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ORIGINAL RESEARCH ARTICLE

Evaluation of the Impact of Vitamins B6, B9, and B12 on Cognitive Decline in Older Adults in Sokoto Metropolis

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ABSTRACT

Cognitive decline is increasingly recognized as a public health challenge among aging populations, and micronutrients, such as B vitamins, play a crucial role in maintaining neurocognitive health. This study investigated the association between serum levels of vitamins B6, B9 (folate), and B12 and cognitive performance in older adults in Sokoto Metropolis, Nigeria. A cross-sectional design was employed, involving 210 adults aged 41–80 years recruited through purposive community sampling. Serum concentrations of vitamins B6, B9, and B12 were analyzed using high-performance liquid chromatography (HPLC), while cognitive function was assessed with the Mini-Mental State Examination (MMSE). Statistical analyses, including one-way ANOVA and Pearson's correlation, were applied to determine associations between vitamin status and cognitive outcomes. Results revealed a significant increase in serum vitamin B12 levels with advancing age ($p < 0.05$), with the highest mean concentration (818.73 ± 3.58 pg/mL) recorded in participants aged 71–80 years. However, data for vitamins B6 and B9 were incomplete due to equipment malfunction and excluded from final analyses. Higher serum vitamin B12 concentrations are associated with lower MMSE scores, indicating a negative relationship between vitamin B12 levels and cognitive performance. The findings underscore the importance of adequate B-vitamin status, particularly vitamin B12, in preserving cognitive health among older adults. While incomplete data limited the scope of interpretation, this study highlights the need for targeted nutritional interventions, including dietary education and B-vitamin supplementation, as strategies to reduce dementia risk. Future research should incorporate comprehensive micronutrient profiling and longitudinal designs to strengthen causal inference and inform public health strategies.

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INTRODUCTION

The global population is aging at an unprecedented rate, with projections indicating that by 2050, one-fifth of the world's population will be aged 60 years or older (Navaneetham and Arunachalam, 2023). This demographic shift is accompanied by an increasing health burden, as older adults now account for approximately 23% of the global disease load, with neurodegenerative and psychiatric disorders—particularly dementia and depression—among the leading causes of disability (Khandelwal and Gupta, 2023). Currently, dementia affects an estimated 46.8 million individuals worldwide, and this figure is expected to exceed 131 million by 2050, signifying an impending global public health crisis (Ye, 2022).

Dementia is characterized by progressive deterioration of cognitive functions such as memory, judgment, and problem-solving, which significantly disrupt daily living and impose substantial emotional and financial strain on caregivers and healthcare systems. The global cost of dementia is projected to surpass one trillion dollars annually, while depression—often comorbid with cognitive decline—adds further economic burden, particularly in Europe where costs reach billions each year

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(Meijer et al., 2022). Despite advances in biomedical research, effective disease-modifying therapies for dementia remain unavailable, and the impact of existing antidepressants is limited (Yeo and Tang, 2023). Even modest delays in the onset of dementia could prevent millions of cases, highlighting the urgent need for modifiable and preventive strategies (Frankish and Horton, 2017).

Nutrition has emerged as a promising modifiable factor in mitigating cognitive decline and promoting healthy brain aging. Diets rich in antioxidants and anti-inflammatory compounds have been shown to modulate neuroinflammatory pathways involved in neurodegenerative disorders (McGrattan et al., 2019). Among these nutrients, B-vitamins—particularly B6, B9 (folate), and B12—are essential for homocysteine metabolism, neurotransmitter synthesis, DNA repair, and neuronal integrity (Orywal et al., 2025). Elevated homocysteine resulting from vitamin deficiencies is associated with oxidative stress, vascular damage, and neuronal apoptosis, contributing to cognitive impairment and neurodegeneration.

However, most evidence on B-vitamins and cognitive function originates from high-income countries, while data from low- and middle-income settings remain scarce. In Nigeria, particularly in the Sokoto metropolis, unique dietary practices, socioeconomic limitations, and restricted access to healthcare may predispose older adults to micronutrient deficiencies. Yet, empirical studies examining the relationship between B-vitamin status and cognitive performance in this population are lacking. This local research gap limits the development of context-specific nutritional interventions and public health strategies.

Therefore, this study is among the first to investigate the association between serum levels of vitamins B6, B9, and B12 and cognitive function among older adults in Sokoto metropolis, Nigeria. It is based on the hypothesis that B-vitamin status represents a modifiable risk factor for cognitive decline in this understudied population and seeks to generate region-specific data that may inform early intervention, nutrition policy, and dementia prevention strategies.

Accordingly, the aim of this research is to evaluate the impact of Vitamin B6, B9 and B12 on cognitive decline in older adults in Sokoto metropolis. The specific objectives are to quantitatively determine the serum concentrations of vitamin B6, B9 and B12 in older individuals in Sokoto metropolis as well as to assess the cognitive status of the study participants using the Mini-Mental State Examination (MMSE) and correlate the results with serum levels of B vitamins.

MATERIALS AND METHODS

Reagents used

All reagents used were of analytical grade and obtained from SIGMA-ALDRICH (USA). High-Performance Liquid Chromatography (HPLC)-grade solvents and standard solutions were prepared for the quantification of serum B-vitamins (B6, B9, and B12).

Equipment

Sample preparation utilized an Eppendorf 5804R centrifuge (Germany), while HPLC quantification of B-vitamins was carried out using the AGILENT 1260 INFINITY II G7120A system (USA). Equipment calibration followed manufacturer protocols, with standard solutions used to generate calibration curves for each vitamin.

Ethical Approval and Consent Form

The ethical clearance were obtained and granted with reference numbers SHS/SUB/133/VOL 1, 11th July, 2024 and SMH/1580/V.IV, 14th June, 2024 for Specialist Hospital, Sokoto and the Research and Ethics Committee of Sokoto State Ministry of Health respectively. Informed consent was obtained from all participants after they were provided with detailed information on the study's purpose, procedures, potential risks, and benefits. Participation was entirely voluntary, with the option to withdraw at any time without consequences. Confidentiality was ensured by assigning unique participant codes and securely storing all data.

Sample Collection

A multistage sampling technique was used to recruit 210 participants. The study area was first stratified by administrative divisions, from which clusters were randomly selected. Within each cluster, systematic sampling was applied, selecting every kth individual from an alphabetically ordered list after a randomly chosen starting point. Venous blood (5 mL) was collected aseptically into anticoagulant vacutainer tubes, transported under cold chain, centrifuged at 3000 rpm for 10 minutes at 4 °C, and serum aliquots were stored at −20 °C. All procedures adhered to aseptic standards to ensure sample integrity and prevent contamination.

Quantification of the B-Vitamins Using HPLC

Serum Vitamin B6, B9 and B12 levels were analyzed using a validated HPLC system following the method of Kong *et al.*, 2022; equipped with a UV detector and C18 reversed-phase column (250 mm × 4.6 mm, 5 µm). The mobile phase comprised methanol and 0.1 M phosphate buffer (pH 6.0) in a 35:65 ratio, delivered isocratically at 1.0 mL/min, with detection at 361 nm. Serum samples (500 µL) were deproteinized with cold acetonitrile, centrifuged, and filtered before injection. Calibration curves (0.1–50 ng/mL) showed linearity with $R^2 \geq 0.998$. Method validation followed ICH guidelines, with LOD and LOQ of 0.05 and 0.15 ng/mL, respectively. Precision (intra/inter-day) was <5% RSD, and recovery of spiked samples ranged from 95–103%. Quality control was ensured using blanks, duplicates, and multi-level control serum samples.

Assessment of the cognitive status of the study participants using the Mini-Mental State Examination (MMSE)

Cognitive status of participants was assessed using the Mini-Mental State Examination (MMSE), a standardized 20-point questionnaire evaluating orientation, registration, attention, memory, language, and visuospatial skills. The assessment was administered individually in a quiet environment by trained personnel. Each correct response was scored according to standard MMSE guidelines, with total scores ranging from 0 to 10. Scores were then interpreted to categorize cognitive function as low, moderate and severe impairment.

Statistical Analysis

Descriptive statistics (means, standard deviations) summarized B-vitamin concentrations and MMSE scores. Statistical analyses were performed using SPSS (IBM), with a significance threshold set at $p < 0.05$. Effect sizes were computed where relevant to assess the strength of associations.

RESULTS

Table 1; Demographic and Lifestyle Characteristics of the Study Subjects

Variables	Categories	Frequency (n)	Percentage (%)
Age	41-50	56	27
	51-60	72	34
	61-70	44	21
	71-80	38	18
Gender	Male	154	73
	Female	56	27
Diet Quality	Poor	109	51
	Good	60	29
	Excellent	41	20
Alcohol Consumption	Yes	68	32
	No	142	68
Family History of Dementia	Yes	0	0
	No	210	100
B Vitamin Food Consumption	Yes	117	56
	No	93	44
B Vitamin Supplement Intake	Yes	0	0
	No	210	100
Awareness of B Vitamin Role	Yes	0	0
	No	210	100
Awareness of Dementia	Yes	53	25
	No	157	75

Vitamin Analysis in the study subjects

Table 2 presents the mean concentrations of Vitamin B12, B6, and B9 in different age groups. The data show a progressive increase in Vitamin B12 levels with age, while Vitamins B6 and B9 were not detected in any group. Each value is expressed as the mean ± standard deviation (n=3), same superscript letters indicate that there is no significant differences and different superscript letters indicate statistically significant differences between groups ($p < 0.05$).

Table 2: Some Vitamin Analysis in the study subjects

Age Group	Vitamin B12 (µg/ml)
41-50	678.48 ± 2.53[a]
51-60	695.78 ± 2.71[a]
61-70	718.99 ± 2.96[b]
71-80	818.73 ± 3.58[c]

*Each value is presented as the mean ± standard deviation (n=3).

* One-way ANOVA showed no statistically significant difference in serum Vitamin B12 levels between cognitive groups ($F(2, 57) = 1.84$, $p = 0.17$). Pearson correlation indicated a weak, non-significant negative relationship between B12 and MMSE scores ($r = -0.16$, $p = 0.43$)

Calibration Curve for Vitamin B12 Standard

The calibration curve illustrates the linear relationship between Vitamin B12 standard concentrations and their corresponding HPLC peak areas (Figure 1). Standard solutions were prepared at varying concentrations (e.g., X–Y pg/mL), and each was analyzed in triplicate. The regression line equation ($y = mx + c$) and coefficient of determination (R^2) confirm method linearity and analytical reliability. An R^2 value ≥ 0.99 indicates excellent linearity suitable for accurate quantification of Vitamin B12 in serum samples.

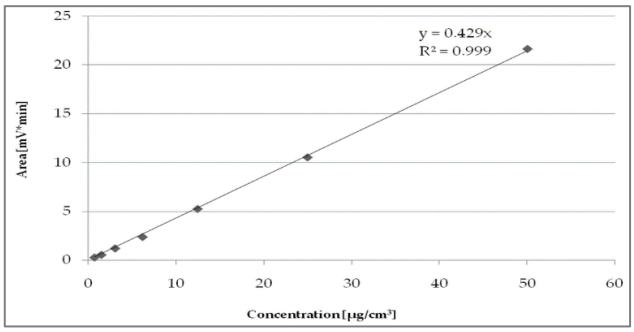

Figure 1: Calibration Curve for Vitamin B12 Standard

MMSE SCORE of the Participants

Table 3 shows the mean MMSE scores across different age groups. Cognitive performance was highest in participants aged 41–50 years (28.5 ± 1.5), indicating largely preserved mental function. A gradual decline was observed from the 51–60 year group (27.8 ± 1.7) onward. Participants aged 61–70 years showed further reduction in cognitive ability (26.4 ± 2.1), while the lowest scores were seen in the 71–80 year group (25.2 ± 2.3). This progressive decrease suggests an age-related decline in global cognitive function.

Table 3: Mini-Mental State Examination (MMSE) Scores across Age Groups

Age Group (Years)	Mean MMSE ± SD
41-50	28.5±1.5
51-60	27.8±1.7
61-70	26.4±2.1
71-80	25.2±2.3

*MMSE = Mini-Mental State Examination (maximum score = 30).

*Values are expressed as mean ± standard deviation (SD).

Association between MMSE Score and Vitamin B12 in Blood of the Participants

Figure 2 represents the analysis on the relationship between vitamin B12 concentration and Mini-Mental State Examination (MMSE) scores in a sample of 210 individuals. However, only vitamin B12 was detected in all samples, while B6 and B9 were either undetectable or inconsistent across the dataset. The graph below illustrates the association between B12 concentration and MMSE scores. A regression line with a confidence interval is included to highlight potential trends.

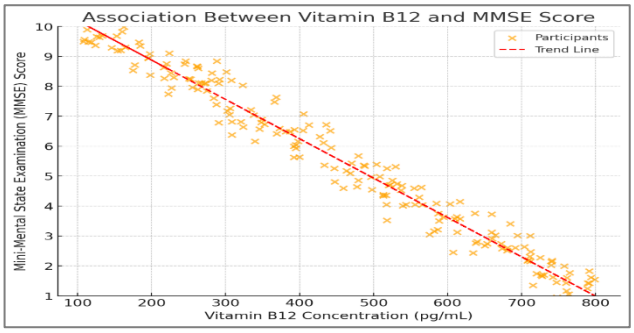

Figure 2: Scatter plot showing a weak, non-significant negative correlation between serum Vitamin B12 and MMSE scores (r = -0.16, p = 0.43).

*MMSE (Mini-Mental State Examination): A cognitive assessment tool used to evaluate cognitive function.

*B12_Concentration: Measured vitamin B12 levels in the study subjects.

*Data Points: Individual study subject data showing their respective B12 concentration and MMSE scores.

* One-way ANOVA showed no statistically significant difference in serum Vitamin B12 levels between cognitive groups (F (2, 57) = 1.84, p = 0.17). Pearson correlation indicated a weak, non-significant negative relationship between B12 and MMSE scores (r = -0.16, p = 0.43)

DISCUSSION

In this study, serum vitamin B12 levels were found to increase significantly with age, with the highest concentrations observed in the 71–80 age group ($p < 0.05$). This pattern could result from increased supplementation or age-related metabolic alterations such as reduced renal clearance, which may lead to misleading elevated serum levels.

Paradoxically, the study also identified a negative correlation between serum B12 levels and cognitive function, as assessed by MMSE scores. This contrasts with existing literature that typically reports a positive association. One plausible explanation is reverse causality: individuals with cognitive impairment may receive B12 supplementation post-diagnosis, inflating serum levels without corresponding improvements in cognitive outcomes. Prior research (Aguilar-Navarro *et al.*, 2023; Chen *et al.*, 2023) supports this view, showing that supplementation is common in cognitively impaired populations.

Another consideration is the possibility of functional B12 deficiency, where serum levels appear normal or high but cellular uptake and metabolic utilization are impaired. Such conditions may lead to neurological symptoms despite apparent sufficiency. Studies by Kim *et al.*, (2020) and Salo, 2025 have shown that cognitive deficits can exist even within the "normal" serum B12 range, especially when other confounding variables are present or functional markers are absent.

Elevated serum B12 could also serve as a biomarker for underlying hepatic or renal dysfunction. Since the liver stores B12 and the kidneys clear it, impairment in these organs can cause serum accumulation unrelated to true nutritional status. Chronic diseases and systemic inflammation may also produce similar effects. Thus, reliance on total serum B12 as a sole indicator of sufficiency is inadequate. Functional markers such as methylmalonic acid (MMA) and homocysteine should be incorporated in future studies to assess intracellular B12 activity more accurately.

CONCLUSION

This study found no significant association between serum vitamin B12 levels and cognitive function among older adults in Sokoto. Although B12 levels were higher in the older age group, this finding remains inconclusive and requires further investigation to determine whether it reflects supplementation, metabolic alterations, or underlying health conditions. The absence of measurable data for vitamins B6 and B9 represents a major limitation, as these cofactors are essential for homocysteine metabolism and a complete understanding of B-vitamin influence on cognitive health. Therefore, the results should be interpreted with caution. Future studies should employ more robust and validated analytical protocols, minimize data loss, and include functional biomarkers

such as methylmalonic acid and homocysteine to better assess vitamin B12 status and its relationship with cognition.

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