RESEARCH PAPER

Association Between Serum Cobalamin (Vitamin B12) Level and Severity of Anxiety in Generalized Anxiety Disorder: A Cross-Sectional Case–Control Study

Original research article

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ABSTRACT

Introduction: Excessive concern is a hallmark of generalized anxiety disorder (GAD), a prevalent mental illness. We still don't know the precise pathophysiology of anxiety. The nutritional hypothesis of anxiety states that anxiety levels can be greatly influenced by one's nutritional status. A balanced diet has a relaxing impact, promotes mental health and helps in mood stabilization. Cobalamin (Vitamin B12) performs a number of neurophysiological tasks, including nerve impulse transmission, neuromuscular conduction, neuroprotection against excitotoxicity and cell death, neuroinflammatory process modulation, brain synaptic creation and maintenance, and mood control. Neuropsychiatric disorders can result from this signalling being disrupted by a cobalamin deficiency.

Aims & Objectives: To assess serum cobalamin levels in patients with generalized anxiety disorder and in healthy individuals, and to determine whether serum cobalamin levels and disease severity are related.

Materials & Methods: The study comprised 100 patients with a diagnosis of generalized anxiety disorder who were enrolled in Psychiatry OPD (cases) and 100 healthy people who were matched for age and gender (controls). Severity of GAD was assessed by Hamilton Anxiety Rating scale and serum cobalamin level in all study subjects were analyzed.

Results: The mean blood cobalamin level in GAD patients was 135.59 ± 19.90 pg/ml, while the mean for healthy controls who did not exhibit anxious behavior was 222.41 ± 35.20 pg/ml. Serum cobalamin levels in the case group decrease as anxiety levels increase.

Conclusion: In the cases, lower cobalamin levels in serum are linked to more severe anxiety, the study found. Thus, it might be essential to measure its level in GAD at an early stage in order to appropriate treatment and lower the chance of developing other anxiety-related morbidities.

Keywords: Generalized anxiety disorder, Neuropsychiatric symptoms, Cobalamin.

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INTRODUCTION

Generalized anxiety disorder (GAD) is characterized by excessive worry, tension, fear and disinterest in activities

that follows an unpleasant event and persists for at least six months. [1] These symptoms can make it harder to function in day-to-day life. These symptoms may recur or occur in a single episode. About 0.57% of individuals in India suffer from this illness. [2] GAD significantly affects patients' and their families' lives, posing a significant public health concern and leading to significant medical costs and financial losses. The aetiology of GAD is complicated and includes environmental factors like emotional, mental, and physical trauma. Anxiety risk is also increased by lifestyle variables such as heavy drinking, smoking, having a type A personality (which is characterized by a fast-paced and anxious nature), and obesity. [3] Neurobiochemical anomalies in the neuroendocrine, neurovascular, neurotrophic, and inflammatory systems are linked to GAD. [4] It's unclear what specifically causes generalized anxiety. According to certain beliefs, anxiety's onset and development are significantly influenced by nutritional deficiencies. [5]

Micronutrients play a crucial role in the synthesis of brain chemicals and the preservation of a sound nervous system. They have a connection to mental wellness as well. Lack of micronutrients in the diet can affect brain function and raise the risk of mental health issues. Our intestinal flora produces the water-soluble vitamin B12, also known as cobalamin. Its main roles include DNA synthesis, brain function, and red blood cell creation. [6] Cobalamin is essential for preserving cognitive and mood balance. **Figure 1** illustrates the action of Cobalamin in controlling cognitive processes. [7]

Mood regulation and Cobalamin: Cobalamin plays a key part in the methylation reaction, which is necessary for the production of neurotransmitters (dopamine, serotonin, and norepinephrine). Methyl-cobalamin, an activated form of cobalamin, is a co-enzyme for Methionine synthase, which induces the conversion of homocysteine to methionine. This conversion does not occur in cobalamin deficiency, causing the blood's levels of homocysteine to rise and methionine to fall. We refer to this as a methyl trap. Reduced synthesis of monoamine transmitters, oxidative stress, mitochondrial dysfunction, and damage to vascular endothelium and dopaminergic neuron death can all result from elevated homocysteine, which can potentially result in anxiety or depression. [8]

Cobalamin contributes to the formation of the myelin sheath. It functions as a cofactor for L-methyl-malonyl-coenzyme A mutase, which changes methyl-malonyl-coenzyme A into succinyl Coenzyme A, which is essential for the critical process of myelin synthesis, which protects nerve fibres and ensures efficient nerve signal transmission. [9] It controls the migration of glial cells and the formation of synapses, both of which are essential for the growth and operation of neurons. It improves many aspects of brain function, including memory and concentration, by aiding in the brain's oxygen supply. According to some research, the proteins megalin and cubilin work in tandem with cobalamin binding proteins like intrinsic factor and transcobalamin to help the brain absorb vitamin B12 and move it across the blood-brain barrier. [10]

Serum levels of cobalamin typically range from 200 pg/ml to 900 pg/ml. Indicator of deficiency include levels below 200 pg/ml and depletion between 200 and 300 pg/ml. [11] The non-vegetarian diet is rich in cobalamin. As a result, vegetarians frequently exhibit this shortage. By altering the expression of cytokines that promote and inhibit inflammation, hyper homocysteinemia may dysregulate innate immunity. The hypothalamic adrenal- pituitary axis, neurotransmitter metabolism, and neuronal activity in the brain regions responsible for emotional regulation can all be dysregulated by persistent chronic inflammation, leading to symptoms of anxiety and sadness. [12,13] Cobalamin deficiency can cause neurological, hematological and mental abnormalities such as sadness, irritability, personality changes, and memory loss. These mental disorders are made worse by the excitotoxic responses brought on by the buildup of homocysteine and may precede hematological or neurological diseases in patients with cobalamin insufficiency, according to research. [14,15] Nevertheless, not much research has looked at the connection between cobalamin and GAD to date. Specifically, there are no investigations on this connection at our institute. The current study set out to measure the level of cobalamin in GAD patients in order to test the hypothesis that these patients would have lower serum levels of the vitamin than healthy people, and be linked to more severe psychopathology.

AIMS & OBJECTIVES

To assess serum cobalamin levels in patients with generalized anxiety disorder and in healthy individuals, and to determine whether serum cobalamin levels and disease severity are related.

MATERIAL AND METHODS

The design and context of the research were the biochemistry and psychiatry departments of the Government Medical College, Chhindwara, M.P. India, where this case control cross-sectional research was conducted with approval from the institutional ethics committee. (CIMS/Ethics Committee/2024/14588, dated 20/12/2024).

Study Population: The study comprised 100 people diagnosed with generalized anxiety disorder (case group) who visited the psychiatry outpatient department of CIMS-affiliated district hospital in Chhindwara, and 100 healthy individuals (control group) who were the same age and gender. Prior to their participation in the study, written consent was acquired.

Inclusion and Exclusion Criteria: Participants were to be of either gender, between the ages of 18 and 50, drug-free for three months and willing to provide written informed permission. Exclusion criteria included being pregnant or nursing, taking B12 supplements, taking antidepressants or anxiety medications, having an active infection, being dependent on alcohol or tobacco, having autoimmune inflammatory disease, having mental retardation, having a serious medical condition (such as cancer or being bedridden), or not wanting to give their consent.

Diagnosis and assessment of severity of anxiety: To identify and grade the intensity of anxiety symptoms, publicly available Hamilton Anxiety Rating scale (HAM-A) [16] was used. Each of the 14 items on the test has a score between 0 and 4, for a total score range of 0-56. According to this scale, 0 denotes no anxiety, 1–7 less anxiety, 8–17 mild anxiety, 18–24 mild to moderate, and > 25 moderate to severe anxiety.

Biochemical Analysis: Using the ADVIA Centaur CP Immunoassay analyzer, the levels of cobalamin were measured in both groups using the Chemiluminescence immunoassay.

Statistical Analysis:

Descriptive analysis: The clinical and demographic variables were compiled using descriptive statistics. The mean ± standard deviation (SD) was used to express continuous variables, such as age, serum cobalamin levels, HAM-A score. Frequencies and percentages were used to display categorical variables such as the distribution of gender and severity categories of depression. Microsoft Excel was used to organize the data, and IBM SPSS version 15.0 was used for analysis.

Inferential statistics: An independent sample t-test was used to compare the serum cobalamin levels between the case and control groups. One-way analysis of variance (ANOVA) was used to compare the mean cobalamin levels among the anxiety severity groups. The association between serum cobalamin levels and the HAM-A scores was evaluated using Pearson's correlation coefficient. Statistical significance was defined as a p-value of less than 0.05.

RESULTS

Clinical and demographic traits: Our study yielded several significant findings: Patients with generalized anxiety disorder were 35.82 years old on average, with a standard deviation of 4.51, while those without the disorder were 34.87 years old on average, with a standard deviation of 4.98. An average of 8.17 months, with a standard deviation of 1.29 months, was the length of illness for patients who displayed worried behaviour. The HAM-A score for anxious patients was 15.87, with a standard deviation of 6.96.

Serum cobalamin levels, case versus control group: GAD patients had an average blood cobalamin level of 135.59 pg/ml with a standard deviation of 19.90, whereas healthy individuals had an average level of 222.41 pg/ml with a standard deviation of 35.20 **(Table 1).**

Levels of serum cobalamin and anxiety intensity, case group: Patients with minor anxiety had a mean serum cobalamin level of 152.02 pg/ml, those with mild anxiety had a level of 121.43 pg/ml, and those with mild to severe anxiety had a level of 101.14 pg/ml. Patients' blood cobalamin levels varied significantly according to how anxious they were. This result showed that as the case group's disease severity grew, blood cobalamin levels dropped **(Tables 2)**.

Relationship between serum cobalamin levels and HAM-A score, Case group: Patients' serum cobalamin levels and HAM-A scores demonstrated a strong negative correlation (R = -0.9673) and were statistically significant **(Table 3).**

Table 1: **Comparison of serum cobalamin between case and control groups**

	Group	N	Mean ± SD	T	P
Serum cobalamin (pg/ml)	**Case group**	100	135.59 ± 19.90	-21.4727	< 0.00001
	Control group	100	222.41 ± 35.20		

Table 2: Mean serum cobalamin levels across case groups with varying anxiety severity

	Case group	N	Mean ± SD	F	P
	Minimal anxiety	51	152.02 ± 7.60		

Serum cobalamin (pg/ml)				169.2759	<0.00001
	Mild anxiety	42	121.43 ± 11.91		
	Mild to Moderate anxiety	07	101.14 ± 2.19		
	Moderate to Severe anxiety	00	--		
	Total	100			

Table 3: Relationship between HAM-A scores and serum cobalamin in the case group

	HAM-A score in Cases	
Serum cobalamin (pg/ml)	**R**	**p**
	-0.9673 (**strong negative**)	<0.00001

DISCUSSION

Anxiety contributes significantly to the burden of mental illness and is a major cause of morbidity and impairment. It affects both genders and is prevalent in all age groups, though it affects women more than men. In order to prevent psychiatric disorders and promote mental health, diet and nutrition are crucial. Preventive measures can improve the treatment of psychiatric disorders and lessen the burden of mental illnesses.

By controlling synaptic development and nerve signal transmission, vitamin B12 influences behavior, memory, and learning **(Figure 1)**. [17] Almost half of all cases of anxiety go undiagnosed for years or receive subpar treatment. Although the precise mechanism linking B12 deficiency to mental health disorders is not yet understood, it may entail changes in one-carbon metabolism, folate metabolism, and hereditary variables. [18] Early measurement of these individuals' serum vitamin B12 levels may alter the course of their illness and lessen associated co-morbidities. In order to determine the severity of generalized anxiety disorder, we looked at the serum vitamin B12 levels of those who had it. According to this study, there is a substantial negative link between the severity score for anxiety in GAD patients and their serum vitamin B12 level. **Tan Y et al.** [19] found a correlation between a higher likelihood of severe anxiety symptoms and lower vitamin B12 levels, which is consistent with our findings. In their study, **Todorov A. et al.** [20] examined decreased serum vitamin B12 levels in anxiety patients relative to healthy people and concluded that reduced vitamin B12 is crucial to its development. Serum cobalamin levels and the intensity of anxiety symptoms in kids and teenagers were found to be negatively correlated by **Esnafoglu E et al.** [21]. In their investigation of a North Indian community, **Victor R et al.** [22] found a strong correlation between anxiety and vitamin B12 insufficiency. Based on their findings, **Erensoy H.** [23] suggested that vitamin B12 levels in the blood could be used to assess how well anxiety and depression treatments are working. According to research by **Skarupski KA et al.** [24] and **Walker JG et al.** [25] respectively, vitamin B12 administration helps mental patients with their cognitive symptoms. **Uygun and others** [26] contrary to our findings, did not find a discernible difference in serum cobalamin levels between healthy controls and patients with anxiety disorders. In their study of the Bhil population in India, **Saraswathy KN and associates** [27] found no evidence of a clear connection between GAD and vitamin B12 deficiency, however, they did propose that elevated serum homocysteine levels in vitamin B12 deficiency significantly increase the risk of both depression and GAD. Despite possible variations in vitamin B12 consumption, **Al Jassem O et al.** [28] reported no discernible difference in GAD scores between vegetarians and omnivores. Therefore, it may be concluded that anxiety and vitamin B12 insufficiency may be related, however their relationship is complicated and treating vitamin B12 deficits may be crucial for mental health. The following information can be proposed based on our study:

(a) Psychiatrists should be aware that vitamin B12 deficiency may result in certain reversible mental disorders in children and adolescents. (b) A healthy dietary pattern that complies with nutrient recommendations may help reduce anxiety. (c) Parents should regularly have their children's serum vitamin B12 levels checked and encourage them to eat foods rich in vitamin B12. (d) Women should consume vitamin B12 in recommended amounts during pregnancy to protect the unborn child from mental problems.

Therefore, screening for cobalamin for this mood illness is necessary. Despite our rigorous approach and adherence to the correct protocols, this study has certain **limitations,** 1. We only had a tiny sample size. 2. The research was a cross-

sectional study, so we couldn't compare vitamin B12 levels before and after treatment. 3. Because our study is a single-centered level institutional study, the findings cannot be applied to other contexts. The intricate relationship between vitamin B12 and GAD requires more investigation, a larger sample size, and a longer follow-up period.

CONCLUSION

The study's findings led us to the following conclusions: (a) Generalized anxiety disorder patients have lower serum cobalamin levels than healthy individuals (below 200 pg/ml). (b) Serum vitamin B12levels decrease as anxiety conditions worsen. (c) Consequently, the severity of the disease in negatively correlated with serum cobalamin levels. The theory that GAD sufferers had lower serum levels of cobalamin than healthy people was validated. Cobalamin deficiency may be a side effect of the condition, resulting in diminished judgment, low mood, and nervous behavior since it directly affects the development and maintenance of complex cognitive functions. New approaches to treating GAD might be created with more investigation into the connection between mental health and vitamin B12 metabolism. However, because its function in treating anxiety is still unclear, vitamin B12 supplements are not currently commonly utilized as a main treatment for generalized anxiety in conjunction with antianxiety drugs. Since anxiety is a common psychiatric condition with a poor prognosis and medical, social, and financial repercussions, our study suggests that measuring blood vitamin B12 levels at the time of diagnosis and offering cobalamin supplements for treatment and prophylaxis could improve the outcome of this clinical condition and reduce its severity and progression.

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REFERENCES

1. Munir, S., Takov, V., & Coletti, V. A. (2022). *Generalized anxiety disorder (nursing)*. StatPearls Publishing. https://pubmed.ncbi.nlm.nih.gov/33760455

2. Jayasankar, P., Suhas, S., Nirisha, N., Lakshmi, P., et al. (2023). Current prevalence and determinants of generalized anxiety disorder from a nationally representative, population-based survey of India. *Indian Journal of Psychiatry*, *65*(12), 1244–1248. https://doi.org/10.4103/indianjpsychiatry.indianjpsychiatry_824_23

3. Nelles, P. A., Singewald, N., Sperner-Unterweger, B., et al. (2025). The conflict avoidance theory of inflammation-induced anxiety (CATIA): A psychoneuroimmunologic hypothesis. *Medical Hypotheses*, *196*, 111580. https://doi.org/10.1016/j.mehy.2025.111580

4. Marazziti, D., Abelli, M., Baroni, S., Carpita, B., Ramacciotti, C. E., & Dell'Osso, L. (2015). Neurobiological correlates of social anxiety disorder: An update. *CNS Spectrums*, *20*(2), 100–111. https://doi.org/10.1017/S109285291400008X

5. Kris-Etherton, P. M., Petersen, K. S., Hibbeln, J. R., et al. (2021). Nutrition and behavioral health disorders: Depression and anxiety. *Nutrition Reviews*, *79*(3), 247–260. https://doi.org/10.1093/nutrit/nuaa025

6. Morris, M. S., Jacques, P. F., Rosenberg, I. H., & Selhub, J. (2007). Folate and vitamin B-12 status in relation to anemia, macrocytosis, and cognitive impairment in older Americans in the age of folic acid fortification. *The American Journal of Clinical Nutrition*, *85*(1), 193–200. https://doi.org/10.1093/ajcn/85.1.193

7. Umekar, M., Premchandani, T., Tatode, A., Qutub, M., Raut, N., Taksande, J., et al. (2025). Vitamin B12 deficiency and cognitive impairment: A comprehensive review of neurological impact. *Brain Disorders*, *18*, 100220. https://doi.org/10.1016/j.dscb.2025.100220

8. Bottiglieri, T. (2013). Folate, vitamin B12, and S-adenosylmethionine. *Psychiatric Clinics of North America*, *36*(1), 1–13.

9. Stabler, S. P. (2013). Clinical practice: Vitamin B12 deficiency. *The New England Journal of Medicine*, *368*(2), 149–160. https://doi.org/10.1056/NEJMcp1113996

10. Kozyraki, R., Verroust, P., & Cases, O. (2022). Cubilin, the intrinsic factor–vitamin B12 receptor. *Vitamins and Hormones*, *119*, 65–119. https://doi.org/10.1016/bs.vh.2022.01.005

11. Allen, L. H. (2012). Vitamin B-12. *Advances in Nutrition*, *3*(1), 54–55. https://doi.org/10.3945/an.111.001370

12. Mikkelsen, K., Stojanovska, L., Prakash, M., & Apostolopoulos, V. (2017). The effects of vitamin B on the immune/cytokine network and their involvement in depression. *Maturitas*, *96*, 58–71. https://doi.org/10.1016/j.maturitas.2016.11.012

13. Hu, P., Lu, Y., Pan, B. X., & Zhang, W. H. (2022). New insights into the pivotal role of the amygdala in inflammation-related depression and anxiety disorder. *International Journal of Molecular Sciences*, *23*(19), 11076.

14. Oh, R., & Brown, D. L. (2003). Vitamin B12 deficiency. *American Family Physician, 67*(5), 979–986. https://pubmed.ncbi.nlm.nih.gov/12643357/

15. Sagar, R., Aggarwal, N., & Jose, N. (2019). A cross-sectional study of serum folate and vitamin B12 levels in psychiatric inpatients. *Primary Care Companion for CNS Disorders, 21*(5), 23219.

16. Hamilton, M. (1959). The assessment of anxiety states by rating. *British Journal of Medical Psychology, 32*, 50–55.

17. Venkatramanan, S., Armata, I. E., Strupp, B. J., & Finkelstein, J. L. (2016). Vitamin B-12 and cognition in children. *Advances in Nutrition, 7*(5), 879–888. https://doi.org/10.3945/an.115. 012021

18. Sahu, P., Thippeswamy, H., & Chaturvedi, S. K. (2022). Neuropsychiatric manifestations in vitamin B12 deficiency. *Vitamins and Hormones, 119*, 457–470. https://doi.org/10.1016/bs.vh.2022.01.001

19. Tan, Y., Zhou, L., Gu, K., Xie, C., Wang, Y., Cha, L., Wu, Y., Wang, J., Song, X., Chen, X., Hu, H., & Yang, Q. (2023). Correlation between vitamin B12 and mental health in children and adolescents: A systematic review and meta-analysis. *Clinical Psychopharmacology and Neuroscience, 21*(4), 617–633. https://doi.org/10.9758/cpn.22.1040

20. Todorov, A., Chumpalova-Tumbeva, P., Stoimenova-Popova, P., Popova, M., Todorieva-Todorova, V., Tzvetkov, D., et al. (2017). Correlation between depression and anxiety and the level of vitamin B12 in patients with depression and anxiety and healthy controls. *Journal of Biomedical and Clinical Research, 10*(2). https://doi.org/10.1515/jbcr-2017-0023

21. Esnafoglu, E., & Ozturan, D. D. (2020). The relationship of severity of depression with homocysteine, folate, vitamin B12, and vitamin D levels in children and adolescents. *Child and Adolescent Mental Health, 25*(4), 249–255. https://doi.org/10.1111/camh.12387

22. Victor, R., Gondwal, R., Avinash, P., et al. (2023). Psychiatric implication of deficiency of vitamin B12 and vitamin D: A cross-sectional study from North India. *Indian Journal of Private Psychiatry, 17*(2), 64–67.

23. Erensoy, H. (2020). Vitamin B12 and folic acid in depression and anxiety: A pilot study. *Journal of Neurobehavioral Sciences, 7*, 164–169.

24. Skarupski, K. A., Tangney, C., Li, H., et al. (2010). Longitudinal association of vitamin B-6, folate, and vitamin B-12 with depressive symptoms among older adults over time. *The American Journal of Clinical Nutrition, 92*(2), 330–335. https://doi.org/10.3945/ajcn.2010.29413

25. Walker, J. G., Batterham, P. J., Mackinnon, A. J., et al. (2012). Oral folic acid and vitamin B-12 supplementation to prevent cognitive decline in community-dwelling older adults with depressive symptoms: The Beyond Ageing Project randomized controlled trial. *The American Journal of Clinical Nutrition, 95*(1), 194–203. https://doi.org/10.3945/ajcn.110.007799

26. Uygun, S. D., Goker, Z., Karaca Kara, F., & Üneri, Ö. (2022). Serum vitamin B12, folate, and ferritin levels in children and adolescents with anxiety disorders: A controlled study. *Van Medical Journal, 29*(3), 260–266. https://doi.org/10.5505/vtd.2022.95867

27. Saraswathy, K. N., Ansari, S. N., Kaur, G., Joshi, P. C., & Chandel, S. (2019). Association of vitamin B12-mediated hyperhomocysteinemia with depression and anxiety disorder: A cross-sectional study among Bhil indigenous population of India. *Clinical Nutrition ESPEN, 30*, 199–203. https://doi.org/10.1016/j.clnesp.2019.01.009

28. Al Jassem, O., Kheir, K., Ismail, A., Abou-Abbas, L., Masri, A., Haddad, C., Nasrallah, K., & El Masry, J. (2024). Vitamin B12 deficiency and neuropsychiatric symptoms in Lebanon: A cross-sectional study of vegans, vegetarians, and omnivores. *PLOS ONE, 19*(4), e0297976. https://doi.org/10.1371/journal.pone.0297976

Figure 1: Role of Vitamin B12 in cognition [17]