RESEARCH PAPER

Vitamin B12 Status in Adults with Type 2 Diabetes Mellitus Attending Regular Follow-Up: Age- And Sex-Specific Findings

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ABSTRACT
Vitamin B12 status may vary with age, particularly in adults with type 2 diabetes mellitus (T2DM). Data describing this pattern in Saudi populations remain limited. This cross-sectional study assessed serum vitamin B12 concentrations in 251 adults with T2DM attending regular follow-up at Qassim University, Saudi Arabia, from January to June 2026. Fasting serum vitamin B12 was measured by electrochemiluminescence immunoassay using the Roche Cobas Pro. Concentrations were classified as deficient (≤107 pmol/L), borderline (>107 to <133 pmol/L), or sufficient (≥133 pmol/L). Participants had a mean age of 59.45±13.17 years, 156 (62.2%) were female. Median vitamin B12 was 262.0 pmol/L (IQR, 200.5-368.5), values ranged from 80 to 823 pmol/L. Deficiency occurred in 2.0% of participants, 4.8% had borderline concentrations, and 93.2% were sufficient. Overall, 6.8% had low vitamin B12 (<133 pmol/L). Concentrations differed significantly among age groups (p<0.001). The median was 634.0 pmol/L at 18-39 years, compared with 249.0 and 252.0 pmol/L at 60-69 and ≥70 years, respectively. Age correlated inversely with vitamin B12 (ρ=-0.223, p<0.001), including among males (ρ=-0.245, p=0.013) and females (ρ=-0.193, p=0.018). In adjusted analysis, neither age per 10-year increase (OR, 1.51; 95% CI, 0.94-2.43; p=0.091) nor female sex (OR, 2.27; 95% CI, 0.72-7.23; p=0.164) was independently associated with low vitamin B12. Thus, biochemical deficiency was uncommon in this T2DM cohort, despite lower circulating vitamin B12 concentrations with increasing age. Assessing the continuous concentration alongside laboratory-defined categories may better characterize vitamin B12 status in adults with T2DM.
Keywords: *Vitamin B12; Cobalamin deficiency; Nutritional biomarker;Type-2 Diabetes; Neurological health*
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INTRODUCTION

Vitamin B12 (cobalamin) is an essential water-soluble micronutrient that serves as a cofactor for methionine synthase and methylmalonyl-CoA mutase, enzymes that are indispensable for DNA synthesis, erythropoiesis, one-carbon metabolism, and normal neurological function. [1-3] Through its role in methionine regeneration and methyl-group transfer, vitamin B12 contributes to nucleic acid synthesis, cellular proliferation, epigenetic regulation, and maintenance of myelin integrity. [2-5] Inadequate vitamin B12 status may therefore result in a broad spectrum of hematological, neurological, cognitive, and metabolic abnormalities, ranging from asymptomatic biochemical deficiency to megaloblastic anemia, peripheral neuropathy, neuropsychiatric manifestations, and irreversible neurological impairment when diagnosis or treatment is delayed. [4] Because body stores are relatively large, clinical manifestations often develop gradually, and biochemical deficiency may remain undetected for prolonged periods before overt symptoms become apparent. [3,4] Consequently, early identification of low vitamin B12 status has become an important component of preventive nutritional assessment and clinical practice.

Vitamin B12 deficiency represents a global public health concern affecting populations across both high- and low-income countries, although reported prevalence varies considerably according to age distribution, dietary habits, socioeconomic factors, assay methodology, and the diagnostic thresholds applied.[6] Population-based studies from Europe have demonstrated that vitamin B12 deficiency becomes increasingly common with advancing

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age, with prevalence estimates ranging from approximately 5% to over 20% depending on the biochemical definition employed.[7-10] Studies from South Asia and the Middle East have reported substantially higher frequencies of biochemical vitamin B12 deficiency, largely reflecting dietary patterns, vegetarian practices, nutritional transitions, and differences in laboratory cutoffs.[11-14] For example, community-based investigations in India have documented deficiency rates exceeding one-third of apparently healthy adults, whereas studies from Iran and Iraq have similarly reported a substantial burden of low vitamin B12 concentrations among otherwise healthy populations.[12,13] These marked differences highlight that direct comparison of prevalence estimates across studies is challenging because serum vitamin B12 concentrations are influenced by demographic characteristics, nutritional intake, laboratory methodology, and locally adopted reference intervals.

Age is among the most consistently investigated determinants of vitamin B12 status, yet published findings remain heterogeneous.[6-10] Advancing age is frequently associated with declining vitamin B12 concentrations owing to reduced gastric acid secretion, food-bound cobalamin malabsorption, atrophic gastritis, reduced intrinsic factor activity, polypharmacy, and increasing gastrointestinal disorders that impair vitamin B12 absorption.[2,3,6,7.] Nevertheless, several population-based investigations have demonstrated variable age-related patterns, with some reporting progressive declines in serum vitamin B12 among older adults, whereas others observed minimal age-associated differences or even relatively greater deficiency among younger adults because of dietary insufficiency.[8-13] Similarly, evidence regarding sex differences remains inconsistent. Large cohort studies have generally reported lower serum vitamin B12 concentrations among men than women, although these observations have not been replicated uniformly across different populations. [13,15,16] Such inconsistencies likely reflect differences in dietary habits, supplement use, lifestyle characteristics, healthcare utilization, laboratory methodology, and population structure rather than true biological variation alone.

Interpretation of vitamin B12 status is further complicated by substantial methodological variability in laboratory assessment and diagnostic classification. [17-22] Although total serum vitamin B12 remains the most widely used first-line biomarker because of its accessibility and cost-effectiveness, no universally accepted biochemical cutoff exists for defining deficiency. [17-20]. International guidelines recommend interpreting serum vitamin B12 concentrations within the context of clinical presentation and, where appropriate, complementary biomarkers such as methylmalonic acid, homocysteine, or holotranscobalamin to improve diagnostic accuracy. [17,19-22] Furthermore, considerable variation exists among laboratories regarding the definition of deficient, borderline, and sufficient vitamin B12 status, contributing to substantial heterogeneity in reported prevalence estimates across epidemiological studies.[18-22] Consequently, transparent reporting of laboratory-specific reference intervals is essential for appropriate interpretation and comparison of study findings.

Despite extensive epidemiological research, several important gaps remain. Many previous investigations have focused primarily on estimating overall prevalence or have examined selected population groups, such as older adults, healthcare workers, women of reproductive age, or individuals with specific clinical conditions.[7-15] Relatively few studies have simultaneously characterized the continuous distribution of serum vitamin B12 concentrations, laboratory-defined deficient and borderline categories, age-specific prevalence across multiple adult age groups, sex-stratified biomarker distributions, and the independent association of demographic characteristics with low vitamin B12 status within a single adult cohort using clearly defined laboratory reference intervals. A comprehensive evaluation integrating descriptive epidemiology with quantitative assessment of age- and sex-related variation may therefore provide a more informative understanding of vitamin B12 status than prevalence estimates alone.

Accordingly, the present study aimed to characterize serum vitamin B12 concentrations among adults using standardized laboratory-specific reference intervals. Specifically, the study sought to determine the prevalence of deficient, borderline, low, and sufficient vitamin B12 status, evaluate age- and sex-related differences in serum vitamin B12 concentrations, examine the association between age and serum vitamin B12 levels using correlation and regression analyses, and describe the distribution of serum vitamin B12 concentrations across empirical quartiles. By providing an integrated assessment of vitamin B12 status within an adult population, this study seeks to contribute clinically relevant epidemiological evidence that may support nutritional surveillance and facilitate interpretation of laboratory-based vitamin B12 assessment.

MATERIALS AND METHODS

Study design and setting: Analytical cohort comprised 251 participants with confirmed T2DM attending regular follow-up were screened for vitamin B12 status at Qassim University, Kingdom of Saudi Arabia, from January through June 2026. The analysis focused on serum vitamin B12 among adults with established type 2 diabetes mellitus (T2DM) presenting for regular follow-up. Vitamin B12 was examined quantitatively and according to laboratory-defined categories, with age and sex considered the principal demographic characteristics of interest. Ethical approval was obtained from the Committee of Research Ethics, Deanship of Graduate Studies and Scientific Research, Qassim University, Kingdom of Saudi Arabia (IRB No. 25-11-02, November 17, 2025) before recruitment. Each participant provided written informed consent. Study data were anonymized to protect participant confidentiality. The research was

conducted in accordance with the principles of the Declaration of Helsinki and subsequent amendments.

Study population and eligibility criteria: Patients with established T2DM presenting for regular follow-up during the recruitment period were screened consecutively. Inclusion required an age of ≥18 years, documented T2DM, consent to participate, and availability of a fasting serum sample with a vitamin B12 result. Records lacking essential demographic or laboratory information, those without a vitamin B12 measurement, and duplicate entries were omitted. The resulting analytical cohort comprised 251 participants.

Sample size: Recruitment covered all eligible participants encountered during the prespecified study interval; therefore, the analytical sample was determined by the number meeting the study criteria rather than by a prospective sample-size calculation. The final dataset contained 251 adults with T2DM.

Data collection: Age and sex were documented at enrolment, together with confirmation of eligibility and established T2DM status. Blood was drawn after an overnight fast by trained personnel. Specimens were subsequently processed for laboratory testing in the clinical laboratory at Qassim University.

Serum vitamin B12 measurement: Serum vitamin B12 was quantified by electrochemiluminescence immunoassay (ECLIA) using the Roche Cobas Pro analyzer (Roche Diagnostics, Mannheim, Germany). Testing and instrument calibration followed the manufacturer's analytical specifications. Routine internal controls were used to monitor assay performance. The manufacturer's interpretive limits were used: ≤107 pmol/L for deficiency, >107 to <133 pmol/L for the borderline range, and ≥133 pmol/L for sufficiency.

Definition of vitamin B12 status: Vitamin B12 was analyzed both quantitatively and categorically. Participants with concentrations ≤107 pmol/L were designated deficient, those with values >107 but <133 pmol/L were designated borderline, and values ≥133 pmol/L were considered sufficient. For binary regression, deficiency and borderline status were combined to define low vitamin B12 (<133 pmol/L).

Study variables: Serum vitamin B12 concentration constituted the primary study outcome. Age and sex were the main explanatory variables. In addition to analysis as a continuous measure, age was grouped as 18-39, 40-49, 50-59, 60-69, and ≥70 years. Vitamin B12 values were also divided empirically into quartiles to explore the distribution of participant characteristics across progressively higher concentrations.

Quality assurance: Trained personnel handled specimen collection, processing, laboratory testing, and data recording under established laboratory procedures. Assay performance was monitored through routine instrument calibration and internal quality-control procedures. Before analysis, the dataset was checked for missing observations, duplicate entries, internal inconsistencies, and data-entry errors.

Statistical analysis: Data were analyzed with IBM SPSS Statistics version 27.0 (IBM Corp., Armonk, NY, USA). Distributional normality of continuous variables was examined using the Shapiro-Wilk test. Data showing an approximately normal distribution are summarized as mean ± standard deviation (SD); non-normally distributed data are expressed as median and interquartile range (IQR). Categorical data are reported as n (%), with 95% confidence intervals (CIs) where relevant. Differences in serum vitamin B12 across age categories were examined with the Kruskal-Wallis test. Categorical comparisons used the chi-square test or Fisher's exact test when cell frequencies required it. The monotonic association between age and vitamin B12 concentration was quantified using Spearman's rank correlation (ρ). Binary logistic regression was used to examine the associations of age and sex with low vitamin B12 status (<133 pmol/L). Age was entered as a continuous predictor and expressed per 10-year increase, consistent with the reported regression estimates. Both unadjusted and mutually adjusted odds ratios (ORs) with 95% CIs were estimated. Statistical tests were two-sided, with $P<0.05$ denoting statistical significance.

RESULTS

Participant characteristics: The analysis comprised 251 participants, including 102 males (40.6%) and 149 females (59.4%). Mean age was 59.45±13.17 years, and the median was 62.0 years (IQR, 52.5-68.0). Age was comparable between males and females (58.89±12.74 vs 59.83±13.49 years; P=0.576; Cohen's d=-0.07). Serum vitamin B12 averaged 306.59±161.47 pmol/L, with a median of 262.0 pmol/L (IQR, 200.5-368.5). Median concentrations were 265.0 pmol/L in males and 260.0 pmol/L in females (P=0.407; rank-biserial correlation=0.06). Of the 251 participants, 5 (2.0%) were deficient, 12 (4.8%) were borderline, and 234 (93.2%) were sufficient. Low vitamin B12 (<133 pmol/L) occurred in 4/102 males (3.9%) and 13/149 females (8.7%); the difference was not significant (P=0.201; OR=0.43, 95% CI, 0.13-1.45) (Table 1).

Table 1: Baseline demographic characteristics and serum vitamin B12 status of the study participants according to sex

Characteristic	Overall (N=251)	Male (n=102)	Female (n =149)	P value	Effect size
Age (years)					
Mean ± SD	59.45 ± 13.17	58.89 ± 12.74	59.83 ± 13.49	0.576	Cohen's *d* = -0.07
95% CI of mean	57.82-61.08	56.39-61.39	57.65-62.01	-	-
Median (IQR)	62.0 (52.5-68.0)	61.0 (49.2-68.0)	62.0 (54.0-68.0)		-
Range	19-85	21-85	19-84	-	-
Serum vitamin B12 (pmol/L)					
Mean ± SD	306.59 ± 161.47	312.49 ± 154.35	302.56 ± 166.57	-	-
95% CI of mean	286.55-326.63	282.18-342.80	275.59-329.53	-	-
Median (IQR)	262.0 (200.5-368.5)	265.0 (208.5-392.8)	260.0 (192.0-354.0)	0.407	Rank-biserial correlation = 0.06
Range	80-823	86-823	80-775		
Vitamin B12 status					
Deficient (≤107 pmol/L)	5 (2.0)	2 (2.0)	3 (2.0)	0.201	Cramer's V=0.12
Borderline (>107–<133 pmol/L)	12 (4.8)	2 (2.0)	10 (6.7)		
Sufficient (≥133 pmol/L)	234 (93.2)	98 (96.1)	136 (91.3)		
Low vitamin B12 status†	17 (6.8)	4 (3.9)	13 (8.7)	0.201	OR=0.43 (95% CI 0.13-1.45)

†Low vitamin B12 status includes participants classified as deficient or borderline

Prevalence of vitamin B12 status: Deficiency was present in 2.0% (95% CI, 0.6-4.6) of the cohort, while 4.8% (95% CI, 2.5-8.2) fell within the borderline range. Combining these categories yielded a prevalence of 6.8% (95% CI, 4.0-10.6) for low vitamin B12. The remaining 93.2% (95% CI, 89.4-96.0) had sufficient concentrations. Borderline cases outnumbered deficient cases by 2.4:1 (Table 2). Figure 1 shows the right-skewed distribution of serum vitamin B12, with most values lying above the laboratory thresholds for low status.

Table 2: Overall prevalence of laboratory-defined serum vitamin B12 status among the study participants

Vitamin B12 status	Laboratory cutoff (pmol/L)	Participants, n	Prevalence (%)	95% CI (%)
Deficient	≤107	5	2.0	0.6-4.6
Borderline	>107 to <133	12	4.8	2.5-8.2
Low vitamin B12†	<133	17	6.8	4.0-10.6
Sufficient	≥133	234	93.2	89.4-96.0
Deficient-to-borderline ratio	-	1: 2.4	-	-

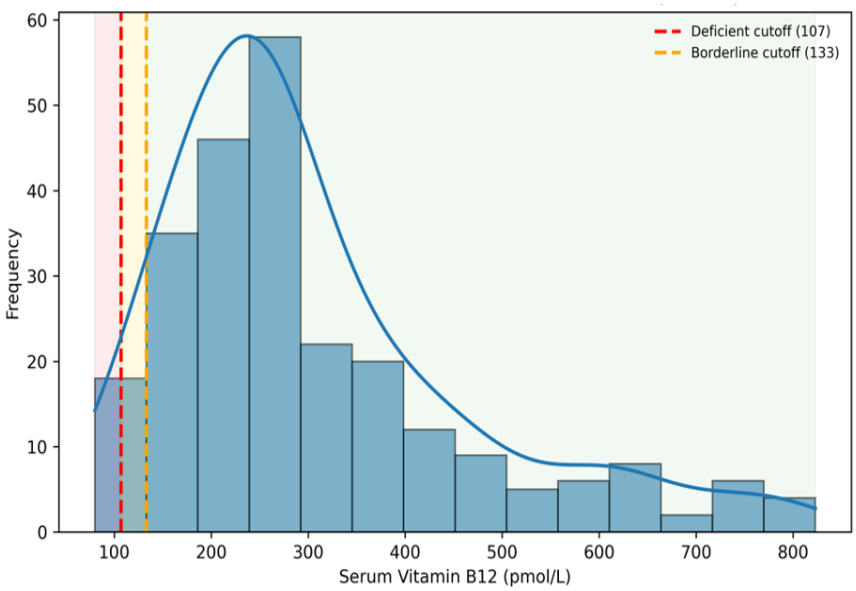

Figure 1: Distribution of serum vitamin B12 concentrations among the study participants

Vitamin B12 according to age: Vitamin B12 concentrations varied across the five age categories (P<0.001) (Table 3). The 18-39-year group had the highest median concentration at 634.0 pmol/L (IQR, 320.5-753.0). Corresponding medians were 265.5, 274.0, 249.0, and 252.0 pmol/L in the 40-49, 50-59, 60-69, and ≥70-year groups, respectively. None of the 18-39-year-old participants had low vitamin B12. Its prevalence was 5.6% at 40-49 years, 4.1% at 50-59 years, 8.1% at 60-69 years, and 10.2% at ≥70 years. These age-group differences in categorical low vitamin B12 were not significant (P=0.284). Figure 2 displays the age-specific proportions of deficient, borderline, and sufficient status (Table 3; Figure 2).

Table 3: Age-specific distribution of serum vitamin B12 concentrations and laboratory-defined vitamin B12 status among the study participants

Age group (years)	Participants n (%)	Serum vitamin B12 (pmol/L), Median (IQR)	Deficient n (%)	Borderline n (%)	Sufficient n (%)	Low vitamin B12 n (%)	95% CI (%)
18-39	18 (7.2)	634.0 (320.5-753.0)	0 (0.0)	0 (0.0)	18 (100.0)	0 (0.0)	0.0-18.5
40-49	36 (14.3)	265.5 (195.0-344.5)	1 (2.8)	1 (2.8)	34 (94.4)	2 (5.6)	0.7-18.7
50-59	49 (19.5)	274.0 (209.0-422.0)	0 (0.0)	2 (4.1)	47 (95.9)	2 (4.1)	0.5-14.0
60-69	99 (39.4)	249.0 (192.0-328.5)	3 (3.0)	5 (5.1)	91 (91.9)	8 (8.1)	3.6-15.3
≥70	49 (19.5)	252.0 (186.0-303.0)	1 (2.0)	4 (8.2)	44 (89.8)	5 (10.2)	3.4-22.2
P value		**<0.001**				**0.284**	

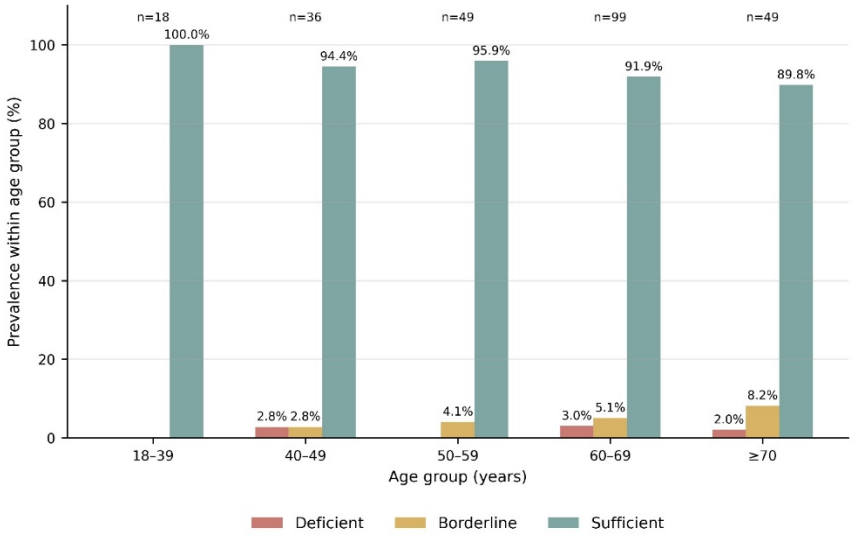

Figure 2: Age-specific distribution of laboratory-defined vitamin B12 status among the study participants

Correlation of age with serum vitamin B12: Age correlated inversely with serum vitamin B12 in the full cohort (ρ=-0.223; 95% CI, -0.345 to -0.096; P<0.001) (Table 4). The association was also evident when the sexes were analyzed separately: ρ=-0.245 (P=0.013) in males and ρ=-0.193 (P=0.018) in females. Figure 3 depicts the continuous relationship between age and serum vitamin B12 (Table 4; Figure 3).

Table 4: Spearman correlation between age and serum vitamin B12 concentration in the overall study population and according to sex

Analysis group	Independent variable	Dependent variable	Participants (n)	Spearman's ρ (95% CI)	P value
Overall	Age (years)	Serum vitamin B12 (pmol/L)	251	-0.223 (-0.345 to -0.096)	<0.001
Male	Age (years)	Serum vitamin B12 (pmol/L)	102	-0.245 (-0.417 to -0.056)	0.013
Female	Age (years)	Serum vitamin B12 (pmol/L)	149	-0.193 (-0.357 to -0.012)	0.018

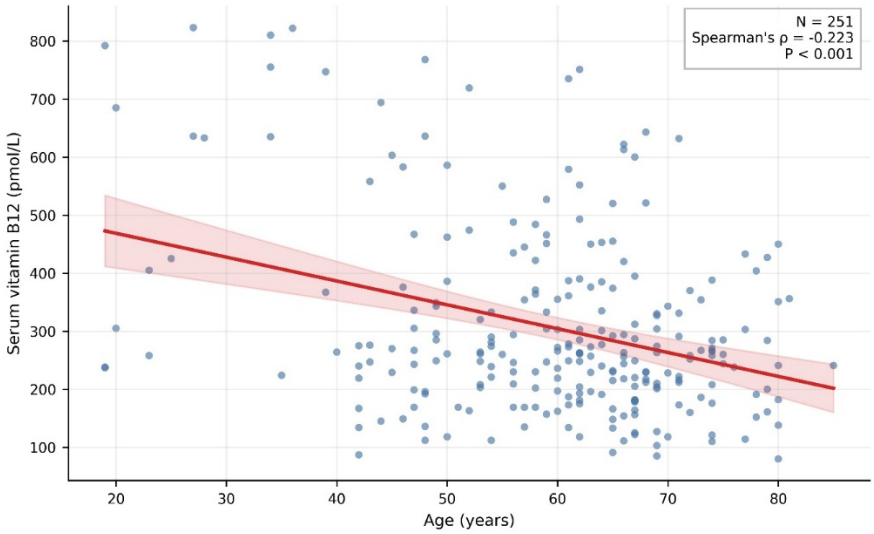

Figure 3: Association between age and serum vitamin B12 concentration among the study participants

Factors associated with low vitamin B12: In univariable logistic regression, the odds of low vitamin B12 increased by an estimated 52% per 10-year increment in age (OR=1.52; 95% CI, 0.95-2.43; P=0.080). Female participants had an OR of 2.34 relative to males (95% CI, 0.74-7.40; P=0.147). Neither estimate reached statistical

significance. Mutual adjustment produced similar estimates for age (adjusted OR=1.51; 95% CI, 0.94-2.43; P=0.091) and female sex (adjusted OR=2.27; 95% CI, 0.72-7.23; P=0.164) (Table 5).

Table 5: Binary logistic regression analysis of factors associated with low vitamin B12 status (<133 pmol/L)

Predictor	Unadjusted OR (95% CI)	P value	Adjusted OR* (95% CI)	P value
Age (per 10-year increase)	1.52 (0.95-2.43)	0.080	1.51 (0.94-2.43)	0.091
Female sex (vs. male)	2.34 (0.74-7.40)	0.147	2.27 (0.72-7.23)	0.164

Age-related distribution according to sex: Within-sex comparisons showed variation in serum vitamin B12 across age categories in males (Kruskal-Wallis H=13.06; P=0.011) and females (H=10.87; P=0.028) (Table 6). In both groups, the highest median concentration occurred at 18-39 years. Figure 4 presents the sex-stratified distributions across age categories, including their dispersion and outlying observations.

Table 6: Sex-specific distribution of serum vitamin B12 concentrations across age groups

Age group (years)	Male, n	Serum vitamin B12 (pmol/L), Median (IQR)	Female, n	Serum vitamin B12 (pmol/L), Median (IQR)
18-39	6	586.0 (410.0-753.0)	12	634.0 (253.0-711.8)
40-49	20	256.5 (213.3-337.8)	16	280.5 (180.3-401.3)
50-59	21	333.0 (208.0-462.0)	28	260.5 (218.0-328.5)
60-69	37	256.0 (201.0-361.0)	62	248.5 (188.0-309.8)
≥70	18	241.0 (214.5-284.8)	31	258.0 (168.5-347.0)
Kruskal-Wallis H statistic[a]	-	**13.06**	-	**10.87**
P value[a]	-	**0.011**	-	**0.028**

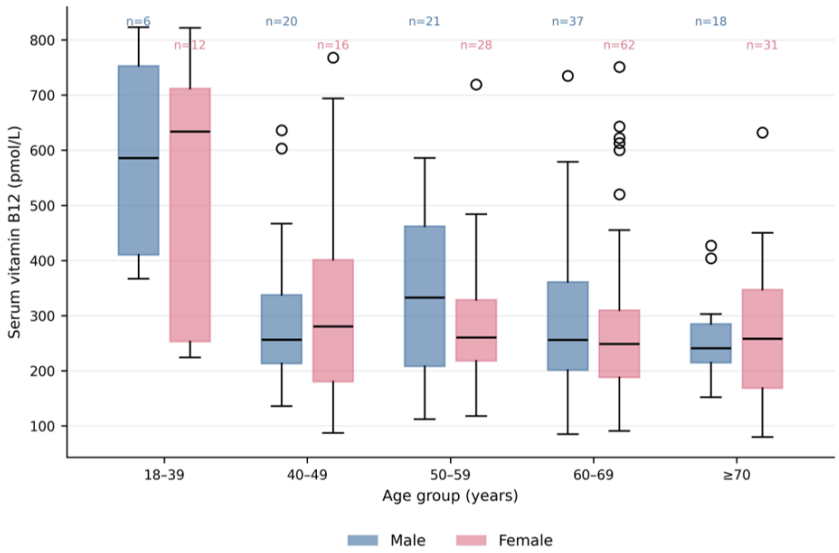

Figure 4. Sex-stratified distribution of serum vitamin B12 concentrations across predefined age groups

Distribution across vitamin B12 quartiles: Age differed across empirical vitamin B12 quartiles (P<0.001) (Table 7). Participants in the lowest quartile (80-200 pmol/L) had a median age of 65.0 years (IQR, 56.5-69.0), compared with 58.0 years (IQR, 46.0-65.0) in the highest quartile (370-823 pmol/L). Sex composition, however, was similar across quartiles (P=0.463) (Table 7).

Table 7: Participant characteristics according to empirical quartiles of serum vitamin B12 concentration

Characteristic	Q1 (Lowest) (80-200 pmol/L) n = 63	Q2(201-262 pmol/L) n = 63	Q3(263-367 pmol/L) n = 62	Q4 (Highest) (370-823 pmol/L) n = 63	P value
Age, years					
Median (IQR)	65.0 (56.5-69.0)	63.0 (54.0-68.5)	61.5 (54.0-69.0)	58.0 (46.0-65.0)	<0.001
Sex					

Male, n (%)	22 (34.9)	28 (44.4)	23 (37.1)	29 (46.0)	0.463
Female, n (%)	41 (65.1)	35 (55.6)	39 (62.9)	34 (54.0)	

DISCUSSION

This study comprehensively evaluated serum vitamin B12 status in an adult population by integrating continuous biomarker assessment with laboratory-defined categories, age- and sex-specific analyses, correlation, logistic regression, and empirical quartile analyses. Three principal findings emerged. First, overt vitamin B12 deficiency was relatively uncommon, with only 2.0% of participants classified as deficient and 6.8% demonstrating low vitamin B12 status. Second, despite the low prevalence of laboratory-defined deficiency, serum vitamin B12 concentrations declined consistently with advancing age, a pattern that was demonstrated across age-group comparisons, correlation analyses and empirical quartiles. Third, no statistically significant sex differences were identified in serum vitamin B12 concentrations or laboratory-defined deficiency, indicating that age rather than sex represented the predominant demographic factor influencing vitamin B12 distribution in this cohort. These findings reinforce the value of evaluating vitamin B12 as a continuous biomarker rather than relying exclusively on categorical diagnostic thresholds, as clinically relevant variation may occur well before concentrations reach conventional deficiency cutoffs.[23] Furthermore, population differences in vitamin B12 status are influenced by demographic characteristics, dietary habits, supplementation practices and regional nutritional patterns, emphasizing the importance of interpreting prevalence estimates within their specific epidemiological context.[24]

The vitamin B12 concentration of 262.0 pmol/L (IQR 200.5-368.5 pmol/L) indicates, generally adequate vitamin B12 status within the study population, although substantial inter-individual variability was observed. Such variability has been consistently reported in population-based studies and reflects differences in age, ethnicity, dietary intake, supplement use, comorbidities and analytical methodology.[25] Variations in laboratory reference intervals further complicate direct comparisons between studies because reference populations, assay calibration and exclusion criteria differ considerably across laboratories. [26] In addition, commercially available immunoassays may yield different absolute vitamin B12 concentrations despite analysing identical samples, highlighting the importance of interpreting biochemical results within the context of laboratory-specific reference intervals.[27]

Only five participants fulfilled the laboratory definition of vitamin B12 deficiency, while an additional 12 exhibited borderline concentrations. Consequently, the overall prevalence of low vitamin B12 status (6.8%) was considerably lower than that reported in several recent population-based studies. Among asymptomatic young women from Saudi Arabia, substantially higher frequencies of vitamin B12 deficiency have been described, reflecting differences in participant characteristics, dietary practices and diagnostic thresholds.[28] Likewise, community-based studies from South Asia have reported markedly greater prevalence of low vitamin B12 status, often attributed to lower consumption of animal-source foods and differing nutritional habits. [29-31] Conversely, studies conducted in European older populations have demonstrated prevalence estimates comparable to those observed in the present investigation when similar serum-based definitions were applied.[32] Considerable heterogeneity among published studies further reflects differences in study design, laboratory methodology and participant selection, underscoring the need for cautious comparison of prevalence estimates across populations. [33]

Although categorical deficiency was uncommon, age demonstrated a consistent association with serum vitamin B12 concentrations. Median vitamin B12 concentrations declined markedly from 634.0 pmol/L among participants aged 18-39 years to approximately 250 pmol/L in individuals aged 60 years and older, while the proportion of participants with low vitamin B12 status increased progressively across successive age groups. This age-dependent pattern was further supported by a significant inverse correlation between age and serum vitamin B12 concentration (ρ=-0.223, $p<0.001$), with comparable findings observed independently in both males and females. Importantly, the consistency of these findings across several complementary analytical approaches strengthens the inference that ageing influences circulating vitamin B12 concentrations even when overt biochemical deficiency remains relatively infrequent.

Several biological mechanisms may explain this progressive reduction in vitamin B12 concentrations with advancing age. Ageing is associated with reduced gastric acid secretion, impaired liberation of food-bound cobalamin, chronic atrophic gastritis, diminished intrinsic factor activity and reduced intestinal absorption, all of which contribute to lower circulating vitamin B12 concentrations. [34,35] In addition, dietary insufficiency, reduced consumption of animal-derived foods and gastrointestinal disorders become increasingly prevalent in older populations, further predisposing individuals to declining vitamin B12 status.[36] Nevertheless, total serum vitamin B12 should not be interpreted as a direct measure of intracellular vitamin availability because circulating vitamin B12 reflects several transport protein fractions and may not accurately represent metabolically active cobalamin.[37] Functional biomarkers such as methylmalonic acid, homocysteine and holotranscobalamin therefore provide important complementary information, particularly among individuals with borderline serum concentrations.[38] Moreover, differences in laboratory cutoffs used to define vitamin B12 deficiency substantially influence reported

prevalence and should be considered when comparing epidemiological studies across different settings.[39]

The age-related trend was further corroborated by the empirical quartile analysis, in which median age decreased progressively from 65.0 years in the lowest vitamin B12 quartile to 58.0 years in the highest quartile. This graded distribution demonstrates that the relationship between age and vitamin B12 extends across the entire concentration spectrum rather than being restricted to individuals meeting laboratory criteria for deficiency. Similar observations have been reported in population-based biomarker studies, where continuous analyses provided greater sensitivity for identifying age-related changes than binary classifications based solely on diagnostic cutoffs.[40] Furthermore, age-related alterations in functional biomarkers such as methylmalonic acid and homocysteine have been shown to occur independently of total serum vitamin B12, highlighting the complex physiological changes accompanying ageing and emphasizing the need for cautious interpretation of isolated serum vitamin B12 measurements. [41] Likewise, modelling studies have demonstrated that clinically meaningful changes in vitamin B12 metabolism occur across a continuum of circulating concentrations rather than at a single threshold, supporting the use of continuous biomarker analyses in epidemiological research. [42]

Although advancing age was consistently associated with lower circulating vitamin B12 concentrations, logistic regression did not demonstrate a statistically significant independent association between age and laboratory-defined low vitamin B12 status after adjustment for sex. Each 10-year increase in age was associated with approximately 50% higher odds of low vitamin B12 status, but the confidence interval included unity and statistical significance was not achieved. This apparent discrepancy is methodologically understandable because dichotomization of a continuous biomarker inevitably reduces statistical information, particularly when relatively few participants fulfil the predefined outcome. In the present study, only 17 participants were classified as having low vitamin B12 status, limiting statistical power and resulting in wide confidence intervals. Moreover, vitamin B12 status is influenced by numerous clinical and nutritional factors that were not incorporated into the regression model. Long-term metformin therapy remains one of the best-recognized causes of acquired vitamin B12 deficiency, particularly among individuals with type 2 diabetes mellitus.[43,44] Similarly, prolonged use of proton pump inhibitors has been associated with reduced vitamin B12 absorption, although recent evidence suggests that this association is modest and influenced by treatment duration, dosage and patient characteristics.[45] Dietary intake, vegetarian dietary patterns, gastrointestinal disorders, previous gastrointestinal surgery and vitamin supplementation also contribute substantially to vitamin B12 status and may partly explain the absence of an independent age effect in the adjusted analysis.[46] Consequently, current expert consensus recommends interpreting serum vitamin B12 alongside dietary history, medication exposure, clinical presentation and additional biochemical markers rather than relying solely on demographic characteristics. [47]

Sex-specific analyses demonstrated remarkably similar vitamin B12 profiles in males and females. Median serum vitamin B12 concentrations differed by only 5 pmol/L, and neither continuous concentrations nor laboratory-defined vitamin B12 categories showed statistically significant sex differences. Although females exhibited a numerically higher prevalence of low vitamin B12 status than males (8.7% versus 3.9%), the adjusted odds ratio was not statistically significant, indicating that the observed difference should be interpreted cautiously. Previous studies evaluating sex-related variation in vitamin B12 status have produced inconsistent findings. A large population-based investigation reported higher frequencies of vitamin B12 deficiency among males [48], whereas other clinical studies have similarly demonstrated greater biochemical abnormalities in men with vitamin B12-related disorders. [49] Differences in dietary habits, supplementation practices, reproductive physiology, healthcare utilization and underlying disease burden may partly account for these inconsistent observations across populations.[50] Importantly, both males and females in the present study demonstrated comparable age-related declines in vitamin B12 concentrations, suggesting that ageing exerted a similar influence irrespective of sex.

The quartile analysis provided additional evidence supporting the use of continuous biomarker assessment. Participants in the lowest vitamin B12 quartile were substantially older than those in the highest quartile, whereas sex distribution remained comparable across quartiles. This finding indicates that the observed age gradient reflects a genuine biological shift in circulating vitamin B12 rather than differences in sex composition. Increasingly, epidemiological studies have shown that clinically relevant associations may exist across the full distribution of vitamin B12 concentrations, even among individuals who do not satisfy conventional laboratory definitions of deficiency.[51,52] Accordingly, continuous analyses and distribution-based approaches may detect early nutritional changes that would remain undetected if vitamin B12 were evaluated solely as a dichotomous variable.

Overall, these findings have important clinical and public health implications. Although overt vitamin B12 deficiency was uncommon within this population, the consistent decline in circulating vitamin B12 across adulthood suggests that older individuals represent an important group for nutritional surveillance. Contemporary laboratory guidance recognizes total serum vitamin B12 as an appropriate first-line biomarker but recommends additional assessment using methylmalonic acid, homocysteine or holotranscobalamin when results are borderline or inconsistent with clinical findings. [53] Interpretation is further complicated by the absence of universally accepted diagnostic cutoffs, as laboratory

reference intervals and assay methodologies differ substantially between institutions.[54] Current NICE guidance similarly recommends integrating biochemical findings with clinical features, dietary history, medication exposure and conditions associated with malabsorption before establishing a diagnosis of vitamin B12 deficiency. [55] Consequently, population-based studies should ideally combine continuous biomarker analyses with functional markers and comprehensive clinical assessment to provide a more complete understanding of vitamin B12 status. The integrated interpretation of the principal findings and their relationship with current evidence is summarized in Table 8.

Table 8: Integrated interpretation of the principal findings of the present study in the context of current evidence

Principal finding	Evidence from the present study	Supporting literature	Integrated interpretation	Clinical implication
Overall serum vitamin B12 distribution	Median serum vitamin B12 concentration was 262.0 pmol/L (IQR: 200.5-368.5 pmol/L), with a broad distribution (80-823 pmol/L).	Population-based studies have consistently reported substantial inter-individual variability in circulating vitamin B12 concentrations influenced by demographic, dietary, genetic and analytical factors [23-27].	Circulating vitamin B12 demonstrates considerable biological variability across apparently healthy adults, supporting evaluation as a continuous biomarker rather than a dichotomous variable.	Continuous assessment of serum vitamin B12 provides a more informative evaluation of population nutritional status than reliance on categorical deficiency alone.
Population prevalence of low vitamin B12 status	Vitamin B12 deficiency was identified in 2.0%, borderline status in 4.8%, and overall low vitamin B12 status in 6.8% of participants.	Published prevalence estimates vary substantially across populations because of differences in participant characteristics, dietary habits, laboratory methodology and diagnostic thresholds [28-33].	The relatively low prevalence observed in the present study likely reflects characteristics of the study population rather than an absence of nutritional vulnerability.	Population-specific epidemiological data are essential for designing appropriate nutritional surveillance and prevention strategies.
Age-related variation in serum vitamin B12	Median vitamin B12 concentration declined from 634.0 pmol/L among participants aged 18–39 years to approximately 250 pmol/L in participants aged ≥60 years ($p<0.001$).	Age-associated reductions in vitamin B12 have been attributed to impaired gastric acid secretion, reduced intrinsic factor-mediated absorption, gastrointestinal disorders and dietary changes accompanying ageing [34-39].	Advancing age is associated with a progressive reduction in circulating vitamin B12 concentrations that may precede overt biochemical deficiency.	Older adults may benefit from closer nutritional surveillance, particularly when additional clinical or dietary risk factors are present.
Association between age and circulating vitamin B12	A modest inverse correlation was observed between age and serum vitamin B12 ($\rho=-0.223$; $p<0.001$), with similar findings in males and females.	Large epidemiological studies demonstrate comparable age-dependent reductions in vitamin B12 concentrations together with progressive alterations in functional biomarkers [40-42].	The influence of age extends across the entire biological distribution of vitamin B12 rather than being confined to individuals meeting laboratory criteria for deficiency.	Continuous biomarker analyses improve understanding of age-associated nutritional changes and may facilitate earlier identification of declining vitamin B12 status.
Factors associated with low	Logistic regression demonstrated a non-significant	Vitamin B12 deficiency is multifactorial and	Progression from declining circulating vitamin B12	Future epidemiological studies should incorporate dietary,

vitamin B12 status	trend towards higher odds of low vitamin B12 with advancing age (adjusted OR 1.51; 95% CI 0.94-2.43; p=0.091). Female sex was not independently associated with low vitamin B12 status.	influenced by medications, gastrointestinal disease, dietary intake, supplementation and other clinical determinants beyond age and sex [43-47].	concentrations to overt biochemical deficiency is likely influenced by multiple interacting biological and clinical factors rather than demographic characteristics alone.	pharmacological, gastrointestinal and lifestyle variables into multivariable prediction models.
Sex-specific findings	No statistically significant differences were observed between males and females in serum vitamin B12 concentrations or laboratory-defined vitamin B12 status.	Previous studies have reported inconsistent sex-related differences, suggesting that observed associations vary according to population characteristics, lifestyle factors and clinical setting [48-50].	Biological sex appears to have a relatively limited influence on vitamin B12 status after accounting for other determinants.	Screening strategies should prioritize established clinical and nutritional risk factors rather than sex alone.
Quartile analysis	Older participants were significantly overrepresented in the lowest serum vitamin B12 quartile (P<0.001), whereas sex distribution remained comparable across quartiles.	Distribution-based analyses demonstrate that clinically relevant information may be identified across the full range of circulating vitamin B12 concentrations rather than only below conventional diagnostic thresholds [51,52].	Quartile analysis confirmed a gradual age-associated downward shift in circulating vitamin B12 across the population.	Population studies should evaluate serum vitamin B12 as a continuous biomarker alongside conventional categorical classification.
Diagnostic interpretation	Continuous analyses demonstrated significant age-associated reductions in circulating vitamin B12 despite the relatively low prevalence of laboratory-defined deficiency.	Recent laboratory recommendations advocate combining serum vitamin B12 with functional biomarkers such as methylmalonic acid or homocysteine when clinically indicated [47,53-55].	Total serum vitamin B12 is an appropriate first-line screening biomarker but should be interpreted alongside clinical findings and, where appropriate, complementary functional biomarkers.	A multimarker diagnostic approach incorporating serum vitamin B12 and functional biomarkers may improve early identification of clinically relevant vitamin B12 deficiency and support personalized nutritional management.

A key strength of this study was the complementary evaluation of serum vitamin B12 using continuous concentrations, laboratory-defined categories, age- and sex-specific analyses, correlation, logistic regression, and empirical quartiles, providing a detailed characterization of vitamin B12 status in adults with T2DM. However, the cross-sectional design precludes causal inference, and the small number of participants with low vitamin B12 status limited the precision of multivariable estimates. Information on dietary intake, vitamin supplementation, gastrointestinal disorders, renal function, and medication exposure, particularly metformin use, was unavailable and therefore could not be incorporated into the analysis. Functional biomarkers, including methylmalonic acid, holotranscobalamin, and homocysteine, were not measured. Finally, the single-center design and inclusion of patients with T2DM attending regular follow-up may limit generalizability to other populations.

Conclusion: Laboratory-defined vitamin B12 deficiency was uncommon among adults with T2DM attending regular follow-up; however, serum vitamin B12 concentrations were consistently lower with advancing age across complementary analyses. These findings highlight age-associated variation in vitamin B12 status within this T2DM cohort while emphasizing that serum concentrations provide information beyond categorical deficiency alone. Prospective multicenter studies incorporating medication exposure, dietary factors, and functional biomarkers are needed to clarify the clinical

significance and determinants of vitamin B12 status in adults with T2DM.

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