Reduced folic acid, vitamin B₁₂ and docosahexaenoic acid and increased homocysteine and cortisol in never-medicated schizophrenia patients: Implications for altered one-carbon metabolism

Anvita Kale, Nilesh Naphade, Swati Sapkale, Marellassv Kamaraju, Anilkumar Pillai, Sadhana Joshi, Sahebarao Mahadik

Abstract

Abnormal one-carbon metabolism has long been suggested as one of the mechanisms for neuropathology and psychopathology of schizophrenia. Variable levels of components of one-carbon metabolism (folic acid and vitamin B₁₂) and consequent altered levels of homocysteine and phospholipid docosahexaenoic acid (DHA) have been independently reported, mostly in medicated patients. This study examined the simultaneous levels of these key components of one-carbon metabolism and its consequences in unique, medication-naïve first-episode psychotic patients (FEP, n = 31) and healthy controls (HC, n = 48) matched for confounds such as race, diet and lifestyle to reduce the variability. Significantly lower levels of folate and vitamin B₁₂ in plasma and folate in red blood cells were observed in FEP compared to HC. These reductions paralleled the significant increase in plasma homocysteine and cortisol levels. Significantly reduced levels of membrane DHA were also observed in FEP compared to HC. This study, using a unique cohort, provided a broader mechanism (disturbed folic acid–vitamin B₁₂–DHA balance) of altered one-carbon metabolism and one of its key consequential components, an increased homocysteine level that together with cortisol, can contribute to the neuropathology of psychosis. These data may have important implications for the amelioration of psychopathology in schizophrenia.

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1. Introduction

It was first suggested by Osmond and Smythies (1952) that certain aberrant methylated compounds may affect mental state and behavior. This hypothesis was later modified by Smythies (1966) as the abnormal “one-carbon metabolism” hypothesis for schizophrenia. Recently, Frankenburg (2007) extensively reviewed the earlier studies and provided possible mechanisms of abnormal one-carbon metabolism and their role in the neuropsychopathology of schizophrenia. This review pointed out a large number of factors such as smoking, alcohol consumption, several types of medications, and genetic that can contribute to folate and/or vitamin B₁₂ deficiency. The primary cause of altered one-carbon metabolism is the altered levels of folic acid and vitamin B₁₂, and one of the most often studied consequences is the increased level of homocysteine, a highly toxic metabolite to neural and vascular development (Lipton et al., 1997; Mattson and Shea, 2003; Muntjewerff et al., 2003; Regland, 2005). Since these key components are often studied separately and in different subjects with contradictory results, the precise mechanism(s) of cause and consequence of altered one-carbon metabolism in schizophrenia are not clear (Muntjewerff and Blom, 2005; Frankenburg, 2007). Such information can be vital to assess the role of each component in the pathophysiology of altered one-carbon metabolism so that proper balance among these factors can be regulated to ameliorate the pathophysiology.

Altered one-carbon metabolism, in addition to increasing the levels of homocysteine, is known to alter the levels of methylation of catecholamines, phospholipids and chromatin (histone and DNA), leading to epigenetic regulation of vital developmental genes in schizophrenia (Sharma, 2005). Particularly, hypermethylation of reelin gene and reduced reelin mRNA expression have been reported in schizophrenia (Abdolmaleky et al., 2005). Altered functions of neurotrophic factors and antioxidant genes (also regulated by methylation) that play vital roles in brain development and function are also reported in schizophrenia (Mahadik et al., 2001; Yao et al., 2001; Shoval and Weizman, 2005; Buckley et al., 2007). Furthermore, low maternal folate and high homocysteine levels are suggested to increase the risk for developing schizophrenia (Picker and Coyle, 2005).
Earlier studies in schizophrenia have reported a large variability in the level of one or more of the key components of the cause (decreased levels of folic acid, vitamin B12, key metabolic enzymes, and docosahexaenoic acid (DHA)) and the consequence (increased homocysteine levels) of altered one-carbon metabolism in patients at different stages of illness (Smythies, 1983; Smythies et al., 1997; Susser et al., 1998; Muntjewerff and Blom, 2005; Regland, 2005; Stahl et al., 2005; Mahadik and Yao, 2006). A few studies have examined the relationship between psychopathology and level of folate, vitamin B12, homocysteine, or DHA in schizophrenia patients (Herran et al., 1999; Goff et al., 2004; Stahl et al., 2005; Haidemenos et al., 2007). However, none of the studies has measured all of the above key constituents together, and most of these studies were carried out on patients who were treated with multiple medications (Stahl et al., 2005), hospitalized (Silver, 2000), epileptic with psychosis (Monji et al., 2005) or treated with nutritional supplements (Kemperman et al., 2006). These studies have clearly indicated the contribution of folic acid, vitamin B12, and homocysteine to altered one-carbon metabolism and their role in the psychopathology-siologie of schizophrenia.

Recently, a few meta-analytic studies have identified a large number of shortcomings in the published experimental designs; those have been suggested to contribute to the reported variabilities in the components of one-carbon metabolism (Muntjewerff and Blom, 2005; Frankenburg, 2007). Particularly, factors such as race, age, gender, medications, and dietary and lifestyle patterns (smoking, use of alcohol, and drug abuse) can significantly affect the intake, absorption, liver metabolism, and blood levels of the above factors in one-carbon metabolism (Skerritt, 1998; Mahadik et al., 1999a,b; Frankenburg, 2007).

Furthermore, folic acid is now found to influence the membrane phospholipid long chain polysaturated fatty acid (LC-PUFA) metabolism, particularly the levels of the major brain omega-3 fatty acid, DHA (Pita and Delgado, 2000). DHA is well established to play a vital role in brain and behavioral development and cognition (Simopoulos, 1981; Wainwright, 1992), which are found to be altered in schizophrenia. The lower levels of DHA in brain and the periphery are consistently reported in schizophrenia even at the onset of psychosis (Assies et al., 2001; Khan et al., 2002; Yao et al., 2002; Arvindakshan et al., 2003a; Mahadik and Yao, 2006; McNamara et al., 2007; Kale et al., 2008).

The present study tested the hypothesis that lower levels of blood folic acid, vitamin B12, and DHA and increased levels of homocysteine exist in unique medication-naive early psychosis schizophrenia patients compared with healthy normal control subjects. Patients and controls were extremely well matched for race, gender, and dietary and lifestyle patterns with no smoking, drug or alcohol use to reduce confounds to intake and metabolism of these key components. In addition, since increased homocysteine level is often associated with increased cortisol level, which is generally elevated by both stress and psychosis, a further confounding factor. Never-medicated, first-episode psychotic patients could have a diagnosis of schizophrenia or schizoaffective or schizophreniform disorder after 6 months follow-up evaluation; all diagnoses were derived from a structured clinical interview for the DSM-IV (American Psychiatric Association, 1994). Both patients and controls were medically healthy.

### 2.1. Study subjects

#### 2.1.1. Enrollment and diagnosis

The patients enrolled were from consecutive admissions to the outpatient treatment unit of the Psychiatry Department of Bharati Community Hospital in Pune, India. The normal control subjects consisted of healthy volunteers from the general population. Both patients and normal subjects were of common racial origin and had similar lifestyle and dietary patterns based on a dietary questionnaire. Almost all the subjects were Hindus, and belonged to the lower socioeconomic group and had minimal school education (up to 9th grade). This is characteristic of the homogenous urban population from the city of Pune, India. Normal subjects were assessed using Structured Clinical Interview for DSM Disorders, Non-Patient Version (SCID-NP).

#### 2.1.2. Inclusion criteria

Both healthy controls and patients were between 18 and 40 years and of either gender. Never-medicated, first-episode psychotic patients could have a diagnosis of schizophrenia or schizoaffective or schizophreniform disorder after 6 months follow-up evaluation; all diagnoses were derived from a structured clinical interview for the DSM-IV (American Psychiatric Association, 1994). Both patients and controls were medically healthy.

#### 2.1.3. Exclusion criteria

Exclusion criteria were the same for both control subjects and patients and included the following: 1) full-scale IQ > 80 (Wechsler, 1981); 2) use of high levels of dietary supplements; 3) severe under or malnourishment; 4) seizure disorder; 5) head injury with loss of consciousness; 6) alcohol or substance abuse which was extremely rare in this sample; 7) type II diabetes; 8) lipid disorders based on laboratory lipid battery that included estimations of cholesterol, triglycerides, HDL cholesterol, and LDL cholesterol; 9) cardiovascular disease; and 10) patient or family history of hyperton. These factors as well as obesity are also rare in these individuals and are known to affect essential polysaturated fatty acid (EPUFA) status. Control subjects with a history of psychosis or major mood disorder or who were using any medication for other medical problems were also excluded.

#### 2.1.4. Clinical assessments

Clinical characteristics of first episode schizophrenia patients are shown in Table 1. Patients were rated for psychopathology using the Positive and Negative Symptom Scale (PANSS; Kay et al., 1987). The assessments were carried out by trained psychologists within 1 week of enrollment. Ratings were performed by two clinicians (inter-rater reliability of ≥ 0.94 over the period of study), one on the study and the other not associated with the study. The research protocols and consent forms were approved by the institutional review board (IRB) of Bharati Vidyapeeth Medical College, Pune, India, and by the IRB (Human Assurance Committee) of the Medical College of Georgia, Augusta. All the subjects consented to participate in the study and signed written consent documents.

#### 2.1.5. Dietary assessments and other confounds

A food frequency questionnaire was used to estimate the frequency of intake of foods with those rich in folic acid and vitamin B12. These foods were identified using ‘Nutritive Values of Indian Foods’ (Gopalan et al., 1996). The questionnaire consisted of 17 food groups and a number of foods (approximately 10) were listed under each of the food groups. The frequency of intake of foods was recorded on an eight point scale from ‘never’ to ‘thrice daily’. Monthly scores were calculated for each food item. For example, an item consumed once a week has a score of 4 while that consumed daily has a score of 3. None of the subjects smoked or consumed alcohol, which is typical of the lifestyle of the cohort in this study.

#### 2.2. Biochemical estimations

All biochemical analyses were performed at laboratories separate from patient recruitment sites. Investigators were blinded to subject identity (patient vs control), which was indicated by a code number maintained by the clinical staff until analysis was completed. Fasting venous blood was collected immediately on enrollment into tubes containing ethylenediamine tetra acetic acid (EDTA). The red blood cell and plasma samples were separated by centrifugation, coded, and stored at −80 °C until further analysis.

#### 2.2.1. Fatty acid analysis

The procedure for fatty acid analysis used in our study was revised from the original method of Manku et al. (1983) and has been used in several earlier reports (Khan et al., 2002; Arvindakshan et al., 2003a; Evans et al., 2003; Ranjekar et al., 2003). Briefly, trans-esterification of the phospholipid fraction was carried out using HCl–methanol. Fatty acid methyl esters were separated and quantified using a Perkin Elmer gas chromatograph (SD 2330, 30 m capillary column, Supelco). Helium was used as carrier gas at 1 ml/min. Oven temperature was held at 150 °C for 10 min, was programmed to rise from 150 to 220 °C at 10 °C/min. and held at 220 °C for 10 min. The detector temperature was 275 °C and the injector temperature was 240 °C. Retention times and quantitation were carried out by comparison with authentic standards.

#### Table 1

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>HC-M</th>
<th>HC-F</th>
<th>FEP-M</th>
<th>FEP-F</th>
</tr>
</thead>
<tbody>
<tr>
<td>20–29</td>
<td>35.05 ± 8.32</td>
<td>35.57 ± 8.35</td>
<td>31.94 ± 7.90</td>
<td></td>
</tr>
<tr>
<td>30–39</td>
<td>4.92 ± 7.00</td>
<td>7.41 ± 8.31</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥40</td>
<td>104.14 ± 21.04</td>
<td>107.65 ± 15.23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PANSS-TOT</td>
<td>NA</td>
<td>NA</td>
<td>28.33 ± 3.98</td>
<td></td>
</tr>
<tr>
<td>PANSS-N</td>
<td>NA</td>
<td>NA</td>
<td>24.88 ± 6.95</td>
<td></td>
</tr>
<tr>
<td>PANSS-G</td>
<td>NA</td>
<td>NA</td>
<td>54.41 ± 7.63</td>
<td></td>
</tr>
</tbody>
</table>

HC, Healthy controls; FEP, first episode of psychosis patients; M, males; F, females; DUI, duration of untreated illness; SES, socioeconomic status; PANSS, Positive and Negative Symptom Scale; PANSS-TOT, PANSS total score; PANSS-P, PANSS positive symptom factor score; PANSS-N, PANSS negative symptom factor score; PANSS-G, PANSS general psychopathology cluster score; NA, not applicable. Values are mean ± S.D.
2.2.2. Folate, vitamin B12, homocysteine, and cortisol determinations

Folate and vitamin B12 were estimated by the fluorescence polarization immunoassay (Abbott Diagnostics) (Lee and Griffiths, 1985). Since the folate in plasma is known to be unstable with long storage times, the blood samples were always collected at the same time from patients and healthy controls, and analyses were done in matched pairs every 3 months. Homocysteine estimation was performed by the micro particle enzyme immunoassay method (Abbott Diagnostics, Abbott Park, IL) (Zighetti et al., 2002). Plasma cortisol was estimated by the chemiluminescence immunoassay (Diagnostic Products Corporation Kit (DPC Kit), Los Angeles, U.S.A.) (Kohen et al., 1980) on the automated instrument DPC Immulite Immuno analyzer.

2.3. Statistical analysis

The data are expressed as mean ± S.D. We used one-way analysis of variance to compare between groups and the post hoc Mann–Whitney U test for bivariate comparisons. Correlation between variables was studied using Pearson’s correlation analysis. Bonferroni correction was applied for 3 comparisons i.e. (Males + Females), (Males) and (Females) and after the correction, the acceptance level for statistical significance was lowered from 0.05 to 0.016. Data are presented for group comparisons between total subjects and male and female patients and their matched normal control groups.

3. Results

Table 1 shows the demographic characteristics of the medication-naive first-episode of psychosis (FEP, N = 31) and the normal control (HC, N = 48) subjects which are matched for their age, gender and socioeconomic status. Similarly, both patients and healthy controls were well matched on their folic acid and vitamin B12 intake based on food frequency data. Food frequency data was available on all subjects. None of the subjects were vegans. Most of the subjects in this study were Hindus and consumed a vegetarian cereal pulse diet that included milk, eggs and green leafy vegetables. The frequency of consumption of both folate (6.0 ± 4.5 in HC vs 6.7 ± 8.5 in FEP) and vitamin B12 rich foods (11.5 ± 10.4 in HC vs 11.1 ± 9.9 in FEP) was similar.

3.1. Plasma folic acid and vitamin B12, and red blood cell folate, DHA, and arachidonic acid (AA) concentrations in patients vs. normal healthy controls

Analysis of variance showed that plasma folic acid levels were significantly lower in patients as compared to controls (F (1,60) = 5.737, P = 0.02, 25/28 patients below the average of HC) (Table 2). Similarly, lower levels of plasma vitamin B12 were observed in the patients as compared to controls (F (1, 51) = 2.876, P = 0.096, 20/25 patients below the average of HC). The red blood cell folate levels were also lower in patients vs. normal controls (F (1, 62) = 8.951, P = 0.004, 25/27 patients below the average of HC) (Table 2). Red blood cell DHA levels but not the AA levels, were also significantly lower (F (1, 75) = 8.453, P = 0.005) in patients vs. normal controls.

When data was analyzed for the sex effect on levels of these measures, folic acid levels were slightly lower in males than females,

<table>
<thead>
<tr>
<th>Table 2</th>
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<tbody>
<tr>
<td>Folate, vitamin B12, homocysteine and cortisol levels in FEP and healthy controls.</td>
</tr>
<tr>
<td>HC-M + F</td>
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<tr>
<td>----------------------------------</td>
</tr>
<tr>
<td>Plasma folate (ng/ml)</td>
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<tr>
<td>(n = 34)</td>
</tr>
<tr>
<td>RBC folate (ng/ml)</td>
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<tr>
<td>(n = 37)</td>
</tr>
<tr>
<td>Plasma vitamin B12 (pg/ml)</td>
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<tr>
<td>(n = 28)</td>
</tr>
<tr>
<td>Plasma homocysteine (µmol/l)</td>
</tr>
<tr>
<td>(n = 34)</td>
</tr>
<tr>
<td>Plasma cortisol (mcg/dl)</td>
</tr>
<tr>
<td>(n = 38)</td>
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</tbody>
</table>

HC, Healthy controls; FEP, first episode of psychosis patients; M, males; F, females. Values are mean ± S.D. One-way analysis of variance between HC-M + F vs. FEP-M + F. **P<0.01; *P<0.05; $P<0.10.
but levels of plasma vitamin B12, and red blood cell folic and DHA levels were predominantly lower (P = 0.05 for both) in males compared with females in patients (Fig. 1).

3.2. Plasma homocysteine and cortisol levels in patients vs normal controls

Plasma homocysteine levels were significantly higher (F (1, 62) = 5.44, P = 0.023; 20/30 patients above the average of HC) in patients as compared with controls (Table 2). Similarly, plasma cortisol levels were also significantly higher (F (1, 67) = 8.47, P = 0.005; 24/31 patients above the average of HC) in patients as compared to controls. When plasma homocysteine and cortisol levels were analyzed for gender effect, levels of both were significantly higher in male patients (Fig. 1).

3.3. Relationship of folic acid and vitamin B12 to red blood cell DHA and AA

Plasma and red blood cell folic acid levels showed a strong trend (P < 0.1) for positive correlation to red blood cell DHA in patients. Since folic acid is involved in methylation of membrane phospholipids containing DHA lower plasma folic acid levels may indicate a causal relationship to the lower red blood cell DHA present. There was a significant negative correlation of plasma folic acid with red blood cell AA (n = 33, r = −0.475, P = 0.005). Vitamin B12 levels did not show such relationship to levels of red blood cell DHA or AA.

3.4. Relationship of folic acid, vitamin B12, DHA, AA and cortisol to plasma homocysteine

In patients, plasma folic acid did not correlate to increased homocysteine level, but vitamin B12 showed a trend for negative correlation to the increased homocysteine level (Table 3). A trend for negative correlation (P = 0.055) was also found between red blood cell levels of DHA and homocysteine. Plasma cortisol as well as RBC AA levels did not show any correlation to homocysteine.

3.5. Relationship of folic acid, vitamin B12, DHA, AA and cortisol to psychopathology

Plasma folic acid levels did not show any significant correlations with the PANSS scores, but vitamin B12 levels showed a trend (P = 0.1) for negative correlation with the positive symptom scores. The plasma homocysteine levels showed a negative correlation (P = 0.02) with the negative symptom scores, which was significant primarily in males, whereas plasma cortisol levels showed a negative correlation with the positive symptom scores primarily in females.

4. Discussion

This study is novel and significant since 1) the patients were medication-naïve first-episode and shared common ethnicity, dietary patterns and lifestyle with matched healthy controls in addition to being matched for age, gender and socioeconomic and educational status, which is critical to reduce confounds on the key components of one-carbon metabolism; 2) the study analyzed the key causal components, plasma folic acid, vitamin B12, red blood cell folic acid and DHA and the consequential components, homocysteine and cortisol to explain better the mechanisms of altered one-carbon metabolism in this unique cohort; and 3) the differences in these components were examined for gender effect and for their relationship to psychopathologic measures.

Our data indicates that significantly reduced levels of plasma folic acid and vitamin B12, and red blood cell folic acid (which represent the storage form of folate intake), DHA, as well as increased plasma homocysteine and cortisol levels exist early in schizophrenia. Fig. 2 shows the key constituents of the one-carbon metabolic cycle, particularly in relation to the constituents analyzed in this study to better discuss their impact on some of the etiopathophysiology of schizophrenia. Both folate (as 5 methyl tetrahydrofolate (5-MTHF)) and vitamin B12, which are reduced in this study, can contribute to the observed increase in homocysteine. Fig. 2 also shows that the reduced levels of DHA may be indicative of reduced levels of phosphatidyl ethanolamine (Horrobin et al., 1991), a major methyl group acceptor to form phosphatidyl choline, consists of almost 40% of the neural phospholipids. The reduced phospholipid methylation will lead to more methyl groups for catecholamine methylation by catecholamine-O-methyl transferase (COMT) enzyme and also for increased nuclear histone and DNA methylation, both of which are reported in schizophrenia (Sharma, 2005).

Earlier studies on folate and vitamin B12 levels in psychiatric patients have reported contrasting findings primarily due to limitations of methodologies, improperly matched patients and normal controls, and difficulty in controlling for smoking, medications, alcohol use and dietary and racial differences, all of which affect the plasma levels of these key components of one-carbon metabolism (Muntjewerff and Blom, 2005; Frankenburg, 2007). Reduced levels of serum folate but not the red blood cell folate are reported in both schizophrenia and depressive disorder patients (Kay et al., 1987; Herran et al., 1999). In contrast increased red blood cell folate concentrations are reported in schizophrenia patients (Muntjewerff et al., 2003). Goff et al. (2004) reported lower folate in chronic outpatients compared to published serum folate levels of control samples. In contrast Reif et al. (2005) did not find any difference in either folic acid or vitamin B12 in an ethnically homogeneous female population with different psychiatric disorders. A meta-analysis of 10 folate studies found lower folate in the majority of patients only in three studies and indicated several methodological shortcomings including not adequately matched patients and controls or using controls from published studies for comparison (Muntjewerff and Blom, 2005).

In addition, only red blood cell DHA levels were lower in this patient cohort as has been consistently reported earlier (Khan et al., 2002; Arvindakshan et al., 2003a; Evans et al., 2003; Kale et al., 2008). This is very important since folic acid has been shown to regulate DHA metabolism through phospholipid methylation (Pita and Delgado, 2000). There are limited studies in which the association between folic acid and EPUFA levels in schizophrenia patients has been examined. Kemperman et al. (2006) examined essential fatty acid (EFA) and B-vitamin status in 61 patients with schizophrenia and concluded that a subgroup of patients with schizophrenia had EFA deficiency. DHA marginality, moderate hyperhomocysteinemia or a combination of these. Our study indicated a positive correlation of folic acid to red blood cell DHA, which showed a negative correlation to homocysteine suggesting a role of DHA in altered one-carbon metabolism in schizophrenia (see Fig. 2). A recent study has shown that abnormal folate metabolism in rats was associated with reduced brain DHA levels (Rao et al., 2006).

The most important finding was the increased levels of homocysteine in patients. Our homocysteine data is comparable to a study done in young male schizophrenia patients (Stahl et al., 2005). Elevated homocysteine levels are also reported in young male or newly admitted schizophrenia patients (Levine et al., 2002; Applebaum et al., 2004).
There are a few inconsistent reports on the levels of homocysteine in schizophrenia (Muntjewerff and Blom, 2005). We found that increased homocysteine was not associated with reduced folic acid levels, but was highly negatively associated with the reduced vitamin B12. Increased homocysteine levels have also been reported without change in folic acid and vitamin B12 levels in chronic schizophrenia patients (Haidemenos et al., 2007). However, Muntjewerff et al. (2003) found a disturbed folate metabolism in schizophrenia patients independent of homocysteine. Goff et al. (2004) did not find a change in homocysteine levels even though patients had significantly lower folic acid levels compared to published values for normal controls. Recently, reduction of hyperhomocysteinemia by oral folic acid, vitamin B-12, and pyridoxine has been reported to be clinically beneficial in schizophrenia (Levine et al., 2006). We also found that the increased homocysteine level was negatively associated with the reduced DHA level. Thus, as seen in Fig. 2, the increased homocysteine level can result from one or more key constituents of one-carbon cycle. The increased levels of homocysteine, a pathophysiological marker may result from deficiency of folate and/or vitamin B12 (Coppen and Bolander-Gouaille, 2005; Monji et al., 2005). It is important to mention that reduced folate levels in the absence of increased homocysteine do not increase the risk of disease (Mattson and Shea, 2003).

We found a very significant increase in levels of plasma cortisol in patients. Reports on high cortisol concentrations in chronic schizophrenia patients have also been reported earlier, as compared to controls (Walder et al., 2000; Yilmaz et al., 2007). There are no studies that have analyzed cortisol levels in relation to components of one-carbon metabolism, particularly homocysteine in schizophrenia.

It is important to point out that the changes in DHA, vitamin B12, and cortisol are more significant in males compared to females. Lower levels of DHA have been consistently reported in male patients (Assies et al., 2001; McNamara et al., 2007; Kale et al., 2008). This is very important since several studies have reported a higher incidence and severity of psychotic disorders in males than in females in all the cultures studied (Vaskinn et al., 2007). Further studies with larger number of male and female patients may be able to address better the gender differences and their role in the differences in psychopathophysiology and treatment.

In our study, since the dietary intake of folic acid, vitamin B12, and DHA was similar in patients and healthy controls, and none of the study subjects consumed alcohol or smoked, the changes observed need further explanation. Reduced folate levels in our schizophrenia patients may be due to the reduced absorption of folate at the intestinal brush border membrane reflecting low “Glutamate carboxypeptidase II (GCP-II)” activity which is known to facilitate folate absorption (Devlin et al., 2000). Recently, liver dysfunction contributing to a large number of metabolic abnormalities has been proposed in schizophrenia (Kraßl, 2001, 2007). This is important since liver plays a vital role in storage and metabolism of micronutrients, particularly vitamin B12, and perhaps also folate. In addition, COMT Val158 carriers have been shown to have significantly higher tHcy levels and high activity variants of COMT that interact with the low activity variant of methylene tetrahydrofolate reductase (MTHFR) to increase tHcy levels and the effect on tHcy could contribute to the reported associations of COMT genotype with psychiatric and neurobiological phenotypes (Tunbridge et al., 2008). In addition, hypermethylation of histone and DNA by methylases is also reported in schizophrenia (Sharma, 2005).

Furthermore, dietary folate is converted in the body to 5 methyl tetrahydrofolate (5-MTHF) by an enzyme methylene tetrahydrofolate reductase (MTHFR). A polymorphism in MTHFR (MTHFR C677T with reduced activity) has been found to be associated with negative symptoms by one study (Roffman et al., 2008) but not by another (Muntjewerff et al., 2008). As seen in Fig. 2, the transfer of methyl from 5-MTHF to homocysteine requires vitamin B12, and results in the synthesis of methionine. Methionine is converted into S-adenosyl-L-methionine (SAM). Methyl groups from SAM are transferred by phosphatidyl ethanolamine-N-methyltransferase (PEMT) to ethanolamine in a series of steps that convert it to phosphatidylcholine and produce homocysteine (Uhm et al., 2005). It is important to point out that lower levels of phosphatidyl ethanolamine (PE) are reported in schizophrenia (Mahdik and Yao, 2006).

This study did not find any association between plasma folic acid, vitamin B12, and homocysteine and any of the psychiatric scores. Similar to our findings, no associations have been reported in hospitalized psychiatric patients where vitamin B12 levels are generally known to be low (Silver, 2000). However, significant negative correlation between serum folate and negative symptoms has been reported in hospitalized psychiatric patients (Silver, 2000), in outpatients with schizophrenia (Goff et al., 2004) and in depressive patients (Sachdev et al., 2005). It is important to note that a significant negative correlation between folic acid and negative symptoms reported by Goff et al. (2004) was found in
a large number of outpatient medicated schizophrenia patients who also had high level of cigarette smoking, which is known to lower blood folate acid levels. Also, the folic acid levels were much lower and negative symptom scores were higher in patients with deficit than non-deficit syndrome whereas, and correlation with negative symptoms was more significant in deficit syndrome patients. We also found a negative correlation between negative symptoms and homocysteine which was puzzling. In our study, the number of subjects is relatively small but none of our study subjects had ever smoked or consumed alcohol. The negative symptom scores in our patients were also variable and low, as has been often reported in early psychotic patients from USA (Evans et al., 2003) and from India (Thara, 2004; Srivinasa and Tirupati, 2005).

Future studies with larger cohort and also with longer duration of follow-up, where stable negative symptoms may develop may help better understand the relationship between homocysteine and negative symptoms.

Although this study is a cross-sectional in nature, our data on the altered relationship between various key analytes (e.g., folate, B12, DHA and homocysteine) supports the altered one-carbon metabolism (see Fig. 2) at the early psychosis (baseline) which may have implications to neurodevelopmental pathophysiology, progression and treatment of schizophrenia. This is timely and important now compared to 1966.

Research to validate the hypothesis of schizophrenia (Picker – 66) which supports the altered one-carbon metabolism (see Fig. 2) at the early psychosis (baseline) which may have implications to neurodevelopmental pathophysiology, progression and treatment of schizophrenia. This is timely and important now compared to 1966.

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