Original Article

Vitamin B12 and homocysteine in patients with major depressive disorder

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Abstract

Background: Alterations in the level of neurotransmitters are evident in patients with major depressive disorder (MDD). Vitamin B12 mediates the synthesis of neurotransmitters, and hence, vitamin B12 deficiency could be associated with depression. **Aims and Objectives:** To assess the levels of serum vitamin B12, homocysteine (Hcy), and haematological profiles in patients of MDD. **Materials and Methods:** Fifty-nine patients with MDD were recruited based on ICD-10 criteria. Severity of depression was assessed by HAM-D scale. Vitamin B12, Hcy levels, and haematological profiles were analysed. **Results:** Vitamin B12 was deficient or depleted in all patients with MDD. The median level of vitamin B12 in serum was 164.2 pg./ml and significantly lower in patients with severe MDD. The mean value of Hcy was 18.34 μmol/L, which was high compared to the normal reference range. The red cell distribution width (RDW-CV) varied significantly between the three groups of MDD patients. Patients consuming non-vegetarian food had a significantly higher median value of serum vitamin B12. **Conclusion:** Vitamin B12 deficiency is found in patients with MDD and varies inversely with severity of MDD. Hcy is found to be higher in patients with MDD. The manifestation of depressive symptoms precedes the more commonly known haematological manifestations of vitamin B12 deficiency in this study.

Keywords: Homocysteine, major depressive disorder, vitamin B12, RDW-CV

Introduction

Major depressive disorder (MDD) is a multi-factorial global mental health problem that is characterised by mood changes, guilt, anxiety, decreased interest, reduced cognitive performance, and disturbed sleep or appetite.[1,2] Around 322 million people in the world are affected by depression.[3] A family physician is often the first point of contact of this commonly encountered disease. MDD has a complex aetiology, with genetic and environmental variables such as physical, mental, and emotional trauma in childhood and nutritional causes being closely linked to the development of MDD.[2] Various hypotheses have been proposed regarding the pathogenesis of depressive disorders. Among them, the "monoamine hypothesis" suggests a lack of or inadequate synthesis of monoamine transmitters such as serotonin, dopamine, and norepinephrine as the causes of depression.[1] Vitamin B12**,** a water-soluble vitamin present largely in non-vegetarian sources, helps in the initial myelination and thus neuronal development and function. It is a cofactor for L-methyl-malonyl-coenzyme A mutase and methionine synthase enzymes.[4,5] It also plays a significant role in DNA synthesis and in haematopoiesis. Serum vitamin B12 levels normally range between 200 pg/ml and 900 pg/ml. Serum B12 <200 pg/ml suggests deficiency, and serum B12 between 200 and 300 pg/ml indicates depletion.[6] Low B12 leads to megaloblastic or macrocytic anaemia, which is

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characterised by enlarged red blood cells and hyper-segmented neutrophils.[7,8] As per the World Health Organisation (WHO) criteria, anaemia is defined by haemoglobin (Hb) levels less than 12 g/dl in women and less than 13 g/dl in men.[9] Low B12 levels are a risk factor for the development of MDD, and they impair S-adenosyl methionine (SAM) production. SAM is involved in the methylation of phospholipids. Vitamin B12 replacement therapy appears to generate significant mental health benefits in severely depressed patients.[10] Homocysteine (Hcy) is a non-essential sulphur-containing amino acid derived by the transmethylation of essential amino acid methionine.[11] The normal plasma value of homocysteine ranges between 7 and 14 μmol/L.[12] A strong correlation between depression and elevated Hcy has been reported.[13] Hcy indirectly mediates dysfunction of the methionine metabolism loop and thus inhibits the formation of the neurotransmitters like dopamine, serotonin, and noradrenaline that are involved in the pathogenesis of depression.[14]

Hence, we aimed to assess the serum levels of vitamin B12 and Hcy in patients with MDD and their haematological profiles.

Method

The study was conducted after the approval by the Institute Ethics Committee and in compliance with ethical standards. The study was designed as a cross-sectional analytical study involving newly diagnosed MDD patients in the age group of 18–65 years. Patients with MDD diagnosed based on the International Classification of Disease 10 criteria[15] were recruited from the Department of Psychiatry. Written informed consent was taken, and history, socio-demographic, and clinical data were collected and noted down in the proforma. Patients suffering from other psychiatric illnesses and patients taking a supplement of vitamin B12 were excluded from the study. The severity of symptoms was assessed by the Hamilton Depression Rating scale.[16] The sample size was estimated based on the assumptions for prevalence estimation. An anticipated 22% prevalence of vitamin B12 deficiency in people with depression, 95% confidence level, and 10% absolute precision were observed; the required sample size is 66.[17] A sample size of 59 could be achieved due to the prevailing coronavirus disease pandemic.

5 ml of blood was collected from MDD patients, of which 2 ml was collected in ethylene diamine tetra-acetic acid tubes and sent for haematological analysis using a SYSMEX XS-1000i Automated Haematology analyser, and 3 ml was collected in plain tubes for analysis of serum vitamin B12 and Hcy. Serum vitamin B12 was estimated by chemiluminescence immunoassay using a unicell DXI 600 Access Immunoassay System manufactured by Beckman Coulter. Serum Hcy was estimated by enzyme-linked immunosorbent assay.

Statistical analysis

Statistical analysis was done by using SPSS 19.0 version. All categorical variables were summarised in terms of frequency and proportions. Continuous variables were summarised as arithmetic mean and standard deviation or median and inter-quartile range. Prevalence was computed in terms of proportion and its 95%confidence interval. A comparison of biochemical and haematological parameters with the severity of MDD was done using analysis of variance.

Result

There were 31 women and 28 men among the 59 MDD patients showing a female preponderance. The severity of MDD was determined by using the HAM-D scale, and patients were classified into very severe, severe, moderate, and mild depression groups. Ten patients had a score ≥25 and were labelled as having very severe depression. Twenty-four patients with a score of 19–22 were labelled as having severe depression. Twenty-two patients had moderate depression with scores between 14 and 18, while three patients were mild with scores between 8 and 13. The mild group was merged with the moderate group for ease of statistical calculations.

The baseline characteristics of patients are summarised in Table 1. The levels of serum vitamin B12 ranged from 107.6 pg/ml to 234.3 pg/ml with a median value of 164.2 pg/ml. (reference range 200–900 pg/ml).[6] All patients either were deficient in B12 or had depleted levels. The median value of vitamin B12 was significantly higher in the moderate group compared to severe and very severe groups (*P* value 0.031) [Table 2]. The mean value of Hcy was 18.34 μmol/L (reference range 5–15 μmol/L).[12] The homocysteine value was higher in the patients as compared to the reference range, which is a marker of B12 deficiency. The group with severe MDD had a higher Hcy value than the moderate MDD group, although it was not statistically significant [Table 2].

Out of 59 patients, 19 had anaemia based on WHO criteria; of them, three were men and 16 women. RDW CV also varied significantly between the three groups (a *P* value of 0.037). There was an increase in the RDW in the group with very severe

Table 1: Descriptive statistics of baseline characteristics of MDD patients

Parameter	Major Depressive Disorder N=59
Age (years)	37.32±12.24
Height in meter	1.62±0.0801
Weight in kg	64.05±11.15
BMI kg/m^2	24.35±3.97
HAM-D SCORE	19.31±3.48
Vitamin B12 pg/ml	164.2 (107.6-234.3)
HCY μmol/l	18.34±6.036
Haemoglobin (g/dl)	12.87±2.115
RBC count x10^9/L	4.63±0.6
MCV (fl)	87.9 (85.1-92)
MCH (pg)	28.1 (26.8-29.2)
MCHC (gm%)	31.52±1.99
RDW CV	13 (12.5-14)

Table 2: Comparison of biochemical and haematological parameters between the severity of MDD

Parameter	Moderate (*n*=25)	Severe (*n*=24)	Very severe (*n*=10)	*P*
Vitamin B12 pg/ml	206.6 (134.35, 262.5)	133.75 (99.92,181.4)	159 (129.7,288.2)	0.031
HCYμmol/l	17.47±6.83	19.5±5.97	17.72±3.61	0.479
HGB	12.4 (11.6, 14.6)	14.1 (12,14.8)	12.25 (9.85,13.65)	0.116
RBC count	4.61±0.57	4.8±0.53	4.2±0.69	0.069
MCV	87.5 (83.55, 91.75)	88.3 (85.3, 94.87)	88.15 (85,92.2)	0.744
MCH	28 (26.65,28.6)	28.5 (27.72, 29.72)	27.95 (26.77,30.22)	0.273
MCHC	31.3±1.7	31.72±2.2	31.59±2.15	0.759
RDW CV	12.9 (12.3, 13.15)	13.15 (12.72,14.67)	13.7 (12.87,15)	0.037

MDD, though values were within normal limits. MCV was found to be higher in the group with very severe MDD as compared to moderate MDD, though the difference was not statistically significant [Table 2]. There was no association between peripheral smear findings with the severity of MDD. Table 3 shows the haematological picture and severity grading of MDD in the study participants.

Table 4 shows that patients consuming a moderate amount of non-vegetarian food had a statistically significantly higher median value of serum B12 as compared to those who were neglecting their diet (a *P* value of 0.031).

Table 3: Haematology picture and grade of severity of MDD

Number of patients	RBC pictures	Presence of anaemia	Grade of MDD in anaemic (HAM-D)
49	Normocytic normochromic	11	3 Very severe 2 Severe 6 Moderate
8	Microcytic hypochromic	8	1 Very severe 3 Severe 4 Moderate
2	Macrocytic normochromic	-	2 Severe

Discussion

Vitamin B12 is involved in the development of the central nervous system and helps in initial myelination. It is a cofactor for L-methyl-malonyl-coenzyme A mutase and methionine synthase enzymes. Vitamin B12 deficiency causes haematologic (megaloblastic anaemia), neurologic (demyelination, paraesthesia), gastrointestinal (anorexia, glossitis), and psychiatric symptoms, which include depression, bipolar disorder, and dementia.[18] Elevated levels of homocysteine are also associated with vitamin B12 deficieny.[5] Psychiatric manifestations precede neurological and haematological ones in many instances in patients with deficiency of vitamin B12.[19]

Fifty-nine patients who were recently diagnosed with MDD were recruited for the study based on the ICD10 criteria.[15] As in our study, MDD is known to occur in women with a frequency twice as high as in men.[2] This difference may arise more from the basic biological differences between the two genders than from culture, diet, race, and other economic and social causes.[20] The patients were categorised into three groups, very severe, severe, and moderate MDD, based on HAM-D scores.[16]

The median level of vitamin B12 in serum was found to be 164.2 (107.6–234.3) pg/ml. A value less than 200 pg/ml is considered as deficient, and values between 200 and 300 pg/ml are considered as depleted levels of vitamin B12 in serum.[6] Hence, these patients with MDD were suffering from deficiency/depletion of vitamin B12. The median value of vitamin B12 was found to be significantly lower in severe and very severe groups as compared to the group having moderate MDD in the present study (*P* value 0.031). A study by Khosravi *et al.*[3] showed an association between healthy dietary pattern and low risk of depression mediated by adequate levels of vitamin B12 and folate. In a study by Penninx *et al.*[10] conducted on elderly women, it was found that subjects with deficiency of vitamin B12 were twice as likely to be severely depressed than the ones who did not have deficiency. Hence, these studies also bring out the association of depression with deficiency of vitamin B12, which is similar to the findings in the current study. This association is probably mediated through SAM, which has anti-depressant properties.[7] Co-factors of vitamin B12, 5' deoxy adenosyl cobalamin and 5 methyl cobalamin, play important roles in metabolism of odd chain fatty acids and ketogenic amino acids.[7] Vitamin B12 is hence involved in synthesis of important neurotransmitters like dopamine and serotonin, lack of which is associated with depression and psychosis.[21]

The mean value of Hcy was 18.34 μmol/L, which is higher compared to the reference range. Increased levels of serum Hcy are commonly seen in vitamin B12 deficiency, but it is not specific as similar increases are seen with deficiency of folate and Vitamin B6.[6] Many human diseases are related to imbalances in metabolism of Hcy. It remains to be seen whether elevated levels of Hcy directly contribute to the pathogenesis of diseases or are a biomarker of altered methyl group metabolism.[11] In a study conducted in Taiwan, it was suggested that depression and anxiety may have a positive association with higher levels of Hcy.[13] In the present study, the groups of patients with severe and very severe depression had higher levels of Hcy in serum as compared to those with moderate depression, which is similar to the findings in the Taiwanese study. The difference was not statistically significant, probably due to the low sample size.

Table 4: Comparison of biochemical parameters among different classifications of diet

Parameter	Neglect of diet	Reduction in the consumption of non-veg	Moderately consumption of non-veg	*P*
Vitamin B12 pg/ml	164.34 (110.75, 245.47)	152 (100.57, 204.75)	186 (128.4, 358)	0.031
HCY µmol/L	17.56 (12.8, 22.17)	19.06 (14.96, 23.82)	16.87 (11.96, 20.66)	0.259

In this study, 19 patients had anaemia based on WHO criteria, of whom 84% were women. Eleven of these patients had a normocytic normochromic RBC picture, while the rest had a microcytic hypochromic RBC picture. Anaemia is a multi-factorial and predominantly nutritional in our country. None of the patients had macrocytic anaemia. In fact, two patients with macrocytic RBC indices did not have anaemia. Of the 19 patients with anaemia, 4 had very severe MDD, 5 had severe MDD, and 10 had moderate MDD. Haemoglobin, MCH, and MCHC did not differ much between the three groups of patients with MDD, but MCV was found to be higher in the group with severe and very severe MDD as compared to moderate MDD, though the difference was not statistically significant. RDW CV varied significantly between the three groups (*P* value 0.037). There is an increase in the RDW in the group with very severe MDD. An increase in MCV and RDW CV, reflecting the higher degree of anisocytosis or variation in the size of RBCs, is a feature of vitamin B12 deficiency. Often these changes precede the actual development of megaloblastic anaemia[5] Moreover, megaloblastic anaemia is a late clinical manifestation of vitamin B12 deficiency.[19] The increase of MCV and RDW CV with severity of MDD is of significance as it corroborates the fact that macrocytic anaemia manifests later than depression in patients with vitamin B12 deficiency.[19] Vitamin B12 deficiency is related to high Hcy, and macrocytosis is not always found to be associated.[7] This finding is very well reflected in the results from the current study.

Patients consuming moderate amount of non-vegetarian food had a statistically significant higher median value of serum B12 as compared to those who were neglecting their diet (*P* value 0.031). This finding is consistent with the results of the study by Khosravi *et al.*,[3] where they found that a healthy diet was associated with low risk of depression. Human beings cannot synthesise vitamin B12 and hence depend on a dietary source or supplementation for maintaining a normal level.[7] It is not always recognised that even little restriction of animal food gives rise to lowering of vitamin B12 status.[7]

Depression and anxiety are serious and common mental disorders in the general population including children and adolescence.[22] Depressive and anxiety symptoms are related to numerous factors, amongst which diet plays an important role in the occurrence and progress of depression and anxiety. Malnutrition of various macro- and micronutrients including vitamin B12 leads to aggravation of symptoms of MDD, especially in women.[23] Although a larger sample size and inclusion of control samples would have helped to consolidate the findings better, this study has brought out the fact that MDD is associated with deficiency of vitamin B12 and it correlated with severity of MDD. It also showed that symptoms of depression manifest before the haematological changes are found. Interestingly, RDW CV showed significant variation with severity of disease, which reflects development of macrocytosis has begun. The study also emphasises that vitamin B12 levels are lowered when consumption of animal food is decreased.

Conclusion

To conclude, we found that the serum vitamin B12 value is low in all patients with MDD. Its deficiency correlated significantly with severity of MDD (*P* value 0.031). The mean value of Hcy is found to be higher in all groups with MDD as compared to the normal reference range. The group with severe MDD has a higher Hcy value than the moderate MDD group, although it is not statistically significant. RDW CV correlates with severity of MDD (*P* value 0.037). Hence, in patients with MDD, deficiency of B12 should be considered even in the absence of haematological changes. These patients may benefit from supplementation of vitamin B12.

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Conflicts of interest

There are no conflicts of interest.

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