

## Are vitamin D, B12, and folate deficiency associated with depressive disorder? A case-control study

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### ABSTRACT

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#### Background:

Depression is a global burden with complex etiopathogenesis. Some nutrients including vitamin D, B12, and folate deficiency have been considered risk factors for depression. Therefore, this study has been contemplated to find out the possible association of vitamin D, B12, and folate deficiency with depression.

#### Method:

This study included 81 case subjects with depression and 95 control subjects without any International Classification of Diseases (ICD)-10 diagnosis. The sociodemographic details were collected from each subject. Beck's Depression Inventory (BDI) was administered to identify the severity of depression. The blood samples were collected and measured for vitamin D, B12, and folate along with other laboratory investigations as per exclusion criteria. The data were obtained and analyzed using descriptive and inferential statistics.

#### Results:

The mean age  $\pm$  standard deviation (SD) of the case and control subjects were  $34.86 \pm 9.25$  and  $33.49 \pm 8.44$ , respectively, without any significant difference ( $P > 0.05$ ). The subjects with vitamin D deficiency were found to have four times higher odds (OR 4.703; 95% CI = 2.378–9.300) for depression compared to subjects with sufficient vitamin D levels. In addition, there was a negative correlation between vitamin D levels and the severity of depression as per BDI scoring ( $r = -.384$ ,  $P < 0.01$ ). However, there was no significant association identified between the case and control group with respect to serum vitamin B12 and folate levels.

## Conclusion:

The results of the study revealed that vitamin D deficiency has an association with depression. However, further research studies are needed to validate its correlation to the etiopathogenesis of depression.

**Keywords:** Deficiency, depressive disorder, folate, vitamin B12, vitamin D

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In the mental health action plan 2013–2020, the World Health Organization (WHO) reported that people with mental health problems have higher disability and mortality. The population with depression has 40–60% more chances of premature death in comparison to the population having a physical illness.[1] Depression alone is responsible for 4.3% of the global burden of disease and is one of the large single causes of disability.[1] In 2017, mental disorders were considered the second leading cause of years lived with disability (YLDs) and the sixth leading cause of disability-adjusted life years (DALYs) in the world.[2] India had 197.3 million people with mental health disorders including 45.7 million people with depressive disorders in 2017. [2] Depressive disorder was the most common mental disorder which contributed to 33.8% of DALYs.[2] Multiple factors affect the development of mental disorders including genetic, stress, social, environmental, physical activity, nutrition in diet, and drugs.[3,4] Among these factors, dietary factors have some role in the modification and progression of mental disorders. It has been recognized that nutritional deficiency in the form of insufficient intake or absorption of nutrients is considered a contributory factor to mental disorders.[5] Studies have reported that daily adequate nutrients effectively reduce a patient's symptoms. The omega-3 fatty acids, niacin, folate, vitamin B6, vitamin B12, and vitamin D are beneficial for mental health.[6] Based on these emerging evidences, the identification of risk factors may be a game-changer for the management of psychiatric disorders.

Vitamin D is one of the essential fat-soluble vitamins for humans. The human body gets vitamin D from the diet as well as local production in the skin by sun exposure.[7] The half-life of 25-hydroxyvitamin D is 2–3 weeks, and that of 1, 25-dihydroxyvitamin D is 2–4 h. Though 25-hydroxyvitamin D is stored in fat tissue, serum 25-hydroxyvitamin D is currently the most accurate indicator of vitamin D level due to its relatively long half-life.[8] Recommendations for “normal range” serum 25-hydroxyvitamin D have been based on what levels are sufficient to avoid rickets.[9] There is evidence that vitamin D has an important role in brain health and disease.[10] Many areas of the brain including the cingulate cortex and hippocampus have receptors for vitamin D on neuronal and glial cells.[11] Vitamin D receptors and 1 $\alpha$ -hydroxylase are found in the hippocampus, an area that is found to be reduced in volume in persons with depression. The function of vitamin D in this area is unclear, but *in vitro* study reported a neuroprotective effect of 1, 25-dihydroxyvitamin D in cells of the hippocampal area when exposed

to glucocorticoids, suggesting that vitamin D could have favorable effects by protecting against stress-induced depression.[12] Clinical studies suggested that a low serum level of 25-hydroxyvitamin D in the body is associated with cognitive decline and features suggestive of anxiety and depressive disorder.[13]

In the deficiency of vitamin B12, there is an impairment of DNA methylation in redox-related genes due to the neurotoxic effects of homocysteine. If the level of homocysteine increases above the standard level, then it affects the redox signaling pathways in neurons through the generation of reactive oxygen species (ROS). The increased level of homocysteine may have a direct neurotoxic effect on N-methyl-D-aspartate (NMDA) receptors. It has been described that a low level of vitamin B12 is attributed to mental illness in the form of depression, mania, psychosis, and cognitive deficit.[14]

Folate deficiency affects the metabolism of monoamines; hence, folate insufficiencies are more commonly observed in mood disorders.[15] L-methyl folate insufficiency may be due to dietary deficiency (malnutrition, poor intake) or methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism. It has been observed that the TT genotype of methylenetetrahydrofolate reductase (MTHFR) C677T gene is largely associated with depression and bipolar disorder.[16]

Depression is a well-known burden on the health system, which causes patient suffering and family distress, and significantly increases the risk of suicide. Nowadays, the health system requires the recognition of risk factors that can screen those individuals who are at risk so that illness can be prevented or treated in advance for better outcomes for patients suffering from the disorder. Therefore, this study was contemplated with the following aims and objectives.

#### Aim

This study was conducted to find out the association between vitamin D, vitamin B12, folate deficiency, and depressive disorder.

#### Objectives

This study was planned with the following objectives:

1. Prevalence of vitamin D, B12, and folate deficiency in depressive disorder.
2. Association of vitamin D, B12, and folate deficiency with presence and severity of depressive disorder.

## MATERIAL AND METHOD

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This is a case-control study planned to carry out in a tertiary care center for the duration of two years from September 2019 to July 2021. Approval of the institutional ethical committee and informed consent of all subjects were taken before the conduct of the study. The study population was composed of male/female outpatients and inpatients with cases of first episode depression (Depressive episode as per International Classification of Diseases (ICD)-10) and matched control subjects from the general population. All individuals who reported to tertiary care psychiatry center were clinically evaluated as per ICD-10 and evaluated for the severity by psychometric scale; Beck's depression inventory (BDI). The BDI range 0–9 was considered as

minimal, 10–16 as mild depression, 17–29 as moderate depression, and 30–63 as severe depression.[17] The control subjects without any psychiatric illness were taken from the general population after clinical evaluation. We decided to take a minimum of 70 case subjects having first episode depression and 70 healthy control subjects as a statistically calculated sample size for the case-control study. The participants were included in this study after fulfilling the inclusion and exclusion criteria.

### Inclusion criteria

1. Age group of 18–60 years of age.
2. The cases with the first episode of depression; diagnosed as depressive episode as per ICD-10 and severity was analyzed using the BDI scale. Age and gender-matched healthy controls were taken from the general population.

### Exclusion criteria

1. Individual has been taking psychoactive substances or alcohol and having a chronic medical ailment or is on any medication in the previous 6 months.
2. Individuals with a past history of chronic medical illness or psychiatric illness and a present history of eating disorders.

The sociodemographic details of all subjects were taken and their blood samples were collected and tested for 25-hydroxyvitamin D, vitamin B12, and folate levels using radioimmunoassay. The serum vitamin D level below 50 nmol/l, serum vitamin B12 level less than 200 pg/ml, and serum folate level less than 3 pg/ml were considered as deficient.[18,19]

The data was collected and analyzed using Microsoft excel (Microsoft Office 2019, Meerut, Uttar Pradesh, India) and SPSS software version 2020 (IBM India Pvt Ltd, Pune, Maharashtra, India). Descriptive statistics (i.e. frequency, percentage, mean, standard deviation) was calculated wherever applicable and inferential statistics (i.e. independent *t*-test, Chi-square test, binary logistic regression) was used to compare the variables. The significant value was set as  $P < 0.05$ .

## RESULTS

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A total of 100 case subjects and 100 control subjects were taken for the study. Out of 100 cases, 15 subjects were having chronic medical co-morbidity and 04 subjects were cases of alcohol dependence syndrome; hence, 19 cases were excluded from the study. Out of 100 control subjects, 05 subjects refused to give blood samples; hence, excluded from the study. A total of 81 cases and 95 control subjects were taken for analysis. The mean age (SD) of cases and controls were 34.86 (9.25) and 33.49 (8.44), respectively. There was no significant difference between cases and control subjects with respect to age (*t* value = 1.026, *P* value = 0.306), age group, gender, religion, residential area, marital status, and occupation [Table 1].

Out of a total of 176 subjects, we found 58 subjects (32.95%) with vitamin D deficiency, 17 subjects (9.65%) with vitamin B12 deficiency, and 30 subjects (17.04%) with folate deficiency. A total of 50.61%, 12.34%, and 22.22% of subjects with depression had vitamin D, B12, and folate deficiency, respectively. The participants with vitamin D deficiency were found to have four times higher odds for depression in comparison to participants having sufficient vitamin D levels. There was a significant difference ( $P < 0.05$ ) between the case and control subjects with respect to serum vitamin D levels. Although, there were more vitamin B12 and folate level deficient subjects in the patient group in comparison to the healthy control group. No significant difference ( $P > 0.05$ ) was noted [Table 2].

On bivariate correlation (Spearman's) analysis, a significant negative correlation was found between BDI severity scoring and serum vitamin D level in all subjects ( $r = -.384$ ,  $P < 0.01$ ). However, no significant correlation was identified between BDI severity scoring and vitamin B12 and folate level in all subjects ( $r = -.048$ ,  $P = 0.530$  and  $r = -.117$ ,  $P = 0.123$ , respectively). The patient group was evaluated separately and found to have a negative significant correlation between vitamin D level and severity of depression ( $r = -.241$ ,  $P = 0.03$ ). No significant correlation was seen with respect to vitamin B12, folate level, and the severity of depression ( $r = .135$ ,  $P = 0.229$  and  $r = .017$ ,  $P = 0.883$ , respectively).

The binary logistic regression analysis was performed to find out the effects of sociodemographic variables on depressive episodes. It was observed that there were no significant effects on depression with respect to age group, gender, and occupation [Table 3].

## DISCUSSION

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There are evidences that polymorphism of vitamin D receptor (VDR) in the brain tissue is found to be associated with depression.[11,20] A meta-analysis revealed mixed results; cross-sectional studies observed no significant association between depression and vitamin D level; however, cohort studies observed a significantly increased hazard ratio in depressive patients with low vitamin D.[18] This study observed that individuals with vitamin D deficiency have significantly higher odds of depression which may be suggestive to be a risk factor for depression.

One RCT and open-label trial revealed that vitamin D as an adjunctive in the treatment of depression was given an oral dose of 1500 IU/day along with 3,00,000 IU at starting and 4 weeks of follow-up. There were no significant differences noted in response and remission rates between the vitamin D and the control group.[21] The dose of vitamin D (1500 IU/day) as an adjunctive compared with placebo during fluoxetine treatment for depression found a significantly lower score on Hamilton Depression Rating Scale (HDRS) and BDI at week 8. At baseline, depressive patients with vitamin D deficiency and serum levels of vitamin D were found to be negatively correlated to HDRS and BDI scores ( $r = -0.63$ ,  $P < 0.01$ ).[22] We also found a significant negative correlation of depression severity with vitamin D deficiency.

The present study has identified no significant association of depression with respect to vitamin B12 levels. In contrast to these findings, few studies have identified a significant association between vitamin B12 and depression.[23-25] A prospective cohort study in Korean persons also observed a significant association of vitamin B12 with depressive disorder over a period of 2–3 years.[26] In accordance with our results, some studies observed no beneficial effect of vitamin B12 supplementation on depressive symptoms.[27,28] A study in middle-aged

adults from Finland observed that vitamin B12 level was associated with melancholic depressive symptoms but not with non-melancholic symptoms of depression.[29] However, the distinction between subtypes of depression was not considered in our study.

A study reported that folic acid improves depressive symptoms as an adjunctive to antidepressants.[30] However, another study identified no significant improvement in major depressive disorder following folic acid administration.[31] This study also showed no significant association between serum folate level and depressive disorder.

The results from past studies indicated an association of vitamin B12 and folate deficiency with symptoms of depression.[32] However, congruent to our findings, a study revealed that serum vitamin B12 and folate concentrations in 224 newly diagnosed psychiatric patients and healthy controls from the Israeli population were compared and showed no significant difference between the groups.[33]

Literature reveals that the percentage of vitamin D deficient psychiatry patients with depression is high. Therefore, screening for vitamin D in patients with depression should be part of the health assessment. A cost-efficient alternative to screening was also suggested that all patients with depression should be treated with a pharmacological dose of vitamin D (ergocalciferol).[34]

US Preventive Service Task Force (USPSTF) also tried to find out the benefits and harms of screening and early treatment for vitamin D deficiency. USPSTF had insufficient evidence for the same in asymptomatic adults. However, the American Association of Clinical Endocrinologists recommended screening for vitamin D deficiency in individuals at risk and did not recommend it in individuals at no risk.[35]

The limitations of this study include that the sample population was taken from a single tertiary care center and the nearby region, with a limited number of the study population. Hence, results might not be generalized to the overall population of India. This was a case-control study; however, a cohort study would have been better to find the exact prevalence of risk, attributable risk, and predictive nature of vitamin D in the causation of depression. The duration of psychiatric illness, lifestyle, and habits of food intake were not taken into account. In addition, genetic variability may be a contributory factor to vitamin deficiency and its presentation, which was not considered in this study. The vitamin concentration was measured in blood serum; however, there are no gold standard tests to measure vitamin concentration in the brain. In addition, there are ambiguities about vitamin D values, which value may be considered as normal in psychiatric illness.[36]

## CONCLUSION

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Based on our findings, 32.95% subject population had vitamin D deficiency and these vitamin D deficient subjects had four times higher odds of depression in comparison to subjects with sufficient vitamin D levels. Therefore, vitamin D deficiency has an association with depression. However, vitamin B12 and folic acid showed no significant association with depression. The findings of this study have recommended that clinicians should investigate the vitamin D level in patients with depression. Further research is needed to find out the modality of vitamin D deficiency causing depression.



## Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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## Conflicts of interest

There are no conflicts of interest.

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Table 1

Distribution of all subjects by sociodemographic variables

	Case <i>n</i> (%)	Control <i>n</i> (%)	Chi-square value ( $\chi^2$ )	<i>P</i>
Age Group				
18-29 years	23 (40.4%)	34 (59.6%)	1.486	0.685
30-39 years	36 (46.8%)	41 (53.2%)		
40-49 years	15 (53.6%)	13 (46.4%)		
50-60 years	07 (50.0%)	07 (50.0%)		
Gender				
Male	41 (42.7%)	55 (57.3%)	0.934	0.334
Female	40 (50.0%)	40 (50.0%)		
Religion				
Hindu	63 (46.0%)	74 (54.0%)	1.088	0.780
Muslim	07 (58.3%)	05 (41.7%)		
Sikh	05 (38.5%)	08 (61.5%)		
Christian	06 (42.9%)	08 (57.1%)		
Residential Area				
Urban	33 (50.8%)	32 (49.2%)	1.268	0.530
Semi-urban	24 (40.7%)	35 (59.3%)		
Rural	24 (46.2%)	28 (53.8%)		
Marital Status				
Unmarried	14 (41.2%)	20 (58.8%)	0.656	0.720
Married	58 (46.4%)	67 (53.6%)		
Widow/Widower	09 (52.9%)	08 (47.1%)		
Occupation				
Unemployed	30 (54.5%)	25 (45.5%)	8.042	0.154
Unskilled worker	09 (45.0%)	11 (55.0%)		
Semi-skilled worker	06 (27.3%)	16 (72.7%)		
Skilled worker	09 (32.1%)	19 (67.9%)		
Arithmetic skill jobs	15 (55.6%)	12 (44.4%)		
Semi-professional	12 (50.0%)	12 (50.0%)		

Table 2

Association of depression with respect to vitamin D, B12, and folate levels

	Case (n)	Control (n)	Odds Ratio (95% CI)	P
Vitamin D				
Deficient	41	17	4.703 (2.378-9.300)	0.001*
Sufficient	40	78		
Vitamin B12				
Deficient	10	7	1.771 (0.642-4.887)	0.265
Sufficient	71	88		
Folate				
Deficient	18	12	1.976 (0.888-4.400)	0.092
Sufficient	63	83		

\*Significant difference (P<0.05)

Table 3

Prediction of depression with respect to independent variables in logistic regression analysis

	B	S.E.	Wald	df	Sig.	Exp (B)	95% C.I. for EXP (B)	
							Lower	Upper
Age Group								
18-29 years	0.391	0.599	0.426	1	0.514	1.478	0.457	4.781
30-39 years	0.130	0.581	0.050	1	0.823	1.139	0.365	3.558
40-49 years	-0.143	0.655	0.048	1	0.827	0.867	0.240	3.130
50-60 years						Reference	-	-
Gender								
Male	-0.294	0.304	0.932	1	0.334	0.745	0.411	1.353
Female						Reference	-	-
Occupation								
Unemployed	-0.182	0.490	0.139	1	0.710	0.833	0.319	2.177
Unskilled worker	0.201	0.607	0.109	1	0.741	1.222	0.372	4.018
Semi-skilled worker	0.981	0.629	2.430	1	0.119	2.667	0.777	9.152
Skilled worker	0.747	0.575	1.690	1	0.194	2.111	0.684	6.513
Arithmetic skill jobs	-0.223	0.563	0.157	1	0.692	0.800	0.266	2.410
Semi-professional						Reference	-	-