Original Article

Cobalamin Intake in North Indians by Food Frequency Questionnaire (COIN-FFQ) – A Development and Validation Study

Swapnil Rawat, Meena Kumari, Jitender Nagpal[1]

Amity Institute of Food Technology, Amity University, Noida Campus, Noida, Uttar Pradesh, [1]Pediatrics and Clinical Epidemiology, Sitaram Bhartia Institute of Science and Research, New Delhi, India

Abstract

Background: Vitamin B_{12} deficiency is widely prevalent in all age groups which is of major concern. However, there is no valid Food Frequency Questionnaire (FFQ) for dietary vitamin B_{12} estimation. Hence, we aimed to develop and validate an FFQ for the estimation of dietary intake of Vitamin B_{12}. **Materials and Methods:** Commonly consumed B_{12}-rich food items were selected from literature and filtered using a market survey. For concordant and discriminant validation, B_{12} and homocysteine levels were estimated. To establish convergent validity, the Cobalamin Intake in North Indians by Food Frequency Questionnaire (COIN-FFQ) and 72-hour dietary recall (72HrDR) were both administered to the same subjects. The COIN-FFQ was readministered after initial administration for test–retest reliability. Internal consistency of the FFQ was then tested using Cronbach's alpha. **Results:** We enrolled 115 adults with a mean age and weight of 31.9 ± 8.7 years and 66.0 ± 11.8 kg, respectively. In total, 19.1% were vegetarian. The dietary B_{12} using COIN-FFQ (n = 60; mean = 4.3 ± 1.8 µg/d) was significantly correlated (r = 0.255; P = 0.049) with serum levels (mean = 120.1 ± 62.6 pmol/L) establishing concordant validity. A significant difference was noted between the dietary, serum B_{12}, and homocysteine levels of vegetarians versus nonvegetarians establishing discriminant validity (mean diff 1.4 (0.5–2.4), P = 0.004; Z-statistic −2.182, P value 0.029, and Z-statistic −2.438; P value 0.015), respectively. FFQ was strongly correlated with 72HrDR and test–retest FFQ (n = 27; r = 0.814, P < 0.001 and r = 0.869, P < 0.001, respectively) establishing convergent validity and test–retest reliability. The internal consistency with Cronbach's alpha was in the acceptable range, 0.631 (n = 115). **Conclusion:** The newly developed COIN-FFQ is valid and reliable in estimating dietary B_{12} intake.

Keywords: Dietary assessment, food frequency questionnaire, India, validation, vitamin B_{12}

Introduction

Vitamin B_{12} (cobalamin) is an essential water-soluble micronutrient necessary for normal neurological and hematological functioning. Deficiency of vitamin B_{12} may lead to pernicious anemia, irreversible neurological damage, psychiatric disorders like depression, irritability, impaired memory, dementia, infertility, and adverse pregnancy outcomes.[1–3] It is naturally found in animal food products including meat, poultry, fish, eggs, milk, and other dairy products.[4] The worldwide prevalence of vitamin B_{12} deficiency is 62% among pregnant women, 25–86% in children, 21–41% in adolescents, and 11–90% in the elderly population,[5] whereas in the North Indian population, the prevalence of B_{12} deficiency (Vitamin B_{12} levels <200 pg/ml) averages 47%[6] Low intake of animal foods in the diet is the key factor in developing B_{12} deficiency.[7–9] Laboratory estimations of vitamin B_{12} biomarkers have been key to a timely identification of deficiency, which in turn allows early intervention and minimizes future consequences.[10] Due to poor awareness and expensive testing for B_{12} biomarkers, the deficiency is widely underdiagnosed, and populations from poorer socioeconomic strata suffer the worst consequences of late diagnosis.

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Address for correspondence: Swapnil Rawat, Amity Institute of Food Technology, Amity University, Noida Campus, Sector-125, Noida, Uttar Pradesh - 201 301, India. E-mail: swapnil.rawat@gmail.com

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The Food Frequency Questionnaire (FFQ) is a useful clinical tool used for estimating the dietary intake of any nutrient and helps in identifying deficiency risk in the early stages. FFQs consist of lists of foods and beverages with the frequency of consumption over a specific period. Also, FFQs estimate the moderate dietary intake of specific/multiple nutrient/s and are, hence, the most popular methods used in epidemiological studies to assess long-term nutritional exposure.[11] Additionally, FFQs are low cost, easy to administer, and have minimal participant burden. While there are several advantages, FFQs have limitations by being specific to the cultural contexts where they are developed and validated. There are several validated FFQs for single and multiple micronutrients like folate, zinc, fiber, calcium, Vitamin A, Vitamin C, Vitamin B_6, and Vitamin B_{12}. However, most FFQs target multiple nutrients. Multinutrient FFQs provide generic information on the intake of many nutrients but are often too tedious for routine use and often less accurate for individual micronutrients (detailed intakes of specific foods related to every nutrient are often not possible).[12–16] Single nutrient FFQs on the other hand can provide estimates for the interest nutrient, are less time consuming, and can be more accurate/reliable for that specific micronutrient.[15] However, very few single micronutrient FFQs are validated for use in India while none were retrieved for B_{12} intake estimation.[17,18] Internationally, Mearns *et al.*[19] developed and validated a semiquantitative FFQ to estimate the dietary B_{12} intake in South Asian women living in New Zealand. This is the only validated FFQ retrieved for a single nutrient B_{12} assessment. However, this FFQ has not been tested in the Indian population and the validation did not include test–retest reliability. Therefore, we conducted this study with the objective of developing and validating a quick and easy-to-apply FFQ for the estimation of vitamin B_{12} intake in North Indians (South Delhi population as represented by the cosmopolitan nature of the Delhi population).

FFQ vs DIETARY RECALL: FFQs typically enlist a series of food items around a specified theme and document an estimate of food frequency in terms of occasions per week/month while also recording a visual cue to quantity. Dietary recall on the other hand involves the recall-documentation of each food item consumed in the specified time period. For macronutrients, FFQs tend to be less accurate and for micronutrients dietary recalls underestimate/inaccurately estimate the intake as tended to report fewer number of foods and fewer mentions of foods consumed.[20] FFQs need customization for locally available foods and dietary practices. Given the objective of estimating only B_{12} intake FFQ was chosen as the tool of choice.

Methods

To develop and validate an FFQ for estimating B_{12} intake in a scientifically robust way, we followed a sequence of steps across two distinct phases, namely, development and validation as summarized in Figure 1.

PHASE1: Development of vitamin B_{12} FFQ

Step 1: Initial construct of draft food list

A draft list was made enlisting all the dietary items rich in B_{12}. Food selection was based on the locally available vitamin B_{12} sources reported in literature to contain Vitamin B_{12}. These food sources included animal sources (dairy, poultry, meats) and B_{12}-fortified foods.

Step 2: Local availability and refinement (Market Survey)

The market survey was conducted in nearby grocery stores to assess the availability of foods enlisted in the draft list. A total of three supermarkets were covered in the National Capital Region area. Online searchers for fortified B_{12} products were also explored for an extensive range of options and diversity in products. Any additional items potentially containing B_{12} were added to the draft list. Special attention was given to packaged eatables labeled as "Fortified with Vitamin B_{12}". The data for this survey were primarily gathered from the nutritional labels provided on these packaged food products, ensuring an accurate representation of their B_{12} content. Formula foods for infants were intentionally excluded from this list. This decision was made considering the unique nutritional requirements and consumption patterns of infants, which are significantly different from the general population. During this survey, it was observed that some staple cereals, specifically wheat flour and rice, were also fortified with vitamin B_{12}, iron, and folic acid. This fortification is a result of the 2016 legislation by the Food Safety and Standards Authority of India.[21] All food items identified as B_{12}-fortified according to their nutrition labels were added to the draft list. This comprehensive approach ensured that all locally available B_{12}-fortified foods were included in the FFQ. After this rigorous market survey and refinement process, a final list of B_{12}-fortified foods was formulated.

Step 3: Standardization of portion size

To estimate the portion size, standardization of homemade recipes was done using household measurements (bowl, glass, spoons). For easy identification of foods by participants, a picture book was prepared. Standardized household measures to allow visual estimation of the food serving size and quantities.

Step 4: Estimation of vitamin B_{12} content in animal source foods

The Indian Council of Medical Research-National Institute of Nutrition (ICMR-NIN) Indian Food Composition Table (2016)[22] does not specify the B_{12} content of Indian foods. Hence, the vitamin B_{12} content in common animal source foods was reconfirmed by laboratory analysis (ELISA (enzyme-linked immunosorbent assay), Eurofins Immunolab Vitamin B12[23] (sensitivity ±0.03ng/mL). The results of the analysis are summarized in Table 1. The presented results were compared with those from the Indian Nutritive values, 1989 by Gopalan.[24]

Step 5: Development of FFQ

The final food list from Step 2 was then converted into a semiquantitative FFQ comprised of three categories of edibles: (i) dairy and dairy products, (ii) nonvegetarian foods,

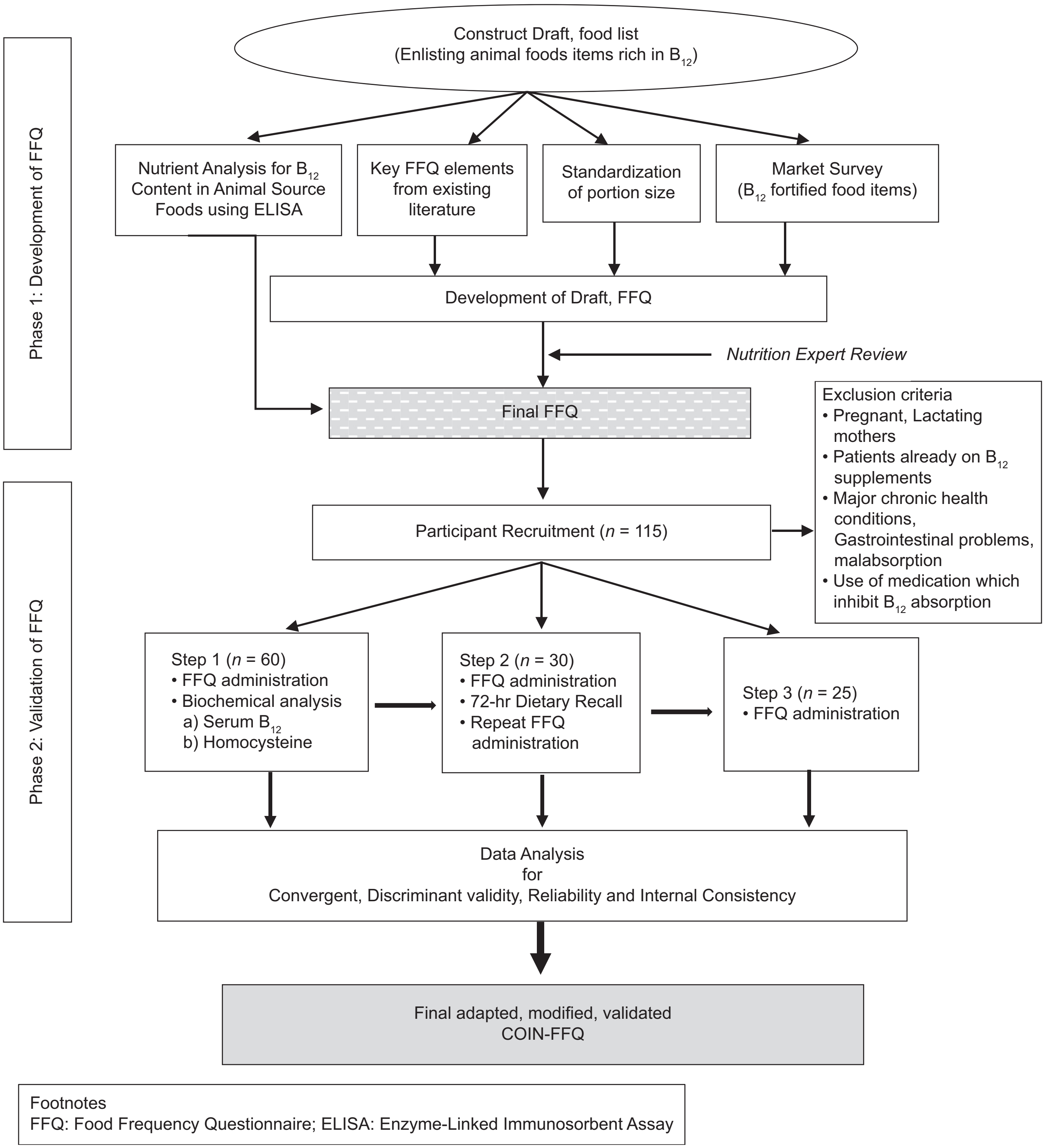

Figure 1: Summary of study design. FFQ: Food Frequency Questionnaire; ELISA: Enzyme-Linked Immunosorbent Assay

and (iii) vitamin B_{12}-fortified foods/drinks. For each item, a commonly used portion size/quantity was listed along with a question frequency of consumption in the past three months. The frequency responses ranged from "Never or less than once per month" to "Four or more times per day." Questions regarding the intake of B_{12} supplements were also included. Open-ended questions recorded brand names of potentially fortified products like protein supplements or breakfast cereals. The draft FFQ was reviewed by nutrition experts to develop a final FFQ (30 food items).

Step 6: Development of database

All final FFQ food items were enlisted in an Excel database. Vitamin B_{12} content of all the standardized recipes was entered.

Table 1: Estimated B_{12} in animal source food (ASF)

Name	Vitamin B_{12} (μg/100 g)
Milk, cow	0.74±0.12
Curd, cow	0.43±0.07
Cream	0.37±0.05
Processed cheese	0.43±0.08
Paneer (cottage cheese)	0.39±0.05
Egg, hen, whole	1.01 ±0.09
Fish, fresh water	2.02±0.31
Red meat, goat	2.45±0.08
Chicken	2.42±0.34

The values for B_{12} content were taken from step 5. For those foods for which the Indian values for B_{12} content were not available, the United States Department of Agriculture (USDA) database[25] was used. The frequency of consumption was multiplied by serving size and B_{12} content to determine the nutrient contribution of each food item and that of the individual by summating all the items.

PHASE 2: Validation of FFQ

Concept of validity

Validity means **"**the main extent to which a concept, conclusion, or measurement is well-founded and likely corresponds accurately to the real world". More specifically in the context of tools, it is the degree to which the tool measures what it claims to measure. Validity is concluded based on the strength of a collection of different types of evidence (e.g. construct validity, reliability, etc.) described in greater detail below-

1. Face and Content Validity: Face validity refers to "the subjective extent to which a particular test/scale appears to measure what it is supposed to measure," while content validity refers to whether the test comprehensively covers all aspects of what the scale is supposed to measure. In our study, the method of development involved the extensive review of literature and market survey establishing face validity, while the FFQ review by nutrition experts established content validity.
2. Construct Validity: "Construct validity refers to how well you translated or transformed a concept, idea, or behavior that is a construct into a functioning and operating reality, the operationalization."[26] Construct validity has two components: convergent and discriminant validity.
   a. Convergent Validity: Convergent validity, a parameter often used in sociology, psychology, and other behavioral sciences, refers to "the degree to which two measures of constructs that theoretically should be related, are in-fact related".
   b. Discriminant Validity: It is the "extent that measures of different constructs diverge or minimally correlate with one another". In the current context, it means the extent to which the questionnaire can differentiate potentially deficient subjects (interpreted as vegetarians) from those at lower risk (interpreted as nonvegetarians).
3. Reliability: It is a measurement that tests whether a scale provides stable and consistent results which is important for the equivalence, stability, and internal consistency of the tool.[26] In our context test–retest reliability is concerned with the repeatability of the same test within a specific interval of time
4. Internal Consistency: Cronbach's alpha is a measure of the internal consistency or reliability between several items, measurements, or ratings. It estimates how reliable the responses of a questionnaire (or domain of a questionnaire), instrumentation, or rating evaluated by subjects, which will indicate the stability of the tools.[27]

Participants

As presented in Figure 1, Phase 2 of the study consisted of three distinct steps toward systematically establishing the validity of the questionnaire.

Pilot Study: A pilot study was conducted on 10 healthy volunteers. Feedback was obtained from the participants on time taken and ease of understanding. Any procedural hurdles/barriers encountered were identified. Learnings were used to modify the workflow and refine the language of the FFQ.

A total of 115 participants were enrolled for the validation exercise in three steps.

In Step 1, 60 healthy adult volunteers aged 18–60 years were enrolled for FFQ administration between July 2021 and May 2022 after an informed written consent. Pregnant women, lactating mothers, patients who are already on B_{12} supplements, major chronic health conditions like gastrointestinal problems, malabsorption, and use of any medication inhibiting B_{12} absorption were excluded. No financial rewards were given for their participation. The study was approved by the Institutional Ethics Committee.

Basic baseline information on socioeconomic status, type of family, food habits, smoking, alcohol status, and anthropometric measurements like weight, height, waist circumference, and blood pressure was recorded. The FFQ consisted of 30 questions that were administered thereafter. All subjects underwent blood sampling (5 ml) for subsequent assessment of serum B_{12} and homocysteine levels.

In *Step 2,* 30 volunteers were sequentially chosen to undergo an FFQ and 72-hour dietary recall assessment (72HrDR).[20] For calculating the 72HrDR, intake data were collected for two weekdays and one Saturday/Sunday to capture the variability in food intake throughout the week. Standardized household measures were used for portion size information. The dietary intake was calculated using the developed database (step 6)

Of the subjects enrolled in Step 2 all were approached for the assessment of test–retest reliability. Two subjects refused consent, while one was Covid Positive. Hence, 27 subjects underwent a repeat assessment (FFQ2) using the COIN-FFQ after a fortnight.

In *Step 3,* an additional 25 volunteers were recruited to achieve a cumulative target number of 115 subjects for internal consistency assessment.

Biochemical assessment: The 5 ml venous blood samples were collected via venipuncture from participants in an serum separator tube vacutainer and centrifuged at 3000 RPM for 15 min; serum was isolated and stored at −70°C. Vitamin B_{12} level was analyzed by Electrochemiluminescence immune assay. Homocysteine (tHcy) was analyzed by competitive immunoassay using the Direct chemiluminescent method. Hemoglobin was estimated from capillary blood by Hemocue Hb 201$^+$.

Sample size consideration

As the study involved multiple independent comparisons, three sample size calculations were conducted to calculate the optimal sample size requirements. For correlations between FFQ-B_{12} and serum B_{12}, it was estimated based on existing literature[19] ($r = 0.50$) that a sample size of 30 subjects will be required assuming an alpha error of 0.05 and a power of 80%. Similarly, for discriminant validity, a sample size of 59 subjects (35% prevalence of vegetarianism) was estimated using a mean difference of 2.6, an SD of 3.3,[19] an alpha error of 0.05, and 80% power. For internal consistency, a sample size of 100 subjects was sufficient to detect and internal consistency of 0.7 with an alpha of 0.05 and 80% power assuming an eigenvalue of <6 for the first component. Therefore, it was decided to recruit an additional 25 subjects (total $n = 115$) in Step 3 to add to the 90 already recruited in the first two steps allowing for errors in estimates.

Statistical analysis

The statistical analysis for the validation of the B_{12} FFQ was performed using IBM SPSS version 25.0. The means and proportions were compared between groups using statistically appropriate tests. For normal continuous variables, the *t*-test was used. For proportions, the Chi-square test was applied. In cases where nonparametric comparisons were required, the Mann–Whitney test was utilized. These comparisons were used in the determination of discriminant validity and test–retest reliability as presented in the results.

To establish convergent validity the Spearman rank correlation coefficients (Spearman's rho) were used for continuous nonparametric data. This method measures the strength and direction of association between two ranked variables, providing insights into the relationship between FFQ responses and actual dietary intake as recorded in the 72HrDR.

Additionally, Cronbach's alpha was calculated to check the internal consistency of the FFQ tool. This statistic measures how closely related a set of items are as a group, providing an indication of the reliability of the FFQ. A high Cronbach's alpha suggests that the items in the FFQ are measuring the same underlying construct of B_{12} intake.

Results

Study groups and baseline characteristics

The cumulative study group consisted of 115 healthy subjects (men = 60, women = 55) working/residing in NCR Delhi who finally completed the COIN-FFQ through the three steps of the study. We excluded three subjects because two were on B_{12} supplementation, (neurological problem ($n = 1$) and refusal ($n = 2$). The baseline characteristics of the recruited subjects are summarized in Table 2. As depicted, 22, 11, and 82 of the subjects reported lacto-vegetarian, lacto-ovo-vegetarian, and nonvegetarian dietary patterns, respectively, whereas 100% were consuming dairy and dairy products. There were no significant differences between the subjects recruited for the three steps of Phase 2 of the study. The participants had a mean age of 31.9 ± 8.7 years and a mean Body Mass Index (BMI) of 24.9 ± 3.7 Kg/m^2. More than 70% of the subjects were nonvegetarian. The estimated mean intake of Dietary B_{12} was 4.0 ± 1.9 µg/day.

Validity and reliability

Convergent Validity: Table 3a and b summarizes the results for the correlation between B_{12} intake by COIN-FFQ and 72HrDR and that with serum B_{12} values. As depicted, the B_{12} intake calculated by the COIN-FFQ was significantly correlated with both the 72HrDR and the serum values for B_{12} and tHcy. The correlation with tHcy levels was negative. This established convergent validity.

Discriminant Validity: Table 3(c) summarizes differences between vegetarians and nonvegetarians in the B_{12} intake calculated by COIN-FFQ. The B_{12} intake of vegetarians calculated by the COIN-FFQ was significantly lower establishing discriminant validity.

Test–Retest reliability: As a next step, we compared the COIN-FFQ calculated intake of Step 2 subjects with a repeat administration after two weeks to study test–retest reliability. As presented in Table 3(d), the test–retest correlation of COIN-FFQ was 0.847 with a *P* value <0.001 establishing test–retest reliability.

Internal Consistency: The internal consistency of COIN-FFQ was evaluated using Factor analysis using all items, yielding a Cronbach's alpha of 0.631, indicating "acceptable" internal consistency.[28]

Clinical Implications: To further understand the clinical implications of the B_{12} intake estimation, we calculated the deciles for B_{12} intake by COIN-FFQ. We then calculated the sensitivity and specificity of these deciles in predicting B_{12} deficiency. Both B_{12} levels and tHcy were sequentially used to determine biochemical B_{12} deficiency as the gold standard for this purpose. As presented in Table 4, it is noted that an intake estimation of <3.26 µg/d was ~95% specific for the detection of low B_{12} levels and elevated tHcy levels. Similarly, a level of >6.98 µg/d was >90% specific for predicting sufficiency or normal B_{12}/tHcy levels.

Discussion

This study aimed to develop and validate an FFQ for the assessment of dietary vitamin B_{12} intake among North Indians and to test its utility in predicting biochemical deficiency. As presented, the newly developed COIN-FFQ had a good face and content validity, good internal consistency, and convergent

Table 2: Baseline characteristics

Characteristics	Overall [%(*n*)/ mean±SD] (*n*=115)	GROUP A [%(*n*)/ mean±SD] (*n*=60)	GROUP B [%(*n*)/ mean±SD] (*n*=30)	Group C [%(*n*)/ mean±SD] (*n*=25)
Age (year)	31.9±8.7	33.9±9.7	29.2±4.7	30.4±8.9
Gender				
Male (%)	52.1 (60)	58.3 (35)	50.0 (15)	40.0 (10)
Female (%)	47.9 (55)	41.7 (25)	50.0 (15)	60.0 (15)
Height (cm)	162.5±8.5	162.9±9.2	163.8±7.1	160.0±8.3
Weight (kgs)	66.0±11.8	67.7±13.1	63.5±8.6	64.9±11.4
Mean BMI (kg/m^2)	24.9±3.7	25.4±4.0	23.6±2.5	25.3±4.0
Waist circumference (cm)	90.5±9.5	92.0±9.4	86.5±6.9	91.7±11.4
Systolic Blood Pressure (mmHg)	122.3±12.9	125.8±13.7	119.8±10.1	116.8±11.5
Diastolic Blood Pressure (mmHg)	83.3±7.9	84.6±8.4	82.2±6.7	81.5±7.8
KSEC*†				
Upper	4.3 (5)	(0)	3.3 (1)	16.0 (4)
Upper middle	72.2 (83)	68.3 (41)	83.3 (25)	68.0 (17)
Lower middle	18.3 (21)	26.6 (16)	10.0 (3)	8.0 (2)
Upper lower	5.2 (6)	5.0 (3)	3.3 (1)	8.0 (2)
Lower	0	0	0	0
Type of family: Nuclear	66.1 (76)	66.7 (40)	66.7 (20)	64.0 (16)
Living in				
Own or family house	41.7 (48)	36.6 (22)	43.3 (13)	52.0 (13)
Rented house	58.3 (67)	63.3 (38)	56.6 (17)	48.0 (12)
Number of family members	4.0±2.1	3.8±1.8	4.0±2.7	4.4±2.1
Smoking use				
Current	13.9 (16)	8.3 (5)	16.6 (5)	24.0 (6)
Former	6.1 (7)	8.3 (5)	3.3 (1)	4.0 (1)
Never	80 (92)	83.3 (50)	80.0 (24)	72.0 (18)
Alcohol user				
Current	44.3 (51)	45.0 (27)	40.0 (12)	48.0 (12)
Former	3.5 (4)	6.6 (4)	0 (0)	0 (0)
Never	51.3 (60)	48.3 (29)	60 (18)	52.0 (13)
Any medical problem*‡	24.3 (28)	23.3 (14)	6.6 (2)	48.0 (12)
Use of supplements*§	13.0 (15)	8.3 (5)	3.3 (1)	36.0 (9)
Food Habits				
Vegan vegetarian	0	0	0	0
Lacto-vegetarian	19.1 (22)	23.3 (14)	(4)	16.0 (4)
Lacto-ovo vegetarian	9.6 (11)	8.3 (5)	(4)	8.0 (2)
Non-vegetarian	71.3 (82)	68.3 (41)	(22)	76.0 (19)

*Correlation is significant at the 0.01 level (2-tailed) between groups. Group A: FFQ + Biochemical parameters; Group B: FFQ + 72HrDR + Repeat FFQ; Group C: Only FFQ. †KSEC: Kuppuswamy Socio-Economic Scale, based on education level,[30] occupation of the head of the family and monthly family income. ‡Any medical problems: Thyroid, Poly Cystic Ovary Syndrome, gastritis. §Use of supplement: Calcium, vitamin C, vitamin D, zinc

and discriminant validity. An intake cut-off of <3.26 µg/d has good specificity in identifying B_{12} deficiency, while intake estimations of >6.98 µg/d are highly predictive of normal B_{12} levels. These values of <3.26 µg/d and >6.98 µg/d could, therefore, help a care provider draw useful inferences on the chances of the patient being deficient or sufficient with a good level of accuracy.

Our study is limited by the lack of information about losses of B_{12} during food preparation, and cooking. The narrow demographics of the study population, including the absence of nondairy consuming vegetarians and recruitment of subjects from a hospital catering to higher-income populace, limit the generalizability of the study. A high prevalence of overweight/obesity in the study population could also limit the general applicability as high BMI is associated with lower B_{12} levels.[29] However, this is the first study from India validating a simple single micronutrient FFQ estimating the B_{12} intake in healthy adult population. The comprehensive market survey conducted in the study population, the use of standard portion sizes, the use of multiple robust markers of validity, and the independent population sets used for different steps are important strengths of the study. Importantly, the study uses estimates of B_{12} content by direct lab analysis[22] of the actual locally available foods.

As presented earlier multiple tools report the assessment of dietary intakes using FFQs. Many focus on multiple micronutrients while others focus on single micronutrients. However, in most of the

Table 3: Validation by different methods

a). Convergent Validity: Correlation between Dietary Intake by FFQ with Serum Values (n=60)

Parameters	Units	Mean±SD	FFQ	Serum B_{12}	tHcy
FFQ	µg/day	4.3±1.8	r=1.000		
Serum B_{12}*	pmol/L	120.1±62.6	r=0.255, P=0.04	r=1.000	
tHcy*	µmol/L	19.7±16.6	r = −0.169, P=0.19	r = −0.529, P<0.001	r=1.000

b). Convergent Validity: Correlation between Dietary Intake by FFQ1 with 72 h DR (n=30)

Parameters	Units	Mean±SD	FFQ1	72HrDR
FFQ	µg/day	3.6±2.0	r=1.000	
72HrDR	µg/day	2.8±1.3	r=0.811, p<0.001	r=1.000

c). Discriminant Validity (n=60)

Method	Overall Mean±SD (n=60)	Vegetarian† (n=20)	Non-vegetarian (n=40)	Mean Diff (95%CI)/ z-score	P
FFQ (µg/day)	4.3±1.8	3.4±1.8	4.8±1.7	1.4 (0.5–2.4)	<0.01
Serum B_{12}* (pmol/L)	120.1±62.6	102.3±32.3	130.1±70.4	−2.182	0.02‡
tHcy* (µmol/L)	19.7±16.6	26.4±19.3	17.0±13.7	−2.438	0.01‡
Deficient serum B_{12} level (%(n))	70.0 (42)	85.0 (17)	62.5 (25)	3.214‡	0.07
Abnormal tHcy level (%(n))	61.7 (37)	80.0 (16)	52.5 (21)	4.266§	0.03
Anemia (%(n))‖	21.6 (13)	30.0 (6)	17.5 (7)	-0.125	0.276

d). Test–retest Reliability (n=27)

Parameters	Units	Mean±SD	FFQ1	FFQ2
FFQ1	µg/day	3.3±1.6	r=1.000	
FFQ2	µg/day	3.4±1.4	r=0.847, P<0.001	r=1.000

tHcy means Homocysteine; *Geometric mean; †Vegetarian includes lacto-vegetarians and lacto-ovo vegetarians; ‡Significance by Wilcoxon nonparametric test; §Chi-square; ‖Anemia defined as <12 g/dl for women and <13 g/dl for men; FFQ1 means initial FFQ and FFQ2 means FFQ administered again after 15 days of interval on the same subjects

Table 4: Specificity and sensitivity

Dietary B_{12} intake (µg/d)	Serum B_{12} level (pmol/L)				Homocysteine level (µmol/L)			
	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
<1.97	14	100	100	33.33	13.51	95.65	83.3	40.74
<2.57	26.19	94.49	91.66	35.41	29.72	95.65	91.66	45.83
<3.26	40.47	94.44	94.44	40.47	45.94	95.65	94.44	52.38
<4.03	47.61	77.77	83.33	38.88	51.35	78.26	79.16	50
<4.24	52.3	55.5	73.33	33.33	54.05	56.52	66.66	43.33
<4.69	59.5	44.44	71.42	32	59.45	43.47	71.42	40
<5.35	71.42	33.33	71.42	33.33	70.27	30.43	61.9	38.88
<5.56	83.33	27.77	72.91	41.66	83.78	21.73	64.58	45.45
<6.98	90.47	11.11	70.37	33.33	91.89	13.04	62.96	50

PPV: Positive predictive value; NPV: Negative predictive value

studies, the validation has been based on fairly lenient criteria with variable definitions. Many such tools were validated for use in India,[15–18] but they do not relate to B_{12} intake and suffer from the same limitations as mentioned above. Another multinutrient FFQ from South India[16] estimates B_{12} intake in pregnant women in addition to energy, protein, fat, carbohydrate B_6, folate, and calcium. However, the tool has 108 items, and the study uses only blood levels correlation and 24HR dietary recall correlation for validation. Also, in the specific instance of single nutrient, FFQ for B_{12} Mearns *et al.*[19] developed and validated a nutrient-specific semiquantitative FFQ. For this purpose, 60 South Asian women aged 18–50 years of age were recruited and the relationships between estimated dietary B_{12} and serum B_{12}/Holo-transcobalamin were evaluated as outcome measures. The authors documented that dietary B_{12} intake was positively correlated with serum B_{12} (r = 0.50; P < 0.001; in comparison our study 0.255; P value 0.049). Insufficient intake was recorded for 18.3% (n = 21) in our study vs 44% (n = 26) in Mearns *et al.*[19] study. Despite similarities, our FFQ has a few numbers of dietary items (30 items compared with 45 for Gael) making it faster and simpler to administer. This is attributable to the lesser variety of nonvegetarian (especially seafoods) and fortified foods available in Delhi. Also, the robust and comprehensive sequence of validation tests followed in the current study means that there is stronger evidence for the validity and reliability of the COIN-FFQ.

Conclusion

The authors conclude that the COIN-FFQ is a valid, easily administered noninvasive tool to estimate the dietary intake of B_{12}. The estimations by COIN-FFQ carry clinically relevant connotations, which are potentially useful for the identification of high-risk individuals for further blood testing and/or supplementation. While the tool has been validated in North India, it may prove to be generalizable to the wider Indian/South Asian population. We recommend this tool be tested in a wider variety of settings/populations for validation.

Abbreviations

72HrDR	72 Hour dietary recall
B_{12}	Vitamin B_{12}
COIN-FFQ	Cobalamin Intake in North Indians-Food Frequency Questionnaire
FFQ	Food Frequency Questionnaire
KSEC	Kuppuswamy Socioeconomic Class
tHcy	Homocysteine

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Conflicts of interest

There are no conflicts of interest.

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