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Vitamin B12 and folate deficiencies, elevated homocysteine and their roles in the biochemical basis of neuropsychiatric diseases in children and adolescents: Case series, review and recommendations

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Abstract

Vitamin B12 and folate deficiencies can be frequently seen in children and adolescents and may manifest with neuropsychiatric symptoms. Vitamin B12 and folate deficiencies and the associated increase in homocysteine are related to one-carbon metabolism (OCM) and may play a role in the pathogenesis of childhood and adolescent psychiatric disorders.

Therefore, a case series and review on OCM will be presented here. Twelve cases with vitamin B12 and folate deficiency and increased homocysteine among those admitted to the child and adolescent psychiatry clinic were retrospectively examined. It was found that vitamin B12 and folate deficiency and increased homocysteine may cause depressive symptoms, anxiety disorders, obsessive compulsive disorder, anger control problems, self-harming behaviors, suicidal thoughts and attempts in children and adolescents.

According to these data, vitamin B12 and folate deficiency and the resulting increase in homocysteine may cause psychiatric symptoms in some children and adolescents. In addition, literature on OCM has been reviewed.

The diagnosis and treatment methods for vitamin B12 and folate deficiencies are summarized for clinicians.

Keyword: Vitamin B12, folate, homocysteine, one-carbon metabolism, child and adolescent psychiatry, anxiety, depression

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INTRODUCTION

Vitamin B12 and folate deficiencies are common vitamin deficiencies in children and adolescents. In a study conducted in Venezuela, vitamin B12 deficiency reached 12% among children and adolescents, while low folate levels were found in 30% of all age groups. In adolescents, this rate may even rise to 82% (1). In vegetarian adolescents, vitamin B12 deficiency can be seen in one third of adolescents (2). While this rate can reach 86% in vegetarian children, it may reach 41% in adolescents (3). In India, while vitamin B12 deficiency was found in 32.4% of adolescents, it was detected in more than half of obese adolescents (4). In a cross-sectional study of adolescents in European countries, serum vitamin B12 was found to be low in 2%, while holotranscobalamin, which forms the active part of total vitamin B12, was found to be low in 5%. However, folate deficiency had rates of 10% (5). It was reported that 37.3% of adolescents in Slovenia receive insufficient vitamin B12 in diet (6). In recent years, vitamin B12 deficiency has been increasing in adolescents and manifests with neuropsychiatric symptoms (7).

Folate and vitamin B12 metabolism, with very common deficiency, may play a role in the etiopathogenesis of some neuropsychiatric diseases. In a previous study we conducted, vitamin B12 levels were found to be lower in children and adolescents with obsessive compulsive disorder. However, although folate levels did not differ significantly, they were found to be low in the patient group and close to significance (p=0.083). Homocysteine (Hcy) was found to be significantly higher in the patient group. Accordingly, it was concluded that biochemical reactions called one-carbon metabolism (OCM), which are related to vitamin B12, folate and Hcy, may play a role in the pathophysiology of neuropsychiatric disorders in childhood and adolescence (8). Again, in a study conducted with 89 children and adolescents with major depressive disorder, vitamin B12 levels were found to be significantly lower in children and adolescents with depression, while Hcy was found to be increased. In fact, while there was a negative correlation between the severity of depressive symptoms and vitamin B12 levels, there was a positive correlation with high Hcy. Folic acid, on the other hand, was found to be almost significantly (p=0.052) low in the patient group. In the same study, the folate levels of the patient group were found to be 11.23% lower than the normal range (9). In a very recent study of adolescents, vitamin B12 deficiency was associated with more severe depression and anxiety symptoms. In this study, the severity of depression and anxiety were correlated with vitamin B12, folate and Hcy, albeit weakly (10). In addition, it was found that taking more B vitamins with diet during adolescence is associated with lower depressive symptoms

(11). Studies conducted with children with attention deficit and hyperactivity disorder (ADHD) also suggested that low vitamin B12 and folate may be risk factors. Studies reported that vitamin B12 and folate are low and Hcy is high in ADHD and autism spectrum disorder (ASD) patients. In addition, in one of these studies, low vitamin B12 was inversely correlated with hyperactivity, impulsivity and oppositional behaviors (12). In a postmortem study, low vitamin B12 was found, as well as increased Hcy and decreased methionine synthase activity,in the brain tissues of people with ASD (13). In another study, low vitamin B12 and folate levels in ASD patients had a negative correlation with the severity of ASD, while a positive association was found with Hcy (14). In a study conducted with children with special learning disability, vitamin B12 was found to be low and homocysteine was increased and close to significance, while folate was found to be significantly lower in the patient group (15). There are only two studies on this subject in children and adolescents with early-onset schizophrenia. According to them, Hcy was increased in the patient group (16,17). It was reported that vitamin B12 deficiency is more common in children with tension-type headache as a neurological symptom compared to the healthy control group. Apart from headache, vitamin B12 deficiency can manifest as non-specific neurological symptoms such as syncope, dizziness, hypotonia, convulsions, hand tremor, ataxia, paresthesia, fatigue, and difficulty concentrating. In addition, these symptoms may occur without hematological findings (18).

One-Carbon Metabolism and pathophysiology

With these findings, it is necessary to examine how OCM, involving vitamin B12, folate and homocysteine, plays a role in neuropsychiatric diseases. OCM includes chain reactions that produce S-adenosyl methionine (SAM), the universal methyl donor used in methylation reactions, which is the best known epigenetic mechanism (19). These biochemical reaction chains essentially involve two cycles: the folate cycle and the methionine cycle. All biochemical reactions in these two cycles are collectively referred to as OCM (Figure 1). Vitamin B12 and folate are cofactors in these chemical reactions. Methionine synthesis occurs by the enzyme methionine synthase, of which vitamin B12 is a cofactor, providing methyl from methyl folate to Hcy. In addition, as a result of OCM, purine-pyrimidine nucleotides and some amino acids are synthesized. One of the most important tasks of OCM reactions is to produce SAM. When SAM used in DNA and histone methylation decreases, gene expression is disrupted. More than 100 methylation reactions, including not only DNA and histones, but also proteins, phospholipids, hormones and neurotransmitters must occur within the cell. In order for them to maintain their normal functions, these reactions require methyl transfer from SAM (Figure 2). When Hcy increases, SAM production decreases in OCM reactions and instead S-adenosylhomocysteine (SAH) formation increases. SAH is an inhibitor of SAM-dependent methylation reactions. Membrane fluidity also depends on the availability of sufficient SAM. SAM is also used in transsulfuration reactions and polyamine synthesis. These have important roles in cell growth, survival and maintenance of normal cell functions. Vitamin B12 and folate are essential in maintaining the normal course of OCM reactions. In addition, OCM produces precursors of DNA bases. Vitamin B12 is required for myelin, neurotransmitter and DNA synthesis in brain tissue. With folate and vitamin B12 deficiency, SAM production decreases and Hcy levels increase. In fact, this increase in Hcy levels is a sensitive indicator of vitamin B12 and folate deficiency. Methyl-folate is required for the conversion of Hcy to methionine-by-methionine synthase, and vitamin B12 acts as a cofactor in this reaction. Therefore, this reaction does not progress in vitamin B12 and folate deficiency and Hcy cannot be converted to methionine. As a result, an increase in Hcy occurs. Increased Hcy, together with methylmalonic acid (MMA), can be seen in neuropsychiatric diseases without hematological findings. Hcy is a toxic substance that damages neurons and vascular endothelium (Figure 3). Increased Hcy can cause DNA damage, mitochondrial dysfunction and apoptotic activation. The most important reason for the increase in Hcy is insufficiency of B vitamins. In addition, chronic renal failure, hypothyroidism, some malignant tumors and some drugs (antiepileptics, methotrexate, L-dopa, lipid lowering drugs, oral contraceptives, antidiabetic drugs) may also cause Hcy increase. Consuming too much coffee, smoking, alcohol and age have effects on Hcy increase. Deficiency or polymorphisms of enzymes that play a role in OCM can also cause Hcy increase. Increased Hcy leads to homocysteinylation of proteins, which leads to disruption of protein functions. In addition to all these, increased Hcy causes synaptic dysfunction and neuronal damage in the following ways: As an NMDA receptor agonist, it causes excitotoxicity, which in turn leads to neuronal DNA damage. Thus, calcium efflux into the cell increases. Endoplasmic reticulum stress occurs. This increases oxidative stress with the formation of reactive oxygen molecules. The apoptotic cascade can be activated. Hcy can cause cytochrome-c release and caspase activation. In the presence of hyperhomocysteinemia (HHcy), Hcy is converted to Hcy-sulfinic acid and Hcy-cysteic acid by oxidation. These two molecules act as endogenous ligands of NMDA receptors and thus cause excitotoxicity (20). In recent studies, it was found that Hcy also activates cytokines and pro-inflammatory molecules. Hcy induces an inflammatory response with the activation of microglia in the brain. It was also suggested that HHcy impairs barrier functions by causing inflammation (21). Vitamin B12 and folate deficiency and pathophysiology of OCM can also cause immune system abnormalities. Deficiency of these two vitamins may cause significant deterioration in immune response. Vitamin B12 deficiency may result in a decrease in the cytotoxicity of natural killer cells and immunoglobulin production and changes in lymphocyte ratios (22).

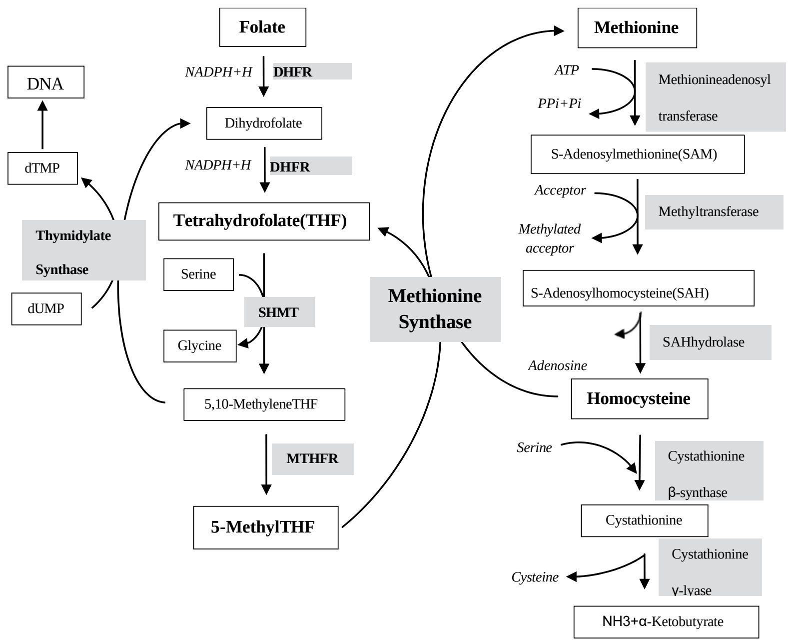

Figure 1. One-carbon metabolism: Folate and Methionine (Homocysteine) cycles
Abb: DHFR: Dihydrofolate reductase; MTHFR: Methylenetetrahydrofolate Reductase SHMT: serine hydroxymethyltransferase

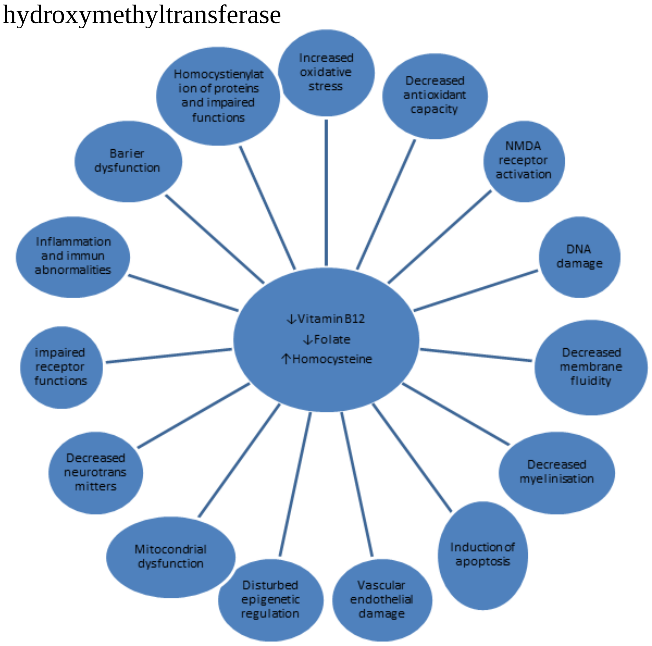

Figure 3. Possible pathophysiological effects of vitamin B12-folate deficiency and increased homocysteine

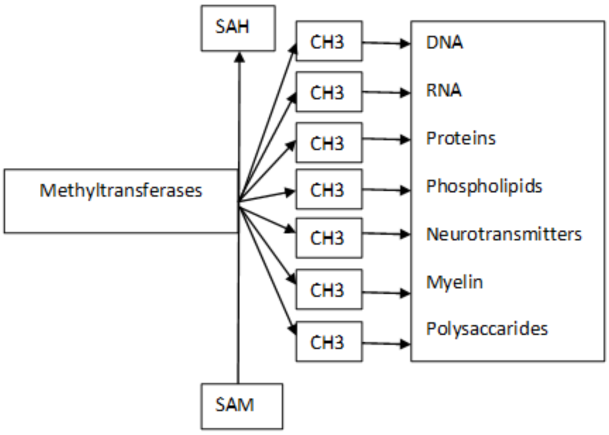

Figure 2. Metyhlation reactions

Adult patients with cardiovascular disease develop depressive disorder significantly if HHcy is elevated. Bjelland et al. (2003) investigated the relationship between vitamin B12, folate, Hcy and depression in 5948 subjects, and stated that the risk of developing depression is twice as high in the case of high HHcy (Hcy level exceeding 15 µmol/l) (23). A decrease in monoamine neurotransmitter metabolites (5HIAA, HVA, MHPG (3-methoxy-4-hydroxyphenyl glycol) was found with HHcy in depressive patients. That is, it is considered that serotonin, dopamine and noradrenaline levels decrease in cases of vitamin B12 and folate deficiency and HHcy. It was stated that Hcy is also associated with schizophrenia and mood disorders in adolescents and may contribute to the pathogenesis (16). In a study conducted in Taiwan with children aged 6-13 years, a positive association was found between Hcy and depression and anxiety levels in boys aged 12-13 years (24).

Vitamin B12

Vitamin B12 is a complex water-soluble vitamin that cannot be synthesized by plants and animals. It is synthesized only in the colons of some animals by some bacterial species. Therefore, most intake of vitamin B12 is only possible with animal foods. Vitamin B12 can be stored in the liver for several years. However, children and adolescents who do not eat meat, vegetarians and those who eat little or have unique eating habits may develop vitamin B12 deficiency. In children and adolescents, vitamin B12 deficiency is the most common cause of inadequate intake and abnormal absorption, excluding inborn errors in transport and metabolism. The main functions of vitamin B12 are hematological red cell formation, neurological functions and DNA synthesis (25).

Folate

Folate (also referred to as folic acid or vitamin B9) is mostly found as a water-soluble vitaminin animal foods (especially liver), eggs, and green leafy vegetables. Since people cannot synthesize folate, they have to obtain it from external sources. Folate can be destroyed during cooking. In addition, folate is not a vitamin that is stored in excess in the body (26). Antiepileptic drugs used for affective disorders and epilepsy may also cause folate and vitamin B12 deficiency and HHcy increases (27). In addition, food processing and storage techniques used in the food industry can cause loss of water-soluble vitamins (28). Therefore, vitamin levels may be low in people who are considered to be eating normally.

Considering the above effects of vitamin B12-folate deficiency and HHcy, they play an important role in childhood and adolescent neuropsychiatric diseases. Adequate amounts of these vitamins are indispensable for healthy brain tissue. Here, it is reported that 12 children and adolescents with various psychiatric disorders had concomitant vitamin B12 and/or folate deficiency and HHcy status. In addition, a review and some recommendations about the diagnosis and treatment methods for vitamin B12 and folate deficiency are presented.

CASE SERİES

Selection of cases and procedure

Cases were selected retrospectively between 2016-2022 among those who applied to Ordu University Medical Faculty Training and Research Hospital Child and Adolescent Psychiatry Outpatient Clinic. Cases with significant vitamin B12 and/or folate deficiency showing psychiatric symptomatology were collected. The study included 12 cases in total. The medical records of the cases were reviewed, and those who did not have medical disease detected (chronic systemic, inflammatory, infectious diseases and neurological diseases) were included. In addition to vitamin B12 and folate, homocysteine levels, hemogram parameters, routine biochemistry measurements, thyroid function tests, ferritin, and CRP values were identified. In addition, Kovacs' Children's Depression Inventory (CDI), State–Trait Anxiety Inventory (STAI–I, STAI–II), and Yale Brown Obsessive Compulsive Disorder Scale (Yale Brown OCD Scale) tests were applied to the participants regarding their psychiatric condition. Diagnoses had been made by an expert child and adolescent psychiatrist, according to DSM 5 criteria, as a result of individual interviews, family interviews, and appropriate psychometric tests. However, the diagnosis was also confirmed by clinical psychologists in our department. Accordingly, the participants were diagnosed with major depressive disorder (MDD), generalized anxiety disorder (GAD), or obsessive-compulsive disorder (OCD). Information about dietary status was collected from patients and their families. Information about whether they had been vegetarian or not, whether they had eaten animal food (such as meat, milk, and egg consumption) and their diet were recorded. They were asked whether they had received nutritional support in the last year before the diagnosis. Psychopharmacological treatment (sertraline or fluoxetine) was administered to the patients in accordance with their diagnosis. In addition, patients were given vitamin B12 and/or folic acid supplementation according to their vitamin deficiency status. Pediatric consultation was requested when necessary. Appropriate supportive interviews were also conducted. These patients were followed up at various intervals from 6 months and 1 year. After 12 participants had shown improvement in their symptoms during this period, their treatment was terminated. Written informed consent was obtained from the participants and their families that their information would be included in an anonymous case report.

Instruments and blood measurements

Children's Depression Inventory (CDI)

This scale, which measures the level of depression in children and adolescents, was developed by Kovacs in 1981. This test is the most commonly used and researched scale in children and adolescents. The Turkish validity and reliability study was performed by Öy et al. in 1991. The 27-item scale is filled in by the child or adolescent themselves. The cut-off point for this scale was determined to be19. A score of 19 and above is considered pathological.

State–Trait Anxiety Inventory (STAI–I, STAI–II)

STAI-I and STAI-II were developed to determine the levels of state and trait anxiety in children and adolescents by Spielberger et al. Öner and Le Compte conducted a Turkish validity and reliability study in 1985. Each scale consists of 20 items (40 items in total). While STAI-I measures the anxiety state of an individual at a certain moment, STAI-II measures the level of anxiety regardless of the current situation. Scores from both scales range from 20 to 80. Cut-off points are 45.

Yale-Brown Obsessive Compulsive Scale (Y-BOCS)

This test was developed by Goodman et al. in 1989. Turkish validity and reliability tests were performed by Karamustafalioglu et al. in 1993. The scale is scored by the clinician. There are 10 items. Each item is rated between 0 (no symptoms) and 4 (maximum symptoms). A score of 8-15 is considered mild obsessive-compulsive symptoms. A score between 16 and 23 indicates moderate disease, while a score above 23 indicates severe disease.

Vitamin B12 measurements

Total vitamin B12 measurements were routinely performed in the laboratory of our hospital. The measurement was made on serum separated from blood taken from patients in the morning after an overnight fast. Vitamin B12 wasmeasured by chemiluminescent micro particle Intrinsic Factor ARCHI-TECT B12 assay with commercial kits supplied by Abbott Laboratory. Normal range was evaluated as 187-883 pg/ml.

Folate measurements

The measurement of this vitamin is routinely done in the biochemistry laboratory of our hospital. Folate was measured by chemiluminescent micro particle folate-binding protein ARCHI-TECT Folate assay with commercial kits fromAbbott Laboratory. Since folate values are affected by food, they were measured after an overnight fast. Normal range was determined as 3.1-20.5 ng/ml.

Homocysteine measurements

Plasma separated from blood taken from the patients after an overnight fast was transferred to the external laboratory contracted with our hospital on the same day, and then the measurement was made. Total homocysteine was measured by chemiluminescent immunoassay method using kits supplied by Abbott Laboratory. Normal range was determined as 4.7-10.3 umol/L.

Case 1

A 15-year and 10-month-old male patient attempted suicide by hanging himself in an impulsive manner. His mother went to him because she heard a voice and saved him. Two weeks previously, he attempted suicide by taking drugs. In the last 3 months, there were thoughts of death, research of death methods, wanting to be alone, concentration of anxiety, being away from friends, distress and unhappiness, and malaise. In detailed interviews with the patient and his family, no obvious psychosocial problems were detected. He played sports and had good friendships. There were no dynamic problems in his family. Depression score was found to be high on the Kovacs'depression inventory. In addition, anxiety levels were found to be high on the STAI-1 and 2 anxiety scales. Thyroid function tests, routine biochemistry and hemogram values were normal in routine blood tests. No pathological finding was detected on cranial MRI. Vitamin B12 level was found to be low at 181 pg/ml. Homocysteine was found to be quite high at 43.5 umol/l. Folate was close to the lower limit at 3.6 ng/ml. Vitamin B12 deficiency did not affect the hematological picture. The patient was diagnosed with MDD and GAD. Antidepressants were started in addition to supportive interviews. Vitamin B12 (first IM, then tablet) and folic acid supplementation were added to this treatment. His condition improved during the following months (Table 1).

Case 2

A 14-year and 8-month-old male patient presented with complaints of shyness, unhappiness, malaise, fear of death, weakness, crying a lot, and seeing the world as a dream in the last 6 months. There were no suicidal thoughts. No significant psychosocial problem was detected that could cause these complaints. In routine measurements, pathologically, vitamin B12 value was found to be significantly low at 131 pg/ml. Folate level was normal at 6.2 ng/ml. Homocysteine level was high at 22.3 umol/l. Vitamin B12 deficiency did not affect the hematological picture. He received high scores on the depression and anxiety scales. The patient was diagnosed with MDD and GAD. Firstly, IM vitamin B12 treatment was administered in addition to antidepressants. Oral supplementation was then initiated. After 2-3 months of treatment, the symptoms clearly disappeared.

Table 1. Characteristics of the cases and laboratory results

Case no	Sex	Age	Clinical presentation	Psychometric test	Vitamin B12 (187-883 pg/ml)	Folate (3.1-20.5 ng/ml)	Homocysteine (4.7-10.3 umol/l)	Diagnosis	Treatment
C1	M	15 years 10 months	Suicide attempt by hanging, concentration of anxiety, wanting to be alone, unhappiness	↑anxiety ↑depression	↓181 pg/ml	3.6 ng/ml	43.5 umol/l	MDD+GAD	Antidepressant Vitamin B12 (IM)
C2	M	14 years 8 months	shyness, unhappiness, malaise, fear of death, weakness, crying a lot	↑anxiety ↑depression	↓131 pg/ml	6.2 ng/ml	22.3 umol/l	MDD+GAD	Antidepressant Vitamin B12 (IM)
C3	M	17 years 3 months	Inattention, forgetfulness, weakness, unhappiness, lack of pleasure, fainting, anger problems	↑anxiety ↑depression	↓<83 pg/ml	3.6 ng/ml	46.8 umol/l	MDD	Antidepressant Vitamin B12 (IM)
C4	F	14.5 years	fears, inability to sleep alone, worrying about unimportant things, tantrums, dizziness, irritability	↑anxiety	↓168 pg/ml	3.2 ng/ml	23.9 umol/l	GAD	Antidepressant Vitamin B12 Folic acid
C5	F	15 years 10 months	unhappiness, introversion, crying, reactivity, sleep problems	↑anxiety ↑depression	↓174 pg/ml	↓1.8 ng/ml	57.5 umol/l	MDD+GAD	Antidepressant Vitamin B12 Folic acid
C6	M	15.5 years	unhappiness, self-harm, attempted suicide by taking medication 5 times, tantrums	↑anxiety ↑depression	↓181 pg/ml	↓2.2 ng/ml	34.6 umol/l	MDD	Antidepressant Vitamin B12 Folic acid
C7	F	17 years	Irritability, outbursts of anger, general anxiety and restlessness, unhappiness, crying a lot, headaches, forgetfulness	↑anxiety ↑depression	↓133 pg/ml	6.8 ng/ml	22.2 umol/l	MDD+GAD	Antidepressant Vitamin B12
C8	M	14 years 10 month	Nervousness, reactivity, distress, depression, feeling lonely, unhappiness, self-harm, suicidal ideation	↑anxiety ↑depression	↓93 pg/ml	4 ng/ml	23.1 umol/l	MDD+GAD	Antidepressant Vitamin B12
C9	M	16 years	palpitations, numbness in hands and feet, tantrums, general anxieties and fears, forgetfulness, allergy to meat	↑anxiety	↓163 pg/ml	↓3 ng/ml	31.8 umol/l	GAD	Antidepressant Vitamin B12 Folic acid
C10	F	16.5 years	Self-harm, suicide attempt, forgetfulness, carelessness, unhappiness	↑depression ↑anxiety	↓90 pg/ml	4 ng/ml	23.2 umol/l	MDD	Antidepressant Vitamin B12 Folic acid
C11	F	15 years 9 months	Unhappiness, suicidal ideation, obsessions, forgetfulness	↑obsessions ↑depression ↑anxiety	↓150 pg/ml	9.3 ng/ml	21.4 umol/l	MDD+ OCD	Antidepressant Vitamin B12
C12	F	16.5 years	Difficulty swallowing solid food, inability to touch other people's belongings, rereading and rewriting, counting	↑ obsession	335 pg/ml	↓3 ng/ml	29.9 umol/l	OCD	Antidepressant Vitamin B12 Folic acid

Footnote: MDD: Major Depressive Disorder; GAD: Generalized Anxiety Disorder; OCD: Obssesive Compulsive Disorder

Case 3

A 17-years and 3-months-old male patient applied to our clinic with complaints of inattention, forgetfulness, weakness, unhappiness, lack of pleasure, fainting, and anger problems. He stated that he was not a vegetarian but ate almost no red meat. He smoked 10 cigarettes a day. In routine examinations, vitamin B12 values were undetectable (<83pg/ml). Folic acid value was close to the lower limit at 3.6 ng/ml. Homocysteine value was quite high at 46.8 umol/l. Among the hemogram parameters, the MCV value was higher than normal at 99.2 fL (normal value: between 80-97). Peripheral smear was requested with pediatric consultation. Hematological findings of vitamin B12 deficiency such as neutrophil hypersegmentation were not observed. No pathology was detected on cranial MRI. Depression and anxiety scores were high in psychometric measures. No significant psychosocial problems were detected in family and personal interviews. A diagnosis of MDD due to organic causes was made. The patient was given antidepressants as well as vitamin B12 supplementation. In the following months, his clinical condition improved, along with his laboratory findings.

Case 4

A 14.5-year-old female patient applied with complaints such as fears, not being able to sleep alone, worrying about unimportant things, unnecessary fears, tantrums, dizziness, and quick temper that emerged in recent months. She ate very little red meat. Depression score from psychometric tests was below the cut-off score. Anxiety scale scores were high. In the measurements, vitamin B12 level was pathologically low at 168 pg/ml. Folate level was also at the lower limit (3.2 ng/ml). Homocysteine level was high at 23.9 umol/l. With the diagnosis of GAD, her treatment was arranged as antidepressants as well as vitamin B12 and folate supplements. In the following months, a significant improvement was observed in her condition.

Case 5

A 15-year and 10-month-old girl applied to our clinic with complaints such as unhappiness, introversion, crying, reactivity, and sleep problems in the last few months. Although she was not a vegetarian, she ate very little red meat. It was determined that she had dynamic problems in the family. Depression and anxiety scores were significantly high. In laboratory measurements, the folate level was found to be very low at 1.8 ng/ml. Vitamin B12 level was also found to be low at 174 pg/ml. Homocysteine level was found to be quite high at 57.5 umol/l. No abnormality was found in the blood count. The patient was diagnosed with MDD and GAD. In addition to antidepressant treatment, vitamin B12 and folic acid support was given. In addition, supportive psychotherapeutic interviews were given to the patient. In this case, not only family dynamic problems, but also vitamin deficiencies contributed to the clinical picture. It was observed that her condition improved during follow-up examinations.

Case 6

A 15.5-year-old male patient came to our clinic with unhappiness, self-harm (in the form of scratching his arm), suicide attempt by taking drugs 5 times, and tantrums. He smoked a pack of cigarettes a day. Although he was not a vegetarian, he consumed very little red meat. Depression and anxiety scores were very high. In laboratory measurements, folate value was 2.2 ng/ml and vitamin B12 value was found to be low at181 pg/ml. Homocysteine was also found to be high at 34.6 umol/l. No significant pathology was detected in other laboratory measurements. With the diagnosis of MDD, vitamin B12 and folic acid were added to the treatment along with antidepressants. His condition improved during follow-up examinations.

Case 7

A 17-year-old female patient was admitted to our clinic with complaints of irritability, outbursts of anger, general anxieties and restlessness, unhappiness, crying a lot, headaches, and forgetfulness. No significant psychosocial problems were detected in herlife. The STAI-1 and 2 anxiety scales, which are psychometric tests, were also high. Kovaks Depression Inventory showed a situation close to the cut-off score of 16 points. However, according to clinical observation, her depressive symptoms were evident. In laboratory findings, folate value was normal at 6.8 ng/ml, while vitamin B12 was low at 133 pg/ml. Homocysteine value was high at 22.2 umol/l. Other laboratory values were normal. As a result, the patient was diagnosed with GAD and MDD. In her treatment, vitamin B12 supplementation was administered in addition to antidepressants. Her condition improved in further interviews.

Case 8

A 14-year and 10-month-old male patient came to our clinic with complaints of extreme nervousness, reactivity, distress, feeling lonely, unhappiness, self-harm, and suicidal ideation. No significant dynamic problems were detected in individual and family interviews. Depression and anxiety scores were high in psychometric tests. In laboratory measurements, pathologically, vitamin B12 was found to be very low at 93 pg/ml, while homocysteine was found to be high at 23.1 umol/l. Folate level was 4 ng/ml. According to clinical observation and psychometric measurements, the patient was diagnosed with MDD and GAD. In addition to antidepressant treatment, vitamin B12 support was given. His psychiatric status improved within 6 months.

Case 9

A 16-year-old male patient came to our clinic with complaints of palpitations, chest tightness, numbness in his hands and feet, tantrums, general anxieties and fears, and forgetfulness, which were occurring for the past year and intensified recently. First of all, pediatric cardiology consultation was requested due to cardiological complaints. No cardiac pathology was detected. The patient had a meat allergy. When he eats meat, redness, swelling and itching develop in his eyes. Therefore, he never consumes red meat. No significant psychosocial problems were detected in family and individual interviews. Depression scores were not high, but anxiety scales were high. He smoked 10 cigarettes a day. In the laboratory tests, folate was at low levels of 3 ng/ml and vitamin B12 was at 163 pg/ml pathologically. There were no hematological findings. His homocysteine level was quite high at 31.8umol/l. With the diagnosis of GAD, folic acid and vitamin B12 support (first IM) was given in addition to antidepressants. In the following months, a significant improvement was observed in his condition.

Case 10

A 16.5-year-old female patient applied to our outpatient clinic with her family with complaints such as self-harming behaviors, suicide attempt by taking pills, unhappiness, forgetfulness, carelessness, desire to cry, suicidal ideation, and decreased academic performance. As a result of individual and family interviews, there were prominent depressive symptoms, although there were no significant psychosocial problems. Depression and anxiety scores were also found to be high in psychometric tests. Complaints about cognitive functions such as forgetfulness, inattention, and decrease in academic success were notable. No hematological abnormality was observed in laboratory measurements. Thyroid function tests and routine biochemical analyses were also normal. Vitamin B12 value was determined to be quite low at 90 pg/ml. Folate value was close to the lower limit at 4 ng/ml. The Hcy value was 23.2 umol/l, more than twice the upper limit. In addition to antidepressants, vitamin B12 IM and oral folic acid were added to the patient's treatment with individual supportive interviews after the diagnosis of MDD. After 6 months of control, her condition was observed to improve.

Case 11

A 15-year and 9-month-old female patient came to our outpatient clinic with depressive symptoms such as suicide attempt, unhappiness, and suicidal thoughts, as well as obsessive symptoms related to uncertainty, controlling behaviors, cleanliness and contagion. She smoked a pack of cigarettes a day. Depression and anxiety scores were found to be high on psychometric tests. The Yale-Brown OCD scale score was found to be high at 21. A diagnosis of MDD and OCD was made as a result of detailed psychiatric examination and family interviews. In routine blood measurements, vitamin B12 was found to be pathologically low at 150 pg/ml. The Hcy value was found to be high at 21.4umol/l. Vitamin B12 treatment was added to her psychopharmacological treatment. Her condition improved in 6 months-1 year follow-up.

Case 12

The female patient was 16.5 years old, and applied to our clinic with complaints such as difficulty in swallowing solid food due to feeling like it would get caught in her throat, not being able to touch or use other people's belongings, dislike of her body, thoughts about her throat looking bad, being unsure, and re-reading and re-writing due to being unsure of what she read and wrote. In psychometric tests, in addition to pathologically high trait anxiety, a moderately increased obsession score (Yale

Brown OCD scale) of 16 points was found. In the blood measurements, folate was found to be pathologically low at 3 ng/ml. Although vitamin B12 (335 pg/ml) was normal, Hcy value was high at 29.9 umol/l. In addition, the ferritin value was low at 4.38 ng/ml (4.63-204). Hematological parameters revealed microcytic anemia. Iron therapy was started with pediatric consultation. Folic acid was urgently added to her psychopharmacological treatment. Although vitamin B12 was within normal limits, we added vitamin B12 orally to the treatment because values were close to the lower limit, and it has no toxic effects. In the following months, her symptoms regressed.

Table 2. Measurement methods in Vitamin B12 deficiency

Methods	Interpretation
Total Vitamin B12 (cobalamin)	It is inexpensive and widely used. But it does not show the active form. False positive and negative values are common.
Holotranscobalamin	It is an expensive and uncommon test. Indicates the active form of total cobalamin. It is the parameter that shows the earliest deficiency. It provides the transport of B12 into cells.
Homosistein	It is sensitive but not specific for vitamin B12. It is affected by kidney and thyroid functions. Age, alcohol, smoking, drugs, and coffee consumption can affect the result. It also increases in folic acid and B6 deficiency.
Metilmalonic asit	It has high diagnostic sensitivity. But it is not a routine measurement. It may be affected by kidney functions. False positive values may occur. It is also measured in urine. It requires advanced technology and is more costly.

DISCUSSION

In this study, cases who applied to a child and adolescent psychiatry clinic with psychiatric symptomatology and found to have vitamin B12 and/or folate deficiency and accompanying Hcy increase in laboratory examinations are presented. As stated in the introduction, there are reports that vitamin B12, folate and homocysteine, which are related to OCM, may play a role in the physiopathology of many psychiatric disorders seen in children and adolescents.

When these cases are evaluated in general, vitamin B12 deficiency and Hcy increase are present in all cases except one, and in some cases, folate deficiency accompanies this. In one case, it was observed that only folate deficiency was accompanied by an increase in Hcy. The common clinical features of all these adolescents are mostly combined anxiety and depressive symptoms. If expanded a little more, it is understood that symptoms of unhappiness, suicidal thoughts or attempts, general concerns, anger control problems, and self-harm come to the forefront. Sometimes these are combined with psychosomatic symptoms such as headache, lethargy, combativeness, weakness and fainting. Sometimes cognitive symptoms like forgetfulness and inattention are observed.

According to the information obtained from the individual and family interviews of the cases, stress that could cause depressive and anxiety symptoms was not common or evident in the cases.

HHcy is evaluated in 3 groups as mild (15-30 umol/l), moderate (30-100 umol/l) and severe (>100 umol/l). The causes of HHcy were mentioned above (29). Considering the causes of HHcy, there is no medical condition that can cause HHcy in each of these cases. All cases were evaluated in terms of thyroid, kidney and liver function tests and no pathology was detected in any of them. Chronic medical conditions were not detected in the registry examinations of the cases. In addition, all cases were within the normal BMI range. Only some cases were smokers. Smoking is also a factor that increases the level of Hcy. Another important common feature seen in most of the cases was that although they were not vegetarian, they consumed very little animal food. Animal foods are very important sources of vitamin B12.

There are some limitations in the evaluation of these cases. First of all, enzyme deficiencies or polymorphisms that may cause HHcy were not examined. If enzyme deficiencies were present, the level of HHcy would be very severe and cause symptoms in young childhood. Polymorphisms may be found in these cases and may contribute to the development of HHcy. Further research is needed in this regard.

In addition, cases should be examined in terms of atrophic gastritis. Further investigations such as autoantibodies and gastric endoscopy were not performed in these cases. However, the fact that not all cases here had gastric complaints reduces this possibility. The fact that vitamin B12 and folate deficiencies accompany HHcy in these cases suggests that deficiencies of these vitamins significantly contribute to the development of HHcy. In addition to the treatment of depression and anxiety with antidepressants, supplementation with vitamin B12 and/or folate, and significant improvement in symptoms during follow-ups within six months to one year suggest that vitamin deficiencies and HHcy may cause neuropsychiatric symptoms. This is also consistent with a report that vitamin B12, folate, and SAM supplementation may have beneficial effects in mood disorders (30). It is also necessary to measure vitamin B6 (pyridoxine) because there may be an increase in Hcy with vitamin B6 deficiency. This study had a retrospective nature. It is difficult to reach a definite cause-effect relationship here. Another missing aspect of the study is the lack of other laboratory measurements that may indicate vitamin B12 and folate deficiency (such as holotranscobalamin and erythrocyte folate level).

The literature includes extensive researched showing that HHcy may cause neurodegenerative diseases and neuropsychiatric findings, mostly in the elderly, and it was thought that this age group may be more sensitive. However, children and adolescents may be more sensitive to these vitamin deficiencies and HHcy due to reasons such as growth, development, cell proliferation, and neuroplastic processes (31). Therefore, the role of HHcy and vitamin deficiencies in neuropsychiatric diseases in children and adolescents needs to be further clarified. In addition, clinicians should consider these vitamin deficiencies and HHcy in adolescents presenting with psychiatric symptoms.

Diagnostic methods forVitamin B12 and Folate deficiency

In this study, only routine measurements were examined. In fact, there is no gold standard test that can show both vitamin B12 and folate deficiency (Table 2). The vitamin B12 values used here are for total cobalamin in plasma. This test may not reflect intracellular vitamin B12 status and by itself is not a completely reliable indicator of deficiency (32). Vitamin B12 is used as a cofactor in two enzymes. One of them is methionine synthase and methionine is synthesized from Hcy, and the other is methyl malonyl coA mutase, which converts methylmalonic acid (MMA) to succinyl coA (32). Therefore, MMA and Hcy increase in vitamin B12 deficiency. These two tests better reflect vitamin B12 deficiency. Holotranscobalamin (Holo-TC) is the active form of vitamin B12 and helps to diagnose deficiency at an early stage. Holo-Tc is present as 6-20% of total cobalamin. Even if the total vitamin B12 level is normal or marginal, evaluating Holo-Tc may provide more accurate results (33). In addition, as seen in the cases here, vitamin deficiencies may not be reflected in blood parameters. In other words, unexplained neuropsychiatric symptoms can be observed without hematological findings. In this regard, iron deficiency anemia, which is common in children and adolescents, should not be forgotten. Because iron deficiency anemia may overshadow the hematological findings of vitamin B12 (32). It should also be considered that even if serum vitamin B12 levels are normal, clinical signs can be seen. This may not reflect actual vitamin B12 deficiency in the tissues. Hcy and MMA measurement may also be required. In addition, routinely-measured serum vitamin B12 levels may be falsely high, and, it is necessary to be careful. As a result, measuring plasma total homocysteine for the evaluation of vitamin B12 deficiency seems more reasonable due to being routine, sensitive but non-specific, and having moderate cost.

Fewer tests are available to assess folate status. The serum or plasma folate level can be measured, but this reflects short-term folate deficiency. Instead, erythrocyte intracellular folate levels can provide information about tissue folate stores. However, this test is not routine (34). In addition, erythrocyte folate levels may decrease in vitamin B12 deficiency. Therefore, measuring serum Hcy levels is a test with moderate cost and high diagnostic utility for detecting folate deficiency.

Treatment of Vitamin B12 and Folate deficiency

Oral vitamin supplements can be given for vitamin B12 and folate deficiency. However, if there is a problem with vitamin absorption, this method may not be very effective. The most common form of vitamin B12 therapy is intramuscular (IM) administration of cyanocobalamin. However, oral administration of vitamin B12 can correct deficiency in children who develop vitamin deficiency due to nutritional deficiency. Oral supportive therapy may be an advantageous treatment method considering the pain of the IM method in children. In adults, no significant difference in efficacy was found between oral and IM therapy (35). Apart from these two methods, vitamin B12 can also be given in the form of sublingual spray or by intranasal administration. Both of these methods were shown to be effective in the treatment of vitamin B12 in children (36). In particular, sublingual spray can be an easy, effective and more practical treatment method. Vitamin B12 levels that improve after treatment should not be misinterpreted. Treatment should be continued even if the levels are within the normal range. In addition, the fact that vitamin B12 levels in serum are in the normal range does not mean that treatment should not be given. However, the absence of hematological findings should not suggest that treatment is not necessary.

Although there are studies conducted in elderly patients, the level of vitamin B12 in brain tissue may be found to be low, even if the serum vitamin B12 level is within the normal range. Here, the CSF/serum vitamin B12 ratio may be a better indicator (37). Of course, this is not an easy method. However, this shows that vitamin B12 values in the normal range may not reflect brain tissue vitamin values. Folic acid (synthetic form of folate) can be easily given as oral treatment. Since these two vitamins are water-soluble, the excess is excreted in urine. There is no report in the literature that vitamin B12 administration causes serious side effects.

Although it was reported that folic acid may contribute to the emergence of conditions such as obesity, insulin resistance and epilepsy in children who are born after excessive doses are given to pregnant women, there is no report that it causes any side effects in children and adolescents. However, when vitamin B12 deficiency is present, adding only folate therapy can mask vitamin B12 deficiency, so care should be taken. However, low folate levels may be associated with poor response to antidepressant treatment. Adding folic acid to treatment increases the effectiveness of antidepressant treatment (38). In addition, adding vitamin B12 to treatment may ensure that neurotrophic factors such as BDNF, which have an important role in the pathogenesis of depression, are maintained at appropriate levels. In animal studies, vitamin B12 supplementation caused an increase in BDNF in the cortex and hippocampus (39). In addition, an increase in Ntrk-2 expression occurs through epigenetic regulation with vitamin B12 supplementation. Ntrk-2 is a BDNF receptor, the tropomyosin/tyrosine receptor kinase B gene. BDNF and its receptor are involved in the pathogenesis of depression and antidepressant response (40). As with folate supplementation, adding vitamin B12 to treatment in depressed patients with inadequate response to antidepressants may provide clinical improvement after supplementation. In many studies, higher vitamin B12 levels were shown to be associated with better response to treatment. Individuals who do not have any clinical symptoms despite having low vitamin B12 levels are also prone to develop symptoms later. Therefore, these individuals should also take vitamin supplements. As a result, it may be beneficial to add vitamin B12 and folate (or their metabolic product, SAM) to treatment for people with mood disorders, and these vitamins should be used to improve treatment outcome (30).

For the treatment of low vitamin B12 in the adolescents in this study, we first attempted to increase the vitamin levels quickly by IM administration. Injections continued once a day for the first 5 days, and then once a month for 6 months. In addition, oral folic acid and vitamin B12 treatments were begun. The intensity and discomfort due to the psychiatric symptoms of the patients indicated the need to start psychopharmacological treatment. It would be unethical not to begin this treatment. Therefore, as a result of vitamin supplementation and psychopharmacological agents, the symptoms of the patients decreased within six months to one year. Of course, it is difficult at this stage to distinguish whether this decrease is due to the psychopharmacological agent or vitamin supplementation, or the positive effects of both.

CONCLUSION

Child and adolescent psychiatrists should suspect vitamin B12 and folate deficiency in patients who attend with different neuropsychiatric symptoms. Some recommendations were made to clinicians in this regard (Table 3). It is important to know the methods for diagnosis and treatment of deficiency of these two vitamins, which are indispensable for healthy brain tissue and functions. Although it is thought that deficiency of these two vitamins is not common in children and adolescents in developed countries, higher deficiency rates than expected can be observed in children and adolescents due to differences in eating habits.

Table 3. Recommendations to clinicians

- It may be beneficial for child and adolescent psychiatrists to measure vitamin B12 and folate, especially in adolescents presenting with depressive and anxiety symptoms.
- In cases where vitamin b12 and folate deficiency is thought, the next test such as homocysteine may give more accurate results.
- Child and adolescent psychiatrists can question their patients' diets, and also make dietary recommendations when necessary.
- Having a normal diet does not mean that vitamin B12 and folate deficiency will not develop.
- Vitamin B12 and folate deficiency are not only seen in the elderly. It can also be seen in children and adolescents.
- Since the vitamins necessary for healthy brain tissue can be taken less, especially in those who consume less animal foods, such as vegetarians, these people may be accompanied by mental symptoms.
- Serum vitamin B12 and folate levels in the normal range should not mean that there is no deficiency. Vitamin values in brain tissue may be lower than serum values. It would be better if vitamin supplementation was done, especially at values close to the lower limit of the normal range.
- Due to brain development, cell proliferation, and increased neuroplastic processes, children and adolescents may be more susceptible to vitamin B12 and folate deficiency and one-carbon metabolism pathologies.
- Existing vitamin deficiencies may not be reflected in the blood picture. Even clinical signs may not have appeared. Supplementation should be given to these risky people.
- Child and adolescent psychiatrists can add vitamin B12 and folic acid to their psychopharmacological treatments. These are non-toxic or negligible, and supplementation therapy is easy and inexpensive. They also increase the effectiveness of antidepressants.

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