CSF AMINES AND THEIR METABOLITES IN FIRST EPISODE DRUG NAIVE SCHIZOPHRENIC PATIENTS AND THEIR CORRELATIONS WITH DIMENSIONS OF SCHIZOPHRENIA

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ABSTRACT

There has been great interest in the neurobiological substrate of the different dimensions of schizophrenia. This has largely focused on structural and functional changes while it has been acknowledged that there is a relation between pharmacological response and neurotransmitter alteration on these dimensions. Atypical anti psychotics which affect both positive and negative symptoms mediate their action predominantly through dopaminergic and serotonergic receptors. The current study extends this further looking at CSF amines. 37 drug naive first episode psychosis patients with the duration of illness less than 2 years were included. Patients were assessed with SAPS (Scale for Assessment of Positive Symptoms) and SANS (Scale for Assessment of Negative Symptoms). Lumbar puncture was done under sterile conditions and CSF was analyzed by HPLC for dopamine, serotonin and their metabolites. Mean CSF 5-HIAA was 25.35ng/dl. Mean CSF 5-HT was 7.72 ng/dl. Mean CSF HVA was 36.99 ng/dl. Mean CSF 5-DA was 3.06 ng/dl. There was significant positive correlation between CSF5-HIAA and Negative and Disorganization dimensions. There was significant negative correlation between CSF HVA and Psychosis dimension. There is evidence to support that the implication of serotonin in Negative and Disorganization dimensions and the Serotonin- Dopamine interaction and dimensions of schizophrenia.

Key words: Dopamine, Serotonin, Schizophrenia

The etiology of the group of illnesses which are referred as 'schizophrenias' continues to be unanswered in various aspects to the researchers in the field of psychiatry and its allied sciences, despite efforts over the past so many decades. The heterogeneity of the schizophrenia phenotype is perhaps the most fundamental problem that clinicians and researchers who study this disorder must face. The heterogeneity is cross-sectional, longitudinal and aetiopathophysiological as well in that different neurochemical abnormalities involving multiple neurotransmitters may be preferentially involved in different patients resulting in the myriad symptomatology of the disease. The study of schizophrenia in terms of subtypes or syndromes can be categorical or dimensional which assumes that a syndrome may be a continuum (as opposed to an all- or-none phenomenon in categorical) and that dimensions can overlap in a given patient.

Dimensional approaches for the study of schizophrenia grew out of early attempts to apply the positive/negative distinction, initially proposed by Hughlin Jackson and later reformulated by Crow as Type 1 and Type 2 (Crow,1980). As rating scales were developed that permitted the

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measurement of a broad range of positive and negative symptoms using continuous measures (Andreasen, 1982; Andreasen and Olsen, 1982), a number of investigators began to apply factor analytic and correlational approaches in order to examine the interrelationships between these symptoms (Andreasen and Grove, 1986; Liddle, 1987; Bilder et al., 1985, Andreasen et al., 1995). Most of these studies have shown that the symptoms of schizophrenia fall into three dimensions: psychoticism, disorganization and negative symptoms (Andreasen et al., 1995). This three-dimensional hypothesis still remains the most robustly replicated of all competing models of schizophrenic psychopathology (Amador and Gorman, 1998).

There is ample evidence from the literature to suggest the involvement of amines such as Dopamine and Serotonin and their interactions in the causation of schizophrenia. After the atypical antipsychotics were introduced attention is being given to neurochemical basis of schizophrenia. These atypical antipsychotics affect both positive and negative symptoms and mediate their action predominantly through dopaminergic and serotonergic receptors. There has been great interest in the neurobiological substrate of the different dimensions of schizophrenia. This has largely focused on structural and functional changes while it has been acknowledged that there is a relation between pharmacological response and neurotransmitter alteration on these dimensions. The current study extends this further looking at CSF amines and their possible relationships with the dimensions of schizophrenia.

Various studies have used techniques of measuring amine metabolites in body fluids and their possible correlations with symptoms of schizophrenia. The present study is aimed at finding a correlation between the concentrations of dopamine and serotonin and their acid metabolites Homo Vannilic Acid (HVA) and 5-Hydroxy Indole Acetic Acid (5-HIAA) in the Cerebro Spinal Fluid in a group of drug naive schizophrenics and three dimensions of schizophrenia.

**MATERIALS AND METHODS**

The subjects for the present study consisted of 37 drug-naive patients fulfilling the Diagnostic and Statistical Manual of Mental Disorders-Fourth Edition (DSM-IV) criteria for schizophrenia or schizophreniform disorder (American Psychiatric Association, 1992). Patients were selected for the study between December 1998 and September 1999. Both sexes between 17-45 years of age were included. The duration of illness varied between 1 month to 2 years. Patients with the history of any general medical disorder, known neurological conditions, head injury, Persistent substance use disorders, concomitant Axis I or Axis II disorders and significant suicidal or homicidal risk were excluded from the study. A written informed consent was taken from the patients and their relatives and the patient was admitted.

Assessment of the psychopathology was carried out using the Scale for the Assessment of Positive Symptoms (SAPS) and Scale for the Assessment of Negative Symptoms (SANS). The scores on each of the three dimensions were calculated based on the method described by Liddle (1987). The score on the psychotic dimension was the sum of items for auditory hallucinations commenting on the patient's behaviour, persecutory delusions and delusions of reference. The score on the disorganization dimension was the sum of the scores for items measuring inappropriate affect, poverty of content of speech and the global rating of positive formal thought disorder. The negative dimension was scored using items assessing poverty of speech, decreased spontaneous movement and the average of four items reflecting aspects of blunting of affect (affective non-responsivity, unchanging facial expression, apacity of expressive gestures and lack of vocal inflections). Thus the maximum score on each of the three dimensions would be 15.

Patients were kept off any kind of medications till next day morning. Lumbar puncture was done between 9.00-9.30 AM with...
overnight fasting. Lumbar puncture were done in left lateral position at L4-5 levels with out using local anaesthetic. About 5 ml of clear CSF was obtained for the analysis. CSF was collected in a bottle and with in 30 minutes was stored at -80° C till the day of the analysis.

Determination of Serotonin (5HT), Dopamine (DA), 5- Hydroxy indole acetic acid (5-HIAA), Homo vanilic acid (HVA) in CSF was done using High Pressure Liquid Chromatography (HPLC) with Electrochemical Detection (ED) (SHIMADZU, JAPAN). Reverse phase C18 column (25cmx4.6cm, pore size-0.5mic) was used for separation of the amines and their metabolites at room temperature. Mobile Phase [20 mM Sodium acetate, 1.9mM Haptane sulphonic acid, 1mM EDTA. 0.002% Dibutylamine with 16% Methanol adjusted at the pH of 3.97] was pumped at 1ml/ min flow rate. Biogenic amines and their metabolites were detected by measuring the current with a glassy carbon electrode at 0.6 volts relative to Ag- AgCl reference electrode. Standards were Serotonin, Dopamine, HVA and HIAA (Sigma, USA). Standards were filtered through 0.2 μ Millipore filters and injected onto the HPLC column. HIAA had a retention time of 12.8 min, HVA had a retention time of 19.1min, DA had a retention time of 11.5min and 5-HT had a retention time of 30.6min. 5 microns of standards were injected with every batch of samples. Sample preparation included extraction with 3M Per Chloro Acetic acid (PCA) and CSF sample were filtered through 0.2 μ Millipore filters and treated with equal volume of 3 M PCA. The precipitate was centrifuged at 10,000 rpm for 15 minutes. The supernatant was filtered using 0.2 μ filters. 20μ L of the prepared samples were injected in to the HPLC in batches along with standard. The values of amine and their metabolites obtained were expressed as ng/ml (Nielsen and Johnson, 1982).

Statistical analysis was done using Statistical Package for Social Sciences (SPSS), version 7.5. A step-wise multiple regression analysis was done to assess the relationship between score on the three dimensions and the levels of monoamines and their metabolites in CSF. Only those variables with significant F values would enter the relationship between score on the three dimensions and the CSF 5 HIAA/HVA ratio. Factor analysis with varimax rotation was done using amine and their metabolites and three dimensional scores.

RESULTS

37 Patients meeting the inclusion criteria were included in the study. Out of these 25 (67.57%) were males and 12 (32.43%) were females. The study sample had a mean age of 28.62 years. 26 patients were diagnosed as Schizophrenia according to DSM IV criteria, and 11 were diagnosed as Schizophreniform disorder. As described in the methodology dimensional scores for the 3 dimensions were calculated. Table 1 shows Socio demographic data, illness characteristics and amine levels.

| Variable                  | Mean | S.D. | Range  |
|---------------------------|------|------|--------|
| Age                       | 28.62| 6.55 | 17-42  |
| Age of onset              | 28.05| 6.44 | 17-40  |
| Duration of illness       | 9.13 | 7.07 | 1-24   |
| Psychosis dimension score | 9.24 | 3.77 | 2-15   |
| Negative dimension score  | 8.11 | 4.05 | 1-15   |
| Disorganization dimension score | 5.81 | 3.11 | 0-11   |
| C-5HT                     | 7.72 | 1.85 | 5.17-12.02 |
| C-HIAA                    | 25.35| 4.39 | 18.89-39.94 |
| C-DA                      | 3.06 | 0.62 | 2.03-4.51  |
| C-HVA                     | 36.98| 5.65 | 28.08-51.49 |

A step-wise Multiple Regression Analysis was done to identify the relationship between the score on three dimensions as independent variable and the levels of Amines and their metabolites in
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CSF as dependent variable. A step wise Multiple Regression Analysis was also done with the levels of Amines and their metabolites in CSF as independent variable, scores on three dimensions as dependent variable. Ratio of 5HIAA/HVA was calculated. A step wise Multiple Regression Analysis was done to identify the relationship between the score on three dimensions as independent variable and 5HIAA/HVA ratio as dependent varibale.

**TABLE 2**

MULTIPLE REGRESSION FOR CSF 5HIAA

| Criteria: | P-ln .0500, P-Out .1000 |
|-----------|--------------------------|
| Dependent Variable: | CHIAA |
| Independent Variable: | Disorganization, Negative, Psychosis |

Variables (s) Entered on Step Number 1. Disorganization

| Multiple R | R Square | Adjusted R Square | Standard Error |
|------------|----------|------------------|----------------|
| 0.49047 | 0.24056 | 0.21886 | 3.87963 |

F=1.08671; Sig F=.0021

**TABLE 3**

MULTIPLE REGRESSION FOR NEGATIVE DIMENSION

| Criteria: | P-ln .0500, P-Out .1000 |
|-----------|--------------------------|
| Dependent Variable: | Negative |
| Independent Variable: | C-5HT, C-DA, C-HIAA, C-HVA |

Variables (s) Entered on Step Number 1. C-HIAA

| Multiple R | R Square | Adjusted R Square | Standard Error |
|------------|----------|------------------|----------------|
| 0.38562 | 0.14717 | 0.12280 | 3.80028 |

F=.03971; Sig F=.0191

Disorganization dimension accounted for 0.241 % of the variance in CSF-5HIAA level as obtained by step-wise multiple regression analysis. Psychosis and negative dimensions did not enter the regression equation (Table 2). Other multiple regression models did not reveal any significant results.

**TABLE 4**

MULTIPLE REGRESSION FOR CSF 5HIAA/HVA RATIO

| Criteria: | P-ln .0500, P-Out .1000 |
|-----------|--------------------------|
| Dependent Variable: | 5HIAA/HVA RATIO |
| Independent Variable: | Disorganization, Negative, Psychosis |

Variables (s) Entered on Step Number 1. Disorganization

| Multiple R | R Square | Adjusted R Square | Standard Error |
|------------|----------|------------------|----------------|
| 0.33012 | 0.10898 | 0.08352 | 0.160524 |

F=.2809; Sig F=.0460

**TABLE 5**

FACTOR ANALYSIS

AMINES AND THEIR METABOLITES AND THREE DIMENSIONAL SCORES ROTATED FACTOR MATRIX

| Variable | Factor 1 | Factor 2 | Factor 3 |
|----------|----------|----------|----------|
| C-5HT    | .115     | .728     | -.137    |
| C-DA     | .036     | .714     | -.053    |
| C-HIAA   | .616     | .392     | .012     |
| C-HVA    | .053     | -.609    | -.416    |
| Disorganization | .909 | .016 | -.049 |
| Negative | .863     | .062     | .032     |
| Psychosis | -.592 | .410 | .032 |
| Eigenvalue | 2.48193 | 1.93700 | 1.56713 |
| Pct of Var | 22.6 | 17.8 | 14.2 |

**LIST OF ABBREVIATIONS**

CSF: Cerebro Spinal Fluid
C-DA: CSF Dopamine
C5HT: CSF Serotonin
C5HIAA: CSF 5 Hydroxy Indole Acetic Acid
CHVA: CSF Homo Vanillic Acid
Std D: Standard Deviation
Pct Var: Percentage of Variance

A factor Analysis with Varimax Rotation was done to find the relationship between the score on three dimensions and the levels of Amines and their metabolites in CSF as variables. Scores of 3 dimensions of schizophrenia and the levels of amines and their metabolites in CSF were used for factor analysis and three factors were extracted. The initial factor solutions were subjected to varimax rotation. CSF 5HIAA,
Disorganization and Negative dimensions load on the first factor. Psychosis dimension had significant negative correlation. In factor two psychosis dimension had negative correlation with CSF HVA. Table 5 shows the factor analysis.

DISCUSSION

Screening of 37 drug naive patients with the presence of strict inclusion and exclusion criteria meant that a relatively 'clean' sample of patients was obtained for the study. Inclusion of drug naive patients for the study ruled out the effect of any psychotropic medication's ability to alter the concentration of biogenic amines and their metabolites in the CSF. The sample had relatively short mean illness duration. The maximum duration of illness was kept at 2 years. This has largely avoided the effect of chronicity on the levels of amines and their metabolites in CSF. Psychopathology was rated on SAPS and SANS. Both the instruments have been well studied and validated. The dimension scores were calculated using the method described by Liddle (1987).

Lumbar puncture (LP) was done at the same time of the day after an overnight fasting for all patients. All LPs were done at the level between L4-5 space in left lateral position. This uniformity in the CSF sampling largely avoided any variation in the level of amines and their metabolites including the site of lumbar puncture, diurnal variation, and diet.

This study attempted to find the possible relationship between three dimensions of schizophrenia and their neurochemical involvement concentrating on Dopamine and Serotonin and their interaction in causing various symptoms of schizophrenia. This study selected measuring both Dopamine and Serotonin and their metabolites since both of these amines are often implicated in the pathophysiology of schizophrenia.

The current study consistently shows a strong correlation between CSF 5-HIAA and disorganization dimension of schizophrenia. Only disorganization dimension entered the multiple regression with CSF 5-HIAA. In factor analysis both CSF 5-HIAA and disorganization loaded in factor 1. Factor analysis provides support to the close association between CSF 5-HIAA and disorganization dimension. Regression analysis also showed significant positive correlation between CSF HIAA/HVA ratio and disorganization dimension. This further supports the relationship between CSF 5-HIAA and disorganization dimension. Bowers et al. (1973) found positive correlation between CSF 5-HIAA levels and scores related to psychotic disorganization of mental content. Bowers & Rozitis (1974) also found positive correlation between CSF 5-HIAA/HVA ratio with first rank symptoms of schizophrenia. King et al. (1985) in a group of schizophrenic patients found that the level of CSF 5-HIAA correlates positively with mannerisms and posturing, but these symptoms co-vary with other negative symptoms. The significant positive correlation between CSF 5-HIAA and disorganization dimension, which emerged consistently from this study, is interesting and provides evidence for the possible involvement of serotonin in the pathophysiology of the disorganization dimension.

The results of this study also show a clear association between CSF 5-HIAA and Negative dimension of schizophrenia. This finding is consistent with previous findings. Kirstein et al. (1976) found inverse correlation between CSF 5-HIAA levels and psychomotor agitation in schizophrenic patients. Bowers (1975) demonstrated inverse correlation between CSF 5-HIAA levels and favorable prognostic signs, acute course and low levels of emotional withdrawal. Csernansky et al. (1990) has also found a positive correlation between CSF 5-HIAA and various deficit symptoms of schizophrenia. Data from literature indicates that higher concentration of 5-HIAA may be associated with negative symptoms and chronicity within a group of schizophrenic patients. Findings from this study add support to the hypothesis that serotonin plays an important role in schizophrenia in particular serotonin function within the normal range may be linked to negative symptoms of this heterogeneous disorder.

This study also shows significant
correlations between psychosis dimension and the levels of amines and their metabolites in the CSF. In factor analysis CSF 5HIAA correlates negatively with psychosis dimension. This is consistent with studies by others showing low levels of CSF HIAA in patients with First Rank symptoms (Post, et al., 1975) and with the presence of delusions (Lindstrom, 1985).

In factor analysis CSF HVA levels correlated negatively with psychosis dimension. This is consistent with the previous studies. Bowers et al. (1973) reported low CSF HVA in patient with first rank symptoms. He also reported low CSF HVA associated with poor prognosis. Post et al. (1975) also demonstrated low CSF HVA in patients with First Rank symptoms. Picker et al. (1990) found negative correlation between CSF HVA and positive symptoms. They suggest CSF HVA levels may relate to frontal cortical dopaminergic activity. The possibility that the frontal cortical dopaminergic system may contribute significantly to HVA levels in CSF could have important implications in interpreting the negative correlation between CSF HVA and psychosis dimension found in this study, since there is evidence to show that CSF HVA at least partially reflect frontal cortex dopamine activity. This is consistent with the "bi-directional" model of psychosis postulated by Picket et al. (1990) that decreased frontal cortical release or metabolism of DA could be coupled with increased sub cortical activity resulting in positive symptoms of schizophrenia.

This study also showed positive correlation between CSF Dopamine and psychosis dimension. Previous studies measured predominantly the levels of HVA rather than Dopamine in CSF. High level of CSF HVA has been positively correlated in one study with paranoid symptoms (Rimon et al., 1971). It can be argued that the CSF Dopamine originates predominantly in the striatum adjacent to lateral ventricles and is thought to reflect nigrostriatal dopaminergic activity. This can explain the sub cortical hyperdopaminergia in producing psychotic symptoms. The negative correlation between CSF HVA and CSF Dopamine in the current study is a contradictory finding in the methodology in measuring Dopamine and its metabolite HVA in CSF.

Measurement of amines and their metabolites in the body fluids to certain extent reflects the central metabolism of the parent amine. The central origin of CSF HVA has been clearly established and to certain extent for CSF 5HIAA as well even though it has significant spinal cord origin. CSF HVA has been clearly established to originate from striatum adjacent to lateral ventricle. Recently it has been suggested following clinical studies the CSF HVA at least partially reflects frontal cortex dopamine activity. This has important implications in interpreting the negative correlation between CSF HVA and psychosis dimension found in this study. This study provides support of the existing hypothesis that hypodopaminergia in mesocortical dopamine neurons results in increased sub cortical dopamine activity result in psychotic symptoms.

Negative symptoms of schizophrenia have been linked to frontal dysfunction. It has also been suggested that this frontal dysfunction reflect at least in part hypodopaminergia in frontal cortex. Serotonin has an inhibitory influence on the dopaminergic neurons at the level of substantia nigra and cortex. The finding of positive correlation between CSF 5HIAA and Negative dimension coupled with the finding of negative correlation between CSF HVA and psychosis dimension provides evidence that increased levels of Serotonin inhibits dopaminergic neurons in frontal cortex results in negative symptoms. This Serotonin-Dopamine interaction at different level is clinically relevant since atypical antipsychotic medications alter the imbalance between Serotonin and Dopamine there by relieves negative symptoms in schizophrenia.

This study has explored the differential effects of Dopamine and Serotonin with dimensions of schizophrenia psychopathology. Serotonin seems to be implicated in the dimension studied with CSF 5HIAA being associated with disorganization and negative symptoms, and
negatively linked with the psychotic dimension. The psychotic dimension is in addition implicated with both Serotonin and Dopamine, and negatively with the Dopamine metabolite HVA. This study suggests that there is an increased frontal serotonergic tone as the neurobiological basis for both disorganization and negative symptoms. A hyperdopaminergic state substrate is suggested for the psychotic dimension. The differential neurochemical involvement in the dimensions of schizophrenia suggests an apt model to explore the mechanism of action of atypical antipsychotic and their wide spectrum of therapeutic efficacy.

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