rating scale was also lowered significantly. These findings suggest that olanzapine play an important role in the improvement of the symptoms of schizophrenia.

Ethical Issue

The study was approved by the University Institutional Review Board (Ref. No. 8803 and Date of issue 27-08-2016). All participants were informed about the nature and purpose of the study in local language (Bangla) and then written consent was taken from each participant.

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