Hyperhomocysteinemia and DNA hypomethylation leading to reduced monoamines synthesis in depression: A case-control study

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Abstract

Background: Numerous studies have shown hyperhomocysteinemia (HHcy) associated with cardiovascular disease, neurodegenerative and neuropsychiatric disease such as clinical depression disorder through DNA hypomethylation and reduced level of monoamines.

Objective: We performed this study to investigate the role of total levels of plasma homocysteine (tHcy), folate vitamin B12, and neurochemicals in depressive patients and compared them with healthy controls and also performed the relationship between HHcy and folate, vitamin B12 and also monoamines in brain.

Methods: In this present study we recruited 223 depressive patients and 273 subjects as healthy controls of the same age and gender. Plasma levels of folic acid, vitamin B12, and total hcy were determined using commercially available ELISA kits (ENZO Life science). Neurochemicals assessment like 5-HT (Serotonin), GABA (by paper chromatography), monoamine oxidase (MAO), nor-epinephrine and epinephrine through HPLC.

Results: Mean plasma levels of folate, vitamin B12, tHcy, 5HT, GABA, nor-epinephrine, epinephrine and MAO 14.34 ± 0.63 in cases were 9.24 ± 1.56, 288.53 ± 22.93, 14.32 ± 0.69, 0.383 ± 0.01, 0.275 ± 0.03, 9.02 ± 0.53, 5.00 ± 0.78 respectively, which showed significant difference in comparison with the controls. In addition, there were significantly tHcy positive correlations with MAO non significantly with depression score but negative correlation with neurotransmitters and folate and vitamin B12.

Conclusions: We concluded that plasma levels of folate and vitamin B12 decreased in depressive patients, but tHcy levels increased significantly and also HHcy reduced the neurotransmitter synthesis through hypo-methionine conversion or DNA hypomethylation and therefore, lead to reduction of 5HT, nor epinephrine, epinephrine and GABA but increased the level of MAO, act as neurotoxin lead to apoptosis in brain. There are some evidences which provided the information that vitamin B12 treatment may regulate and manage the level of HHcy and maintain the concentration of neurotransmitter. Therefore, recent evidence supports the role of tHcy as a potential sensitive biomarker in depressive disorder and attention has been directed towards the utilization of Ayurvedic plants in the prevention and management of depression.

Introduction

Neuropsychiatric disorders such as depression, are a foremost public health issue in developed countries and also are harsh disabiling or mental illnesses. It is an etiologically heterogeneous group of chronic psychiatric infirmity associated with significant morbidity, mortality, and disability [1]. Approximately, one in five individuals is affected by significant symptoms of depression. Recently, the World Health Organization (WHO) estimates that major depression is the fourth most important cause worldwide of loss in disability adjusted life years, and will be the second most important cause by 2020 [2,3]. Clinically, depression is characterized by a wide range of significant symptoms, imitate alteration in cognitive, psychomotor, biological, motivational, behavioural and emotional processes and include an inability to concentrate, insomnia, loss of appetite, feelings of extreme sadness, guilt, helplessness and hopelessness, and thoughts of death and suicidal tendency [4]. Depression is associated with a wide range of different diseases such as cardiovascular disease. As we know the manifestation of depression is in the form of a behavior, cognitive, and physiological changes. The behavioral changes are generally associated with reduction in social and family activity. Recently, its prevalence differs based on geographic latitude of countries [5,6].The definite mechanism of depression is not well understood, but interaction between poor nutritional diet and environmental factors is a well-known causative factor [1,7-9].

It is known that depression more typically depends on biochemical level or monoamines neurotransmitter. Few studies have shown the
bonding between nutritional deficiencies and depression. Nutrition may play a key role in the pathogenesis of mild to moderate depression [10,11]. Innovative discipline is nutritional neuroscience, focusing on the fact that nutritional factors are interacting with cognition, behavior, and emotions. Some nutritional supplement such as folic acid and vitamin B12 & B6 may be determinants of mental illness or depression and deficiencies of these vitamins have been implicated in the etiology and treatment of both depression and other neuropsychological disorders, however, folate and vitamin B12 have fundamental roles in central nervous system (CNS) function [12-16].

In brief, both folic acid and vitamins are essential components in the synthesis pathways for the monoamine neurotransmitters 5HTs, epinephrine, GABA, norepinephrine, and dopamine [2]. Because the synthesis of methionine from homocysteine requires a supply of methyl groups from methyl folate, and also vitamin B12 as a cofactor. Methionine is the immediate precursor of S-adenosylmethionine (SAM), the methyl donor in innumerable methylation reactions in the brain for the synthesis of neurotransmitter. Thus, functional deficiency of either folate or vitamin results in elevated of homocysteine [17,18]. On the other hand, elevation in the total plasma homocysteine which is present in proteins found in meats, poultry, dairy products, eggs, and fish. Hcy is an amino acid derivative from the metabolism of the sulfur-containing amino acid methionine, is a sensitive marker for low folic acid and vitamin B12 in the depression and other neurological disorder [19]. However, hyperhomocysteinemia may have a neurotoxin effect that activates aspartate receptor, which leads to cell death [19]. Therefore, it seems necessary to illuminate the potential mechanisms of evolving depression and correct them for preventing more disability. It may lead to diminishing the societal burden of this disease. However, the current study was designed to investigate the relationship between total plasma tHcy levels and cognitive function, plasma vitaminB12 level and folate and also how the elevated levels of tHcy affect the synthesis of neurotransmitters, especially serotonin (5HT), MAO, GABA epinephrine, norepinephrine, across in Indian patients with depression and compared them with healthy controls. The results will then resolve whether tHcy lowering therapy may be useful to maintain healthy cognitive function by vitamin B12 therapies and prevent depression in this population.

Materials and methods

Subjects

This case control study was conducted at Advanced centre for Traditional and Genomic Medicine, Faculty of Ayurveda Institute of Medical Sciences, affiliated to the Banaras Hindu University, Institute of Medical Sciences, and Varanasi, India. The institutional medical ethics committee approved the study, and all patients and controls enrolled after signing their written informed consent. Two hundred twenty three patients with a diagnosis of depression were enrolled as cases (n = 223). The diagnosis of depression was based on revised Diagnostic and Statistical Manual of Mental Disorders-IV (DSM IV) diagnostic scheme and using Yesavage Geriatric Depression Scale (YGDS) [20]. Age- and sex-matched controls were selected sequentially from healthy volunteers. Inclusion criteria included-Elderly, both male and female above 25-60 years age, all the cases were screened for depression by using YGDS and the subject scored above 5, were selected under this study. None of the cases and controls had received vitamin B12 and folate supplementary products, drugs that increase serum homocysteine level and corticosteroids in the past 2 to 3 months. Demographic, medical history, family background and history were questioned. The demographic variables were studied by age, gender, and body mass index (BMI) was collected for the following parameters: age, gender, and body mass index (BMI). Further bio-chemical measures were determined as described below.

Blood collection and biochemical analysis

After overnight fasting, venous blood (8-10 ml) was collected from diagnosed patient and control centrifuged immediately, collected plasma were transferred to the laboratory in the ice box and stored -20ºc until used. Plasma levels of folic acid, vitamin B12, and total tHcy were determined using commercially available ELISA kits (ENZO Life science), Neurochemical assessment like 5HT (serotonin) [21], GABA (through paper chromatography), MAO [22], norepinephrine and epinephrine through HPLC. Statistical analyses

All the values were calculated as mean ± SD (standard deviation). Correlation of the total tHcy with neurotransmitter, folic acid and vitamin B12 were calculated by Spearman’s correlation. Mean values obtained for the different subgroups were compared using one-way ANOVA. Data were analyzed using the Statistical Package for Social Sciences (SPSS) version 16 software. We consider p < 0.05 as statistically significant.

Result

A total of 223 depressive patients with a mean age of 22.73 ± 0.54 years entered the study; 139 female (62.33%) and 84 male depressive patients (37.66%). We included 273 healthy controls with the same age and sex (p = NS). Table 1 showing the comparative data of biochemical, neurotransmitter, demographic parameter and depression score between control and cases. Depression score obtained following Yesavage Geriatric Depression Scale (YGDS) [20] was applied among young-elderly people, a high depression score was recorded in female than male during screening.

In the present study, mean plasma levels of Hcy, folate and vitamin B12 in patients were 14.32 ± 0.69 µM/L, 9.24 ± 1.56 ng/ml and 288.53 ± 22.93, respectively, which showed significant difference in comparison with controls. The mean level of Hcy was higher in female subjects, and the mean level of vitamin B12 was lower in female subjects; however, these differences were not significant. Patients with higher level of Hcy and lower levels of vitamin B12 and folate were associated with significant behaviour symptom of depression. YGDS scores were higher in cases with low levels of folate and vitamin B12 and high level of Hcy, but these associations were not significant.

In the present series of study, certain neurochemicals such as 5HT (serotonin), GABA, norepinephrine and epinephrine were significantly lower in cases than control, whereas high MAO was found to be associated with significant symptom of depressive patients when compared with control voluntarily.

Table 2 presents the means of the biochemical parameters and neurotransmitter with tHcy and decreases in plasma vitamin B12 and folate levels along with increases in the levels of the Hcy were observed when comparing the means of the control groups (Table 2). Significant negative correlations between plasma tHcy levels and folate levels (r = -0.223**, P < 0.01), vitamin B12 levels (r = .580**, P < 0.00), Nor epinephrine (r = 181**, P < 0.01), epinephrine (r = 0.589**, P < 0.01) and GABA (r = -0.473** P < 0.01) and were determined in the study population whereas positive correlation with MAO (r = 0.423**, P < 0.05) as well as depression (r = .011 P- NS) scores were observed. Sky
was showing a negative correlation with serotonin with non-significant (r = -0.19; P - NS).

**Discussion**

The rationale of this present case-control study was to elucidate the role of total plasma Hcy, (tHcy), folate, and vitamin B12 levels in depression and the effect of level of Hcy on neurotransmitter concentration. We observed lower levels of vitamin B12 and folate and elevated levels of Hcy - (Hyperhomocysteinemia-HHcy). HHcy was also affecting the level of neurotransmitters such as serotonin, norepinephrine, epinephrine, and GABA [23] and decrease in the level of this neurotransmitter whereas increased the level of MAO in depression [24].

As we know the inequal level of biogenic amines or monoamines as neurotransmitters are involved in the pathogenesis of several psychological disorders such as phobias, anxiety and panic disorders and also depression [2]. However, balance of brain chemicals or neurochemicals is involved in improvement of symptoms or cognitive and behavioural function. In the central nervous system (CNS), neurochemicals have been concerned in regulation of sleep, sadness, anxiety, aggression, enthusiasm, temperature, sexual behaviour, and pain sensation [13,24-26], whereas MAO is a mitochondrial bound enzyme, which catalyzes the oxidative deamination of dietary amines, monoamine or neurotransmitters and hormones and is also involved in metabolism of monoamines, is essential for the proper functioning of synaptic neurotransmission and regulates the motor and cognitive function, buthigh level of MAO leads to more degradation of the monoamines into the consequent aldehydes and at the presence of aldehyde dehydrogenase (ALDH) oxidized into acids by or converted into alcohols or glycols. Its by-products (hydrogen peroxide and ammonia) act as potentially neurotoxic species and can trigger the production of reactive oxygen species (ROS) and induce mitochondrial damage and neuronal apoptosis [25]. However, author hypothesized that HHcy may also disturb the level of MAO in the brain.

Numerous studies have shown that the synthesis of neurochemicals are associated with the nutritional supplement. On the other hand low level of neurotransmitter is to be linked with poor nutritional support [10,11, 13,22,26-28]. A countless amino acid acts as a precursor for the synthesis of neurochemicals in the brain, such as tryptophan is an essential amino acid and acts as a precursor to the serotonin (sadness, anxiety, aggression, enthusiasm) and hormone melatonin (regulates sleep- wake cycle) in the presence of folic acid and vitamin B12, act as a cofactor for mention cycle. Furthermore, deficiencies in these essential dietary constituents (omega-3 fatty acids, folate, vitamin B12) adversely affect mood by mechanisms that are still not entirely understood. Several studies have supported the essential supplements (nutrients) are involved in mood regulation and suggest a potential role for supplemental nutrients in mitigating the symptoms of depression. Sometimes tyrosine acts as a precursor to phenylalanine and are converted into dopamine and norepinephrine [2,12,29-31]. Some other studies have suggested the amino acids tryptophan, tyrosine, phenylalanine, and methionine are often helpful in treating many mood disorders including depression through increasing the concentration of monoamines in the brain [11,14,32].

We know that folate or B12-vitamin both are required per the definition of a vitamin, but cannot be synthesized de novo pathway, therefore, it must be derived from supplementation and folates found in leafy green vegetables, legumes, beans, liver, citrus fruits, and yeast. Therefore, folic acid is essential dietary supplement, involved in the transformation of one carbon group or a methyl group (methylolation) in numerous biochemical pathways, including neurotransmitter synthesis, DNA biosynthesis, regulation of gene expression, amino acid synthesis, metabolism, and myelin synthesis, repair and proper cell signalling pathways. After the multiple biochemical conversion reaction (MBCR), dietary folate to become, the more

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Cases</th>
<th>Healthy control</th>
<th>p-value</th>
</tr>
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<tbody>
<tr>
<td>Number</td>
<td>223</td>
<td>273</td>
<td>NS</td>
</tr>
<tr>
<td>Gender-F/M</td>
<td>139/84</td>
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<td>Age (years), mean (SD)</td>
<td>23.59 ± 6.4</td>
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<tr>
<td>BMI</td>
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<td>Duration of disease (min-max) in years</td>
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<td>YGDS, mean (SD)</td>
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<tr>
<td>Biomarkers, mean (SD)/Plasma folate (ng/ml)</td>
<td>9.24 ± 1.56</td>
<td>11.98 ± 3.82</td>
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<td>Plasma vitamin B12 (pg/ml)</td>
<td>288.53 ± 22.93</td>
<td>396.26 ± 27.54</td>
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<td>Plasma homocysteine (µM/L)</td>
<td>14.32 ± 0.69</td>
<td>11.63 ± 1.37</td>
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<td>Serotonin (5HT)</td>
<td>0.383 ± 0.01</td>
<td>0.064 ± 0.02</td>
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<td>GABA</td>
<td>0.275 ± 0.03</td>
<td>0.489 ± 0.074</td>
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<tr>
<td>Norepinephrine</td>
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<td>11.12 ± 5.12</td>
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<td>Epinephrine</td>
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<td>MAO</td>
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<tr>
<td>Depression score</td>
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*p-value<0.05 significant; NS is non-significant.

<table>
<thead>
<tr>
<th>Biological parameters</th>
<th>Correlation coefficient value (r value)</th>
<th>Significance (P value)</th>
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<tbody>
<tr>
<td>Plasma folate (ng/ml)</td>
<td>-.223**</td>
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<tr>
<td>Plasma vitamin B12 (pg/ml)</td>
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<td>Serotonin (5HT)</td>
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<td>MAO</td>
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<td>Norepinephrine</td>
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<tr>
<td>Epinephrine</td>
<td>.473**</td>
<td>.000</td>
</tr>
<tr>
<td>GABA</td>
<td>.423**</td>
<td>.000</td>
</tr>
<tr>
<td>Depression (score)&lt;0.05</td>
<td>-.011</td>
<td>0.87</td>
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</table>

*pCorrelation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed); r value is spearman’s correlation coefficient; NS is non-significant.
metabolically active and tissue-useable forms [25,26]. In MBCR, methylenetetrahydrofolate reductase (MTHFR) enzymecatalyse the conversion of methylenetetrahydrofolate to the active form of 5-methyltetrahydrofolate (5-MTHF, L-methylfolate) and it became to be highly absorbed (85-95%) compared to the dietary form (50%) [15].

During folate metabolism, methionine is converted into S-adenosylmethionine (SAMe), which participates in methylation (CH₃) reactions in the presence of methionine synthetase and vitamin B12 and is involved in the synthesis of an essential phospholipid (phosphatidylcholine) and monoamines such as (serotonin, melatonin, epinephrine norepinephrine, dopamine etc). After methylation, SAMe becomes S-adenosylhomocysteine, then converted into tHcy and at this point, tHcy must either be metabolised in to cysteine, taurine, and glutathione - a B6 -dependent process through transsulfuration or remethylated to become methionine again must either be further metabolized via transsulfuration to become cysteine, taurine, and glutathione - a B6 -dependent process or remethylated to become methionine again [26]. However, deprived intake of essential nutrition, which supply the methyl groups from methyl folate or vitamin B12 and cause hyperhomocysteinemia or high level of tHcy. HHcy in depression indicates a breakdown in the methylation of homocysteine to methionine or cysteine, taurine, and glutathione - a B6 -dependent process and causes oxidative stress resulting in neurological and vascular damage and lead to disturbance in the optimal biosynthesis of neurotransmitters and cell signalling or cell repair and also disturbed the level of MAO in brain, its higher concentration as neurotoxic [24]. It is associated with various cardiovascular and other neurological disorders, including cognitive impairment and dementia conversely, accumulation of tHcy involved in the path physiology of depression and reduces the level of neurotransmitters such as serotonin, dopamine, noradrenalin, and y aminobutyric acid (GABA) are often associated with depression [10,12-14,25,32-34].

Conclusion

In conclusion, we have recognized that folate or Vitamin B12 deficiency, caused HHcy and leads to breakdown of methylation reaction, disrupted the monoamine neurotransmitter metabolism and break the nerve cell signalling pathway. We have concluded that tHcy levels were found to negatively correlate with folate levels, vitamin B12 levels, and neurotransmitter or positive co-relation with MAO in this Indian study. We have also seen a high incidence of folate deficiency and HHcy may reflect the degree of depressed patients (mild to moderate). The patients in this study were not entered into a treatment section, whereas other studies have claimed the antidepressant can regulate the low folate or raised homocysteine concentrations of depressed patients. Furthermore, the effectiveness of B vitamin therapy towards countering cognitive impairment and depression, associated with HHcy and low folate in this study population and may ultimately help reduce theincreasing burden of depression within the country.

On the other hand, as pointed out earlier that deficiency of certain neurochemicals and nutritional supplements are responsible for depressive symptoms. Therefore, attention has been directed to search of plant based drug that can be beneficial in the prevention and management of such neuropsychiatric disease.

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