Methylenetetrahydrofolate reductase polymorphism, folic acid, and B12 in a sample of patients with depressive and anxiety symptoms

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Introduction
Both anxiety and depression are common symptoms or disorders with a major impact on public health. There are several theories regarding potential associations between depression and levels of vitamin B12 and folate. Vitamin B12 and folate are associated with the synthesis of monoamines and are involved in single carbon transfer methylation reactions associated with the production of monoamine neurotransmitters. This study was conducted to investigate the relationship between depression and other components of 1-carbon metabolism, such as vitamin B12, folate, and the methylenetetrahydrofolate reductase 677C\textsuperscript{-}T polymorphism, and to compare the associations among folate, vitamin B12, and the methylenetetrahydrofolate reductase C677T polymorphism, in anxiety and depression.

Methods
After obtaining approval from the ethics committee in Kasr El Aini hospital, 90 participants were randomly selected in a comparative cross-sectional study. The sample consists of three groups: a group of depressive disorders without psychotic symptoms (\(n=30\)), a group of anxiety disorders (\(n=30\)), and a control group (\(n=30\)). The patients were recruited from the psychiatric out-patient clinic. Patients were diagnosed by a lecturer of psychiatry according to DSM-IV criteria. Psychometric procedure: Beck depression Inventory for severity of depression, Hamilton rating scale of depression, and Hamilton rating scale of anxiety. Laboratory: simultaneous assay of vitamin B12 and folic acid by radioimmune assay technique and analysis of methylenetetrahydrofolate reductase (C677T) by means of PCR and RFLP.

Results
Both anxiety and depression groups have the same percentage of gene mutation (33.3\%). Folic acid and vitamin B12 mean values were the highest in the control group, followed by the anxiety group; the least was in the depression group. Within the depression group, there is a negative correlation between the severity of depression and folic acid. Within the depression group, patients with mutant gene have lower levels of both folic acid and vitamin B12 than patients with nonmutant gene. Within the anxiety group, patients with mutant gene have lower levels of both folic acid and vitamin B12 than patients with nonmutant gene.

Conclusion
Folic acid and vitamin B12 were lower than normal in both patients with anxiety and with depression and this was combined with gene mutation.

Keywords:
anxiety, depression, folic acid, vitamin B12

Middle East Current Psychiatry 2011, 18:118–125
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DOI: 10.1097/01.XME.0000395563.70945.52

Introduction
Both anxiety and depression are common symptoms or disorders with a major impact on public health [1]. A possible role of nutritional factors in the pathogenesis of neuropsychiatric disorders has long been debated [2]. There are several theories regarding potential associations between depression and levels of vitamin B12 and folate. Vitamin B12 and folate are associated with the synthesis of monoamines and are involved in single carbon transfer methylation reactions associated with the production of monoamine neurotransmitters. Low levels of 5-hydroxyindole acetic acid in cerebrospinal fluid have been found in patients with depression with folate deficiency [3]. Clinical studies have shown an inverse relationship between folate status and depression [4]. Such a relationship has been inferred from studies showing an increased frequency of folate deficiency among patients with depression [5]. More severe and
prolonged depressive episodes and weaker treatment response to antidepressants in patients with low folate status and enhanced antidepressant response with folic acid supplementation have been observed [6]. In contrast, the possible role of vitamin B12 status in neuropsychiatric disorders has been motivated by the central nervous system damage caused by overt or subtle vitamin B12 deficiency. Data regarding the association between vitamin B12 status and depression are scarce [7]. Vitamin B12 is also required in the synthesis of S-adenosylmethionine, which is needed as a methyl donor in many methylation reactions in the brain. It has also been suggested to have antidepressant properties. The action of methyltetrahydrofolate reductase (MTHFR) is associated with the formation of tetrahydrobiopterin. This compound is an important enzyme cofactor for tryptophan hydroxylase, the rate-limiting enzyme for the synthesis of 5-hydroxytryptamine (serotonin). Similarly, tetrahydrobiopterin is a cofactor for the rate-limiting enzyme tyrosine hydroxylase (tyrosine 3-monoxygenase) for the synthesis of dopamine and norepinephrine. The three monoamines, dopamine, norepinephrine, and serotonin are neurotransmitters. It is generally accepted that boosting the synthesis or the availability of these compounds results in an antidepressant effect. Thus, MTHFR plays a crucial role in neurotransmitter biosynthesis and in the concentration of monoamines in the synaptic cleft [8]. Single nucleotide polymorphisms in MTHFR have been reported, including a C→T transition at nucleotide 677 in exon 4. For the C677T polymorphism, homozygote variants have 30% enzyme activity in comparison with homozygotes for the wild-type C allele, whereas heterozygotes retain 65% of wild-type MTHFR enzyme activity. Both of these polymorphisms are functional and result in diminished enzyme activity. The consequences of the C677T polymorphism have been demonstrated in population studies, in which lower levels of red blood cell folate, plasma folate, and vitamin B12 have been reported among nondiseased individuals with the 677 TT genotype in comparison with individuals with other genotypes [9].

The aim of this study is to examine the associations among folate, vitamin B12, and the MTHFR C677T polymorphism in anxiety and depression.

**Patients and methods**

After obtaining approval from Research Ethics Committee Review in Kasr El Aini hospital, 90 patients were randomly selected in a comparative cross-sectional study. All patients gave consent to participate in the study after full explanation of procedures was provided. The sample consists of three groups: a group of major depressive disorders (MDDs) without psychotic symptoms (n = 30), a group of generalized anxiety disorders (n = 30), and a control group (n = 30). The patients were recruited from a psychiatric outpatient clinic. Control cases (healthy volunteers among medical and paramedical personnel staff of Kasr El Aini university hospital) were chosen from an alphabetical computer list of employees of the hospital. All the scales showed an absence of psychopathology in the control group. They were matched in age and sex. This was conducted over 6 months. Patients were diagnosed by a lecturer of psychiatry according to *Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV)* [10] criteria. Both sexes were included and the age limit was 20–50 years. We excluded patients with other psychiatric disorders such as mixed anxiety–depressive disorder, mental retardation, organic brain disorders, and substance-induced psychiatric disorders, in addition to general medical condition (any condition affecting folic acid or vitamin B12 levels).

**Psychometric tools**

*Semi-structured interview*

A specially designed semi-structural interview derived from the Kasr El Aini psychiatric sheet was used to cover demographic data, personal data, history, and family history.

*Structured Clinical Interview for DSM Axis I Disorders:* It provides broad coverage of axis-I psychiatric diagnosis according to DSM-IV [11].

*Hamilton depression rating scale* [12]

This scale was designed by Hamilton [12,13]. The original version consisted of 17 items and was later increased to 24 items by Klerman *et al.* [14]. The scale is not meant to be a diagnostic instrument [15]. Hamilton depression rating scale was found to distinguish between different groups of patients drawn from general practice, day-patient care, and in-patients [16]. The concurrent validity is high [17]. The interrater reliability of Hamilton depression rating scale is also consistently high [12]. This is an objective test.

*Hamilton anxiety rating scale* [18]

Similar to the depression rating scale by the same investigator, the anxiety rating scale was specially developed to rate clinical anxiety in patients already diagnosed as suffering from an anxiety state (it is for use by a trained rater after an ordinary clinical interview). Hamilton took 12 groups of symptoms that were regularly observed in anxiety states as his starting point. The addition of a rating of behavior at an interview made 13 items. Each was rated on a five-point scale from 0 to 4 in an ascending order of severity [16].

*Beck depression inventory* [19] (Arabic version)

It is a self-report scale designed to assess DSM-IV-defined symptoms of depression such as sadness, guilt, loss of interest, social withdrawal, increase and decrease in appetite or sleep, suicidal ideation, and other behavioral manifestations of depression over the previous 2 weeks. It can also be used over time to monitor symptoms and to assess response to therapeutic interventions. The inventory is composed of 21 groups of statements on a 4-point scale with the patient selecting the one that best matches his or her current state. Each statement group corresponds to specific behavioral manifestation responses and is scored 0–3, corresponding to no, mild, moderate, or severe depressive symptoms. The score range varies from 0 to 63 in which higher score indicates greater depression severity. Score in the range of...
0–13 indicates no or minimal depression; 14–19, mild depression; 20–28, moderate depression; and 29–63 indicates severe depression. It is translated into Arabic by Gharib Abdel Fattah and is used in many studies. We used this test for severity ranking and it is a subjective test.

**Laboratory**

(1) Fasting samples were collected on plain tubes for the assay of serum vitamin B12 and folic acid. Serum was separated and frozen at −20°C until time of analysis. Simultaneous assay of vitamin B12 and folic acid by the radioimmunoassay technique was carried out using SimulTRAC-SNB supplied by MP Biomedicals (Diagnostics Division Orangeburg, New York, USA) [20].

(2) Three milliliter of blood was collected using sterile EDTA vacutainer tubes for DNA extraction and analysis. Samples were stored at −70°C until the assay date. Genomic DNA for MTHFR C677T gene polymorphism was analyzed using PCR, followed by restriction fragment length polymorphism analysis [21].

Genomic DNA was extracted from whole blood by the standard salting-out technique [22]. In brief, the forward and reverse primers supplied by (Fermentas, USA) were used in the following sequence:

\[ 5'\text{-TGAAGAGAAGGTGTCTGCGGGA-3'} \text{ (forward)} \]

\[ 5'\text{-AGGACGGTGCGGTGAGAGTG-3'} \text{ (reverse)} \]

Amplification was performed using Master Taq polymerase enzyme and a hybird thermal cycler (Promega Corporation, USA). The mixture was denatured at 95°C for 10 min, and the PCR reaction was performed for 35 cycles under the following conditions: denaturation at 95°C for 1 min, annealing at 65°C for 30 s, and extension at 72°C for 1 min and a final extension cycle of 72°C was for 7 min. Amplified bands were detected by electrophoresis on 1.5% agarose gel containing ethidium bromide. Amplified PCR products were digested with HinfI (Fermentas, USA) and analyzed on agarose gel (3.5%) for the identification of the point mutation in the MTHFR gene. A single fragment of 198bp was identified as homozygous (CC); a single fragment of 175bp was identified as homozygous (TT) genotype, and two fragments of 198 and 175bp were identified as heterozygous (CT).

**Results**

**Sociodemographic data (Tables 1 and 2)**

**Table 1 Mean age in the three groups**

<table>
<thead>
<tr>
<th>Age</th>
<th>Depression (N=30)</th>
<th>Anxiety (N=30)</th>
<th>Control (N=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean SD</td>
<td>Mean SD</td>
<td>Mean SD</td>
<td>Mean SD</td>
</tr>
<tr>
<td>32.1 7.7</td>
<td>32.33 7.7</td>
<td>31.2 5.7</td>
<td></td>
</tr>
</tbody>
</table>

SD, standard deviation.

**Table 2 Sex and family history of depression or anxiety disorders**

<table>
<thead>
<tr>
<th></th>
<th>Depression</th>
<th>Anxiety</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>N Percentage</td>
<td>N Percentage</td>
<td>N Percentage</td>
</tr>
<tr>
<td>Male</td>
<td>13 43.3</td>
<td>15 50</td>
<td>17 56.7</td>
</tr>
<tr>
<td>Female</td>
<td>17 56.7</td>
<td>15 50</td>
<td>13 43.3</td>
</tr>
<tr>
<td>Family history of depression or anxiety disorder</td>
<td>N Percentage</td>
<td>N Percentage</td>
<td>N Percentage</td>
</tr>
<tr>
<td>Positive</td>
<td>9 30</td>
<td>8 26.7</td>
<td>4 13.3</td>
</tr>
<tr>
<td>Negative</td>
<td>21 70</td>
<td>22 73.3</td>
<td>26 86.7</td>
</tr>
<tr>
<td>Total</td>
<td>30 100</td>
<td>30 100</td>
<td>30 100</td>
</tr>
</tbody>
</table>

N, number.
P<0.05 is statistically significant.

**Beck depression inventory (Table 3)**

**Table 3 Severity of depression in the three groups**

<table>
<thead>
<tr>
<th>Beck depression inventory</th>
<th>Depression</th>
<th>Anxiety</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>N Percentage</td>
<td>N Percentage</td>
<td>N Percentage</td>
<td>P</td>
</tr>
<tr>
<td>No</td>
<td>0 0</td>
<td>19 63.3</td>
<td>30 100</td>
</tr>
<tr>
<td>Minimum-to-mild</td>
<td>17 56.7</td>
<td>11 36.7</td>
<td>0 0</td>
</tr>
<tr>
<td>Moderate-to-severe</td>
<td>13 43.3</td>
<td>0 0</td>
<td>0 0</td>
</tr>
<tr>
<td>Total</td>
<td>30 100</td>
<td>30 100</td>
<td>30 100</td>
</tr>
</tbody>
</table>

N, number; No, no depression.
P<0.05 is statistically significant.
of folic acid and vitamin B12, which was statistically significant.

Within the depression group (Table 9), patients with mutant gene have lower levels of both folic acid and vitamin B12 than patients with nonmutant gene and this difference was statistically significant.

Within the depression group (Table 10), patients with more severe depression have lower levels of folic acid and vitamin B12 than patients with minimal-to-mild depression. This was statistically significant with the vitamin B12 level and did not reach a statistically significant difference with the folic acid level (Table 11).

**Table 11 Correlation between gene mutation and family history in depression group**

<table>
<thead>
<tr>
<th></th>
<th>Positive FH</th>
<th>Negative FH</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>Percentage</td>
<td>N Percentage</td>
<td>N Percentage</td>
</tr>
<tr>
<td>Gene mutation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mutant</td>
<td>2</td>
<td>778</td>
<td>3</td>
</tr>
<tr>
<td>Nonmutant</td>
<td>2</td>
<td>22.2</td>
<td>18</td>
</tr>
<tr>
<td>Total</td>
<td>4</td>
<td>100</td>
<td>41</td>
</tr>
</tbody>
</table>

FH, family history; N, number. 
*P<0.05 is statistically significant.*
Correlations within the anxiety group (Tables 12–14)

Table 12 Correlation between folic acid and vitamin B12 with depression and anxiety

<table>
<thead>
<tr>
<th></th>
<th>Beck depression</th>
<th>Vit B12</th>
<th>Hamilton depression</th>
<th>Hamilton anxiety</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>R</td>
<td>P</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>Folic acid</td>
<td>0.02 – 0.399</td>
<td>0.001</td>
<td>0.57 – 0.15</td>
<td>0.76 – 0.05</td>
</tr>
<tr>
<td>Vit B12</td>
<td>0.33 – 0.181</td>
<td>0.56</td>
<td>0.11</td>
<td>0.59 – 0.100</td>
</tr>
</tbody>
</table>

R, correlation coefficient; Vit, vitamin.

Table 13 Correlation between the folic acid and vitamin B12 and gene mutation within the anxiety group

<table>
<thead>
<tr>
<th></th>
<th>Mutant gene</th>
<th>Non mutant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>Folic acid</td>
<td>3.4</td>
<td>3.7</td>
</tr>
<tr>
<td>Vit B12</td>
<td>421.3</td>
<td>205</td>
</tr>
</tbody>
</table>

SD, standard deviation; Vit, vitamin. 
P<0.05 is statistically significant.

Table 14 Correlation between gene mutation and family history in anxiety group

<table>
<thead>
<tr>
<th>Gene mutation</th>
<th>Positive FH</th>
<th>Negative FH</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N Percentage</td>
<td>N Percentage</td>
<td>N Percentage</td>
</tr>
<tr>
<td>Mutant</td>
<td>7</td>
<td>87.5</td>
<td>3</td>
</tr>
<tr>
<td>Nonmutant</td>
<td>1</td>
<td>12.5</td>
<td>19</td>
</tr>
<tr>
<td>Total</td>
<td>8</td>
<td>100</td>
<td>22</td>
</tr>
</tbody>
</table>

FH, family history; N, number.

Discussion

An association between depression and folate status has been demonstrated in clinical studies, whereas data are sparse on the relationship between depression and other components of 1-carbon metabolism, such as vitamin B12, homocysteine, and the MTHFR 677C→T polymorphism. The relationship between anxiety and these components is less well known [6]. Hence, this study was conducted to examine the associations among folate, vitamin B12, and the MTHFR 677C→T polymorphism, and anxiety and depression in a case–control comparative study.

As regards vitamin B12, mean value was highest in the control group followed by the anxiety group; the least was in the depression group and the difference did not reach statistical significance between all groups. As regards folic acid, mean value was highest in the control group followed by the anxiety group; the least was in the depression group and the difference did not reach statistical significance, except between the depression and the control group.

Our finding was proved by the finding of Coppen and Bolander Gouaille [23] who reported that both low folate and low vitamin B12 have been found in studies of patients with depressive disorders. An association between depression and low levels of the two vitamins is found in studies of the general population. Low plasma or serum folate has also been found in patients with recurrent mood disorders treated by lithium. A link between depression and low folate has similarly been found in patients with alcoholism.

Our results were consistent with the results of Alpert and Fava [24], who reported that a low folate level was relatively common (18%) among patients with MDD. Recently, it was reported that low dietary folate and depressive symptoms are associated in middle-aged Finnish men [25].

Nevertheless, a low folate level was not detected in German or Chinese patients with major depression. Hong Kong and Taiwan populations with traditional Chinese diets (rich in folate), including patients with major depression, have high serum folate concentrations. However, these countries have very low life-time rates of major depression and the low folate levels are linked [23]. This contradiction in the previous results could be explained by the fact that culturally defined dietary habits influence the relationship between the folate status and depression in different societies [26]. In addition, sex, smoking, and creatinine could cause this contradiction [27].

We also detected that both anxiety and depression groups have the same percentage of gene mutation (33.3%), whereas the control group reported 20% mutation, and the differences were not significant.

Absence of significant differences between both groups of depression and anxiety may be explained by the high degree of comorbidity between both disorders. Physicians often attempt to separate depression from anxiety. Unfortunately, such distinctions are often challenging and artificial as anxiety symptoms are common in patients with major depression. Moreover, the National Comorbidity Survey indicates that comorbid depression and anxiety is the rule rather than the exception in up to 60% of patients with MDD [28].

In addition, in our study the control group reported 20% with gene mutation; this can be explained by the fact that approximately 10% of the population is homozygous for the 677 C→T polymorphism of the MTHFR gene. In a meta-analysis of studies investigating the association between depression and MTHFR genotype, overall TT carriers had a 22% increase in the odds of depression compared with CC carriers [29]. Another study conducted by Bjelland et al. [6] from 1996 to 1997 on a large population group found a strong relationship between the T/T MTHFR genotype and depression, and the association was present for both cutoff levels of depression. Associations were observed between the lowest level of vitamin B12 (< 230.0 pmol/l (< 312 pg/ml)) and depression with high cutoff (HADS-D score ≥ 11).

Our results show that within the depression group there is a negative correlation between the severity of depression and
folic acid and this was just statistically significant. Moreover, there is a negative correlation between depression severity and vitamin B12, which was statistically significant. There is also a negative correlation between Hamilton depression and both folic acid and vitamin B12, which was statistically significant. Our findings agree with Penninx et al. [30] who found that older, physically disabled women with metabolically significant vitamin B12 deficiency have been found to have a two-fold higher risk of depression than women with normal plasma levels of vitamin B12.

However, our study disagrees with the finding of Hintikka et al. [3] who detected no correlation between the severity of depression and the level of vitamin B12 at baseline. Furthermore, Bjelland et al. [6] failed to detect a relationship between depression and folate.

Only a weak relationship or no relationship was seen between anxiety disorder and folate or vitamin B12 level or MTHFR genotype. In contrast, an inverse relationship between the level of folate and severity of depression has been reported in some other studies [31].

Finally, one of the important factors to be considered is that, whether the deficiencies in B12 and folate are primary and have a role in depression and anxiety or are secondary because both depression and anxiety affect the diet intake through loss of appetite, it is possible that safe augmentation strategies for antidepressive treatments could be advised. It is proved by Hintikka et al. [3] who applied his study by the determination of hematologically significant vitamin B12 deficiency have been found to be associated in major depressive disorder. BMC Psychiatry 2003; 3:17.


(2) We did not investigate different types of depressive disorders and anxiety disorders.

(3) The sample size was small.

There is no conflict of interest to declare.
دراسة مقارنة بين مرضى بمرض القولون litep الميلينيتي تيتراهيودرو فولات ومرضى أخرى في حالة من مرضى الاكتتاب والقلق في مواجهة تجربة إذا كان له دور في سبب هذين المرضين استردادًا من ذلك في الخطة العلاجية.

الطريقة والإدوات البحثية

في هذه الدراسة، قسم 30 من مرضى الاكتتاب و30 من مرضى القلق الممارسين على الجراحة النفسية، مكونين كمطرأ 30 من عينة إجمالية. وتم تطبيق سؤال للاكتتاب والقلق ومقابلات باهتة للمرضى. كما تم قياس مستوى فيتامين ب12 ومضاعف القولون litep في الدم. ودقيقة تعداد المريض الميلينيتي تيتراهيودرو فولات في مرضى الاكتتاب والقلق كل على حدي.

ووجد أن مستوي فيتامين ب12 ومضاعف القولون litep أكثر في المرضى المصابين بالاكتتاب، ثم مستوي القلق ثم أقل مستوي في مرضى القلق. إلا أن هذه النتائج لم تكن ملائمة للبديل العلاجية. وهي تشير إلى أن هناك علاقة عكسية عصبية بين الاكتتاب والقلق ومستوي فيتامين ب12 ومضاعف القولون litep في كل من مرضى الاكتتاب والقلق. وهذه العلاقة تميزنا 

كلا على حدة بقرار 20% من الخطة العلاجية. و لا يوجد أي فروق معينة بين القلق والاكتتاب والحركة العصبية.

الخلاصة

إن هذه النتائج في مرضى القولون litep الميلينيتي تيتراهيودرو فولات في مرضى الاكتتاب، إضافة إلى أن تلك الخطة العلاجية، فإن هذه العلاقة عكسية بين فيتامين ب12 ومضاعف القولون litep، يجب أن يأخذ في الاعتبار في الاقتراحات والقلق، هذا يفتح في طريق إمكانية إعطاء فيتامين ب12 ومضاعف القولون litep مع مضادات الاكتتاب والقلق. كما ينبغي أن تكون مزيد من البحوث في مجال الجينات للعلاج بالحيوت.