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CLINICAL STUDY

A Preliminary Study to Validate a Novel Screening Tool "B12-DETECT" for Detecting Vitamin B12 Deficiency

S KRISHNA DEEPAK*, ARCHANA TOPPO†, RICHA MISHRA‡

ABSTRACT

Background and objective: Vitamin B12 deficiency is a significant but often undiagnosed public health concern. Currently, its diagnosis relies on expensive laboratory tests with limited access. The present study aimed to develop and validate a 10-item screening questionnaire as a potential tool to identify inapparent vitamin B12 deficiency. **Methods:** This prospective, observational, real-world study was conducted among Indian physicians across multiple specialties. A self-administered, 10-question screening tool, B12-DETECT (Deficiency Evaluation Tool for Early Clinical Testing), was developed based on literature review and expert input, with scores ranging from 0 (indicating optimal vitamin B12 status) to 10 (indicating the most severe deficiency). It was validated using laboratory-based testing, with serum vitamin B12 levels (<200 pg/mL) considered as deficiency. The validity of the questionnaire was also assessed using sensitivity, specificity, and receiver operating characteristic (ROC) curve analysis. Statistical significance was set at $p < 0.05$. **Results:** Among 181 physicians evaluated, the screening tool identified 38.1% at risk for vitamin B12 deficiency (score ≥3), with commonly reported symptoms such as weakness, tiredness, fatigue, low mood, depression, while laboratory testing confirmed deficiency in 28.7%. It demonstrated moderate-to-high sensitivity (78.8%) and specificity (78.3%), with an area under the curve (AUC) of 0.77 (95% confidence interval [CI]: 0.703-0.850, $p = 0.000$). An optimal cut-off of 2.5 maximized the predictive value of the tool. **Interpretations and conclusions:** This simple questionnaire is a promising, cost-effective screening tool for health care professionals to detect vitamin B12 deficiency, particularly in resource-limited settings. Future studies should validate its applicability in diverse populations and explore its integration into primary health care screening programs.

Keywords: Deficiency, physicians, questionnaire, screening, vitamin B12

Vitamin B12 deficiency represents a widespread global health issue that poses significant public health challenges[1,2]. Vitamin B12 level of <200 pg/mL (150 pmol/L) is universally considered to be the threshold of deficiency[3]. The deficiency is reported to be more common in low- and middle-income countries, affecting 50% to 80% of the population in South Asia[2]. In India, over 650 million individuals (approximately 75% of the population) have vitamin B12 deficiency, indicating a relatively high prevalence[4]. A comprehensive review in the Indian population, conducted between 2000 and 2019, has also reported a high prevalence of vitamin B12 deficiency: 78.5% in adults and 61.7% in the elderly, varying according to the diagnostic cut-off used while a retrospective, cross-sectional study based on electronic medical record database (2015-2018) documented a prevalence of 47% among north Indian population[5,6].

In 2018, another study from Haryana, India noted vitamin B12 deficiency among 32.4% adolescent school-going children[7]. Although vitamin B12 deficiency affects people of all ages, elderly individuals and those with multiple comorbidities are disproportionately affected[1,8,9]. Moreover, the deficiency can also occur in the absence of anemia with no remarkable impact of folic acid supplementation on vitamin B12 levels[10].

The clinical manifestations of vitamin B12 deficiency typically vary among individuals, encompassing a

*Consultant Physician
Dr RS Raju Clinic, Alkapur Township, Hyderabad, Telangana, India
†Medical Advisor
‡Manager - Marketing
Biological E Ltd., Hyderabad, Telangana, India
Address for correspondence
Dr S Krishna Deepak
Consultant Physician
Dr RS Raju Clinic, Alkapur Township, Hyderabad, Telangana, India
E-mail: skrishnadeepak@gmail.com

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broad range of symptoms, including neurological, hematological, psychiatric, gastrointestinal, cardiovascular, and cerebrovascular diseases[1,11]. However, many individuals with vitamin B12 deficiency are asymptomatic or have subtle, nonspecific symptoms overlapping with other diseases[9,12]. This often leads to delayed diagnosis, misdiagnosis, and inappropriate treatment or late treatment, resulting in serious issues that eventually become irreversible[9]. Nonetheless, it has been observed that the deficiency is reversible at early stages with effective and affordable therapeutic intervention[12]. Hence, an accurate and early diagnosis of vitamin B12 deficiency is imperative[9].

Currently, the diagnosis of vitamin B12 deficiency is mainly based on expensive laboratory tests and clinical expertise. However, high costs and limited accessibility of these tests, particularly in resource-limited settings such as India, pose significant challenges[13]. Few micronutrient deficiency screening tools exist for conditions such as vitamin D deficiency and iron deficiency anemia, demonstrating the feasibility of questionnaire-based approaches[14,15]. Despite the high burden of vitamin B12 deficiency, validated screening tools are lacking; therefore, the current study was undertaken to develop and validate B12-DETECT (Deficiency Evaluation Tool for Early Clinical Testing) questionnaire as a novel screening tool for vitamin B12 deficiency.

This study serves as a precursor to broader population validation as the testing was initially conducted among physicians to allow for early refinement of the health care professional (HCP)-facing screening tool. This ensures that the tool is medically robust, user-friendly for clinicians, and diagnostically accurate before it is deployed for HCPs to screen their patients for B12 deficiency in routine clinical practice.

METHODS

Study Design, Sample Size, and Population

This real-world, prospective observational study recruited physicians from various medical specialties throughout India, including general physicians (GP, primary care providers) and health care professionals across different specialties (gynecology, general medicine, pediatrics, ophthalmology, general surgery, otorhinolaryngology, general pathology, and microbiology). The list of physicians was collected from the government and private associations, and subsequently, they were approached to participate in this study. The study spanned from December 2024 to January 2025.

The study included practicing physicians who had completed their medical degree, aged up to 75 years with normal hemoglobin levels as per the World Health Organization (WHO) recommendation (≥120 g/L, nonpregnant women; ≥130 g/L, men) and agreed to provide written informed consent[16]. Physicians were excluded from the study if they were currently undergoing treatment for vitamin B12 deficiency or had chronic and debilitating conditions influencing vitamin B12 levels, including pernicious anemia, atrophic gastritis, gastric or small intestine resections, and inflammatory bowel disease. Exclusion criteria also encompass use of metformin (for more than 4 months), proton pump inhibitors or histamine-2 blockers (for more than 12 months), vegans or strict vegetarian diet, and adults older than 75 years[17,18]. Additionally, female physicians who were pregnant or breastfeeding were also excluded.

Study Procedure

This study was conducted in two phases. The first phase involved developing and validating the screening questionnaire with experts. The second phase focused on the clinical validation of the screening tool against the gold-standard laboratory values in the study population.

Development of the questionnaire

A self-administered questionnaire, B12-DETECT was developed in the English language based on an extensive literature review and expert input. The questionnaire contained two components. The first component consisted of the demographic data of the physicians, including their age and gender. The second component (screening tool) included 10 questions in a dichotomous format (yes/no) related to the symptoms and risk factors associated with vitamin B12 deficiency (Supplementary Table S1). The questionnaire was structured to determine if physicians had experienced symptoms for at least 1 month.

The total questionnaire score ranged from 0 (indicating optimal vitamin B12 status) to 10 (indicating the most severe deficiency). A total score of ≥3 classified an individual as vitamin B12 deficient. Responses with no symptoms were given a score of 0, whereas those indicating possible symptoms of vitamin B12 deficiency received a score of 1. Each additional response reflecting more severe symptoms increased the score by 1 point. The study also documented physicians' feedback on the questionnaire's effectiveness as a vitamin B12 deficiency screening tool. This questionnaire was reviewed and validated prior to conducting the study (Cronbach's alpha coefficient = 0.83; intraclass correlation score = 0.92).

Data collection

Trained pharmaceutical field force served as study coordinators and recruited physicians based on pre-defined inclusion and exclusion criteria after obtaining informed consent. Questionnaires were distributed to enrolled physicians through multiple channels: electronically via a survey link (SurveyMonkey) sent through email/messaging, or in-person delivery by study coordinators, who provided guidance and assistance in completing the forms. The overall data collection was coordinated by the study coordinators.

Blood sample collection

A certified central laboratory (Dr Lal PathLabs; NABL, ISO 9001:2000, CAP) in India (metro cities pan India) determined all the blood parameters. Fasting venous blood samples were collected (~5 mL) from each physician and transported (at 2-8°C) for analysis to the nearest laboratory by the lab phlebotomist associated with Dr Lal PathLabs. Thereafter, the samples were processed at the aforementioned lab facility. The results were coded and shared with the principal investigator for further analysis, post which the results were also shared with the respective physicians.

Blood sample analysis and validation of the screening tool

The samples were processed within 1 hour of blood collection. The serum separation was done by centrifugation (1100-1300 RPM, 10-15 min). Serum vitamin B12 concentration was determined using an electrochemiluminescent method on a Cobas E analyzer (Roche Diagnostics). The cut-off was considered to be 200 pg/mL, and values beyond 2,000 pg/mL were not quantified but were instead categorized as >2,000 pg/mL. The linearity of the method was between 50 and 2,000 pg/mL. Hemoglobin level was measured by sodium lauryl sulfate photometry. The cut-off for normal hemoglobin was considered to be ≥120 g/L (12 g/dL) for adult nonpregnant women and ≥130 g/L (13 g/dL) for adult men[16].

Study Endpoints

The primary endpoint was the proportion of physicians correctly identified to be at risk for vitamin B12 deficiency based on the questionnaire. The secondary endpoint was the correlation between the questionnaire results (positive responses) and laboratory measurements of serum vitamin B12, as well as the determination of the diagnostic accuracy of the screening questionnaire. The exploratory endpoint involved the correlation between serum vitamin B12 and hemoglobin levels of the physicians.

Statistical Analysis

Based on the findings of a pilot study conducted in 2024 among 25 HCPs from India, vitamin B12 deficiency was observed in 15% of HCPs (data on demand). Considering a confidence level of 95%, the estimated sample size was 196. Hence, this prospective study included a total of 200 HCPs.

Data analysis was conducted using the IBM Statistical Package for the Social Sciences Statistics for Windows (version 25.0. Armonk, NY: IBM Corp., Released 2017). Continuous variables were represented as mean ± standard deviation (SD)/median and interquartile range (IQR). Categorical variables were summarized as frequencies and percentages. The normality of the data was determined using the Kolmogorov-Smirnov test. Since the data was not normally distributed, Spearman's rank correlation was conducted to assess relationships between questionnaire results, vitamin B12 levels, and hemoglobin values. A p-value of <0.05 was considered to be statistically significant for all analyses.

The effectiveness of the screening tool was assessed by calculating its sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (PLR), and negative likelihood ratio (NLR), relative to laboratory results. The cut-off for serum vitamin B12 was taken as <200 pg/mL. A receiver operating characteristic (ROC) curve analysis was performed to determine the optimal cut-off score for disease classification.

Ethical Approval and Informed Consent

This study was conducted in compliance with the ethical principles outlined in the Declaration of Helsinki and the International Conference on Harmonization Guidelines for Good Clinical Practice. Ethical approval was obtained from the local ethics committee (Ethical approval no. PDCEC/OBS-09/25 Nov 2024). Informed consent was sought from the physicians before enrollment.

RESULTS

A total of 203 physicians were enrolled in the study. However, the final analysis included 181 physicians; 22 physicians were excluded as they did not meet the WHO criteria for hemoglobin levels[16]. The mean age of the physicians was 51.5±11.1 years, with most being men (78.5%, 142/181). General physicians comprised majority of the study population (35.9%, 65/181), followed by

gynecologists (18.2%, 33/181), and pediatricians (13.3%, 24/181). The rest comprised physicians from other specialties (32.6%, 59/181) (Table 1).

Vitamin B12 levels were significantly lower in deficient physicians compared to those with normal levels (127.39 ± 45.75 vs. 549.44 ± 351.67 pg/mL; Mann-Whitney U = 0.000, p = 0.001). Similarly, hemoglobin levels were significantly reduced in the deficient group versus the normal group (13.94 ± 1.77 vs. 14.72 ± 1.53 g/dL; Mann-Whitney U = 3391.000, p = 0.007).

Questionnaire scores demonstrated a clustered distribution in the lower range, with 22.7% of physicians scoring 0, 39.2% scoring 1-2, and 38.1% scoring ≥3. The frequency distribution showed a progressive decrease in the number of physicians for scores exceeding 3 (Fig. 1). Frequently reported symptoms were weakness, tiredness, or fatigue, experienced by 68 physicians (37.6%), followed by low mood or depression in 44 physicians (24.3%), and tingling or numbness in extremities reported by 38 physicians (21%) (Table 2). Questionnaire-based screening identified 69 physicians (38.1%) as vitamin B12 deficient, while laboratory testing confirmed deficiency in 52 physicians (28.7%) (Supplementary Table S2). The total questionnaire scores showed a weak negative but statistically significant correlation with vitamin B12 levels ($\rho = -0.22$, p = 0.003), indicating that lower vitamin B12 levels were associated with higher scores or more symptoms.

The criterion for validity analysis of the questionnaire is detailed in Supplementary Table S3. At the optimal cut-off of 2.5, the questionnaire demonstrated a sensitivity of 78.8% and specificity of 78.3%. The PPV was 59.4%, while the NPV was 90.2%. The PLR was 3.6, and the NLR was 0.3. The ROC curve analysis yielded an AUC of 0.776 ± 0.038 (95% CI: 0.703-0.850, p = 0.000), indicating fair discriminative ability. The optimal cut-off of 2.5 maximized sensitivity (78.8%) and specificity (78.3%), balancing true positive and false positive classifications (Fig. 2).

Physician feedback regarding questionnaire utility was positive, with 120 physicians (66.3%) rating it as extremely beneficial and 43 physicians (23.8%) finding it very useful as a screening tool. The remaining physicians expressed varied opinions on the utility of the questionnaire: 11 (6.1%) rated it as moderately useful, 4 (2.2%) as slightly useful, and 3 (1.6%) did not find it useful. Additionally, correlation analysis revealed a weak positive correlation between serum vitamin B12 levels and hemoglobin concentrations ($\rho = 0.20$, p = 0.007).

Table 1. Demographic Characteristics of the Study Population (N = 181)

Variables		
Age (years), mean (SD)	51.5 ± 11.1	
Gender, n (%)	Men: 142 (78.5)	
	Women: 39 (21.5)	
Specialties, n (%)	General physicians	65 (35.9)
	Gynecology	33 (18.2)
	Pediatrics	24 (13.3)
	General medicine	20 (11.0)
	Ophthalmology	15 (8.3)
	General surgery	10 (5.5)
	Ear, Nose, and Throat	7 (3.9)
	General pathology	4 (2.2)
	Microbiology	3 (1.7)

N = Total number of physicians; n = Number of physicians; SD = Standard deviation.

DISCUSSION

This is one of the first studies to develop and validate a novel questionnaire-based screening tool (B12-DETECT) for vitamin B12 deficiency in the Indian population, which showed promising potential as a preliminary screening approach prior to laboratory testing in real-world settings, particularly valuable in those with cost and accessibility constraints[19,20].

A key observation from this study is that the questionnaire identified 38.1% of physicians with a score ≥3

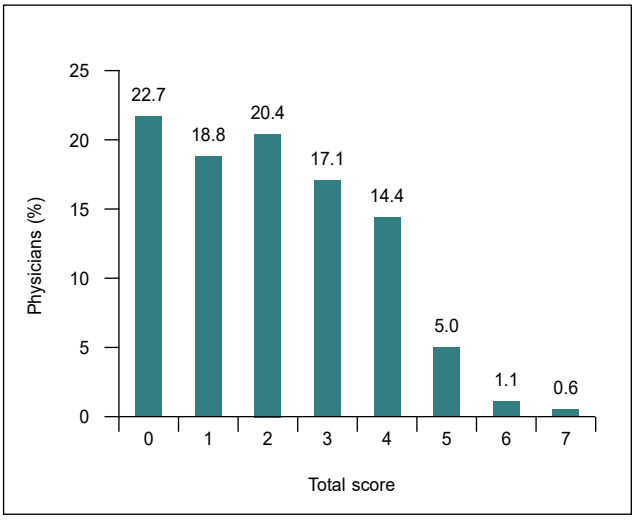


Figure 1. Total questionnaire score distribution of the physicians. The percentage of physicians with scores ranging from 0 to 7 is represented in the bar diagram. The calculations of the percentages were based on responses from 181 physicians.

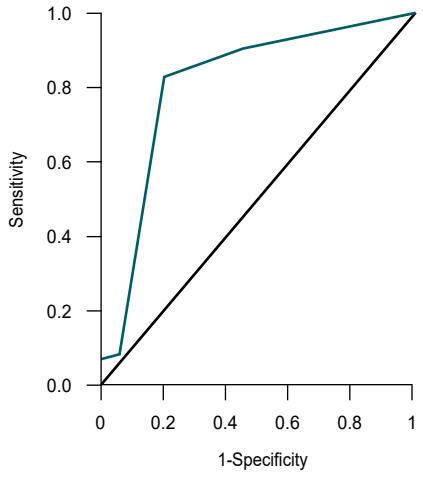
Table 2. Comparison of the Questionnaire Responses and the Laboratory Diagnosis

Questionnaire	Responses*	Healthy n (%)	Vitamin B12 deficient n (%)	Total N (%)
Do you suffer from weakness, tiredness, or fatigue?	Yes	34 (50)	34 (50)	68 (37.6)
	No	95 (84.1)	18 (15.9)	113 (62.4)
Do you experience tingling/numbness or pins and needles sensation in your hands or feet?	Yes	19 (50)	19 (50)	38 (21)
	No	110 (76.9)	33 (23.1)	143 (79)
Do you have a low mood/depression?	Yes	20 (45.5)	24 (54.5)	44 (24.3)
	No	109 (79.6)	28 (20.4)	137 (75.7)
Do you have a poor memory or difficulty concentrating?	Yes	15 (53.6)	13 (46.4)	28 (15.5)
	No	114 (74.5)	39 (25.5)	153 (84.5)
Do you experience frequent or even daily headaches or dizziness?	Yes	8 (33.3)	16 (66.7)	24 (13.3)
	No	121 (77.1)	36 (22.9)	157 (86.7)
Are you a strict vegan or vegetarian?	Yes	35 (68.6)	16 (31.4)	51 (28.2)
	No	94 (72.3)	36 (27.7)	130 (71.8)
Do you regularly drink alcohol (more than 2-3 times per week)?	Yes	11 (64.7)	6 (35.3)	17 (9.4)
	No	118 (72)	46 (28)	164 (90.6)
Do you have difficulty sleeping? Or do you wake up from sleep feeling unrested?	Yes	17 (60.7)	11 (39.3)	28 (15.5)
	No	112 (73.2)	41 (26.8)	153 (84.5)
Do you have difficulty losing weight?	Yes	22 (78.6)	6 (21.4)	28 (15.5)
	No	107 (69.9)	46 (30.1)	153 (84.5)
Do you have indigestion, gas, bloating, diarrhea, or constipation?	Yes	25 (58.1)	18 (41.9)	43 (23.8)
	No	104 (75.4)	34 (24.6)	138 (76.2)

n = Number of physicians; N = Total number of physicians.

*The responses were collected in form of "always agree", "agree", which were classified as "Yes", and "do not agree", "rarely agree", which were classified as "No". The total response count/symptoms does not necessarily correspond to the final vitamin B12 deficiency count.

A



B

Coordinates of the Curve		
Test Result Variable(s): Score		
Positive if greater than or equal to[a]	**Sensitivity**	**1-Specificity**
–1.00	1.000	1.000
0.50	0.953	0.698
1.50	0.906	0.460
2.50	0.828	0.201
3.50	0.422	0.137
4.50	0.078	0.058
5.50	0.063	0.000
6.50	0.016	0.000
8.00	0.000	0.000
The test result variable(s): Score has at least one tie between the positive actual state group and the negative actual state group.		
[a]The smallest cut-off value is the minimum observed test value minus 1, and the largest cut-off value is the maximum observed test value plus 1. All the other cut-off values are the averages of two consecutive ordered observed test values.		

Figure 2. (A) Receiver operating characteristic curve (ROC) of the vitamin B12 questionnaire. **(B)** Coordinates of the ROC.

as vitamin B12 deficient. Most physicians with the deficiency reported symptoms such as weakness, tiredness or fatigue, low mood or depression, and tingling or numbness in extremities, which align with the previously reported common symptoms of vitamin B12 deficiency[1,21]. However, our study showed a higher proportion of physicians as vitamin B12 deficient (38.1%) compared to that identified by laboratory measurements (28.7%). This discrepancy suggests a tendency of overestimation, likely due to the inclusion of nonspecific symptoms such as weakness, tiredness, difficulty in losing weight, that may overlap with other conditions, including anemia, and vitamin D deficiency[14,21]. It is consistent with an earlier study, which documented a prevalence of 26% among a large cohort of HCPs at an Indian tertiary eyecare center[11]. Moreover, both hemoglobin and vitamin B12 deficiency can cause overlapping symptoms and signs, which makes clinical diagnosis challenging. Hence, this study attempted to achieve optimal screening precision by excluding physicians with hemoglobin concentration below WHO-recommended thresholds for anemia while employing a comprehensive questionnaire that captures diverse symptoms and lifestyle factors associated with vitamin B12 deficiency.

In addition, our study also demonstrated a weak negative correlation between vitamin B12 levels and total questionnaire score ($\rho = -0.22$, $p = 0.003$), suggesting that lower vitamin B12 levels were associated with higher symptom burden. As vitamin B12 deficiency worsens, the severity and number of symptoms tend to increase, significantly impacting overall health and quality of life[21]. Hence, the developed questions are relevant to identifying physicians with vitamin B12 deficiency.

The questionnaire demonstrated moderate-to-high sensitivity (78.8%) and specificity (78.3%), making it an effective preliminary screening tool. The optimal cut-off of 2.5 was found to maintain a balance between sensitivity and specificity, minimizing misclassification errors, thus establishing it as a useful first-line tool before confirmatory testing. The high sensitivity of this cut-off ensured that the tool accurately identified the majority of the physicians with actual vitamin B12 deficiency; in contrast, the specificity of 78.3% means that some false positives (21.7%) may also be included. Moreover, the high NPV (90.2%) suggests that physicians classified as not at risk are highly unlikely to have a vitamin B12 deficiency. However, the PPV of 59.4% indicates that physicians classified as high-risk for vitamin B12 deficiency still have a relatively high likelihood of actually having vitamin B12 deficiency. This suggests that the tool is moderately reliable for preliminary screening, necessitating further confirmatory laboratory testing for a definitive diagnosis. As a preliminary tool with high sensitivity and NPV, it is intended to maximize case detection and minimize false negatives, even at the cost of some false-positive classification. This approach is appropriate in the context of a relatively high underlying prevalence, where prioritizing early identification and subsequent confirmatory testing can improve case detection while optimizing resource utilization.

A PLR of 3.6 indicates that physicians with a positive screening result are approximately four times more likely to have a vitamin B12 deficiency than those without deficiency. Conversely, an NLR of 0.3 suggests that a negative result significantly reduces the probability of deficiency. The validation of our screening tool demonstrates its potential utility in clinical practice, aligning with the results of earlier studies on vitamin and micronutrient deficiency and neurological problems[14,15,22]. Additionally, the present vitamin B12 screening questionnaire also manifested a fair discriminatory ability, as evident from the AUC of 0.776. Furthermore, the majority of the physicians also agreed that the questionnaire was extremely beneficial, indicating that it likely represents a valuable tool in clinical practice.

A weak but statistically significant positive correlation ($\rho = 0.20$, $p = 0.007$) was observed between vitamin B12 and hemoglobin levels, consistent with prior research[23,24]. However, a conflicting report had failed to establish a significant relationship between the two parameters, particularly among elderly populations[25]. These discrepancies highlight the complex interplay between vitamin B12 metabolism and hematopoiesis, warranting further investigation.

STRENGTHS AND LIMITATIONS

Conducting the study among physicians offered the advantage of a controlled and medically informed population. Their clinical expertise enabled accurate recognition and reporting of symptoms, thereby minimizing recall and reporting bias commonly seen in self-reported data from the general population. This enhanced the reliability and internal validity of the findings. Given their familiarity with clinical assessment terminologies and tools, physicians are well suited to directly adopt and implement this questionnaire as a practical screening tool in routine outpatient practice. Nevertheless, the study is not without limitations. Factors such as age, gender, dietary habits

(e.g., vegetarianism), and alcohol consumption could act as confounders, potentially affecting vitamin B12 levels and screening tool performance.

CONCLUSION

In conclusion, this study presents a novel, easy-to-administer screening tool for vitamin B12 deficiency with moderate-to-high sensitivity and specificity. This simple, cost-effective screening tool showed strong potential for identifying physicians at risk of vitamin B12 deficiency. Importantly, the tool holds strong future potential as a ready-to-use screening questionnaire, which could be readily implemented by the physician in routine clinical practice, particularly given that serum vitamin B12 testing can be expensive, thereby facilitating timely assessment and early detection of vitamin B12 deficiency in outpatient settings while enabling more judicious use of diagnostic resources. Future studies could further explore the influence of gender and age on the prevalence of vitamin B12 deficiency, which may help refine the screening tool and enhance its applicability across diverse demographic groups in clinical practice.

Key Message

A novel 10-item screening questionnaire was developed and validated to identify physicians at risk of vitamin B12 deficiency in the Indian scenario. This questionnaire-based tool demonstrated moderate-to-high sensitivity and specificity, making it a promising, cost-effective option for early screening of inapparent vitamin B12 deficiency, facilitating optimization of health care approaches, especially in resource-limited settings. Further research is needed to validate its applicability by the physicians in routine clinical practice as a preliminary screening tool and integration into primary health care screening programs.

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