Association between low vitamin B-12 status and latent tuberculosis infection among adults

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ABSTRACT

Background and Objectives: Tuberculosis (TB) remains a major public health threat worldwide, but most of the presumed infected individuals remain asymptomatic and contain *Mycobacterium tuberculosis* (*MTB*) in a latent tuberculosis infection (LTBI), and some of them will progress to active tuberculosis. Vitamin B-12 is crucial to maintain immune function, and play a role in the metabolism of MTB, while few studies investigated the impact of vitamin B-12 deficiency on tuberculosis. Therefore, we carried out the study to explore the association between vitamin B-12 deficiency and LTBI using the National Health and Nutrition Examination Surveys (NHANES). **Methods and Study Design:** A cross-sectional study was conducted by using data from NHANES 2011-2012. Adults (aged ≥18 years) who had available data on serum Vitamin B-12, serum Methylmalonic Acid (MMA) and QuantiFERON-TB Gold In-Tube (QFT-GIT) results were included in the analysis. Multivariable logistic regression was used to assess the association between Vitamin B-12 deficiency and LTBI. **Results:** A total of 4773 subjects were included in the present study, of whom 479 were screened as LTBI. The LTBI group had a higher proportion of participants with low Vitamin B-12 status. After adjusting for the possible confounders, Vitamin B-12 deficiency was independently associated with a 37% increased odds ratio of LTBI in the participants (OR: 1.37; 95% CI: 1.01-1.85). Similar correlations remained in subjects aged ≥35 years and female subjects by further stratified analysis. **Conclusions:** Vitamin B-12 deficiency was significantly associated with higher prevalence of LTBI in US adults. Maintenance of optimal Vitamin B-12 status has potential benefits for LTBI prevention. Future studies are needed to assess the roles and clinical implications of Vitamin B12 in *MTB* infection.

Key Words: vitamin B12, methylmalonic acid, latent tuberculosis infection, adults, National Health and Nutrition Examination Survey

INTRODUCTION

Tuberculosis (TB) remains a major public health threat worldwide, among the leading cause of death and its associated mortality is the highest in infectious diseases. According to the Global Burden of Disease Tuberculosis Collaborators, there were approximately 6.4 million new incident TB patients in 2021 around the world.[1] Several strategies have been developed to eradicate TB, and to identify individuals at high risk in the early stage may contribute a lot.[2]

Mycobacterium tuberculosis (*MTB*) is one of the most challenging pathogens at present, resulting in significant difficulty in clinical treatment. After infecting a host and progressing through the active stage of an infection, *MTB* settles into a dormant state that can last for decades, which is called latent tuberculosis infection (LTBI). When an individual with LTBI became immunocompromised, the infection would be reactivated and active tuberculosis occurs. Although they themselves are both asymptomatic and non-infectious, individuals with LTBI represent a reservoir of MTB infection.[3, 4] Therefore, reducing this reservoir through targeted testing and corresponding treatment may contribute to TB control, especially concerning the public health issue.

Vitamin B-12, an essential water-soluble vitamin with a complex molecule structure, plays a crucial role in DNA synthesis and normal cell growth which may be of significance to maintain immune function while impaired host immunity increased the risk of active tuberculosis. Notably, methylmalonic acid (MMA), a byproduct of propionate metabolism pathway, serves as a functional marker of vitamin B-12 status, offering a more accurate assessment than serum vitamin B-12 levels alone.[5] Elevated serum MMA concentrations reflect vitamin B-12 deficiency with high specificity, as a deficiency in vitamin B-12, dysregulation of the factors involved in its uptake, transport, and processing, as well as increased flux through the propionate pathway beyond its enzymatic capacity lead to MMA accumulation.[6] In addition, vitamin B-12 has been reported to be particularly important in the metabolism of *MTB*.[7, 8] Although only a few studies are available, in Chinese Han and Korean populations, vitamin B-12 levels were found to have significant clinical relevance of tuberculosis, significantly lower in TB patients than controls.[9,10] Detecting serum vitamin B-12 levels could be used as an effective measure for identifying active TB, as well as monitoring the efficacy of TB treatment.[11] Furthermore, several SNPs in vitamin B-12 metabolic genes such as *TCN2*, *CUBN*, and *MUT* were reported to be associated with important clinical manifestations in TB including hypoproteinemia, risk of drug resistance, and sputum smear-positive.[9]

Mounting evidences support the interaction between nutrition and MTB infection, and individual nutritional status is one of the most important determinants of immune response to the bacteria. Micronutrients, such as vitamin A, D, and E, have been reported to play important roles in maintaining immune function under various infectious diseases, including tuberculosis,[12, 13] and the levels of which have been found to be significantly lower in TB patients.[14] However, the association between vitamin B-12 deficiency and *MTB* infection, the progress to active tuberculosis has rarely been reported.

The National Health and Nutrition Examination Survey (NHANES) is a large, representative, population-based survey which provides estimates of various disease prevalence in the United States. The 2011-2012 NHANES contained the result of LTBI based on the tests of tuberculin skin test (TST) and Interferon-Gamma Release Assay (IGRA), the QuantiFERON-TB Gold In-Tube (QFT-GIT), as well as vitamin B-12 status. Thus, we conducted this study to investigate the association between vitamin B-12 deficiency and LTBI using the data, aimed to investigate the potential benefits for LTBI prevention by maintaining optimal vitamin B-12 status.

MATERIALS AND METHODS

Study population

Data from NHANES survey (2011-2012) with QFT-GIT tests were utilized. The NHANES collected information regarding health and nutrition which are released to the public every 2 years. Using a complex, stratified sampling design, it selected representative samples of US non-institutionalized civilians including Mexican Americans, African Americans, older adults, and those with a lower socioeconomic status. Due to the availability of data for QFT-GIF in NHANES cycle 2011-2012, vitamin B-12 and methylmalonic acid (MMA) in NHANES cycles 2011-2012 and 2013–2014, finally, data from NHANES cycle 2011-2012 was analyzed in the present study. We included participants with available results of QFT-GIT, as well as serum vitamin B-12 and MMA.

This cycle included 9756 subjects, of whom 5864 aged 18 years and older. Of them, 632 were excluded due to one or more missing values among the three required measurements in QFT-GIT (TB antigen, mitogen, nil) or indeterminate QFT-GIT results. Among the 5232 persons remained, participants with active TB (N=33), missing data on serum MMA (N=604), missing data on serum vitamin B-12 (N=3), suffering from AIDS (N=17) were also excluded. Finally, 4773 participants were included in the analysis. The flow chart of participant inclusion is shown in Figure 1.

NHANES study protocols were approved by the Institutional Review Board of the National Center of Health Statistics (NCHS), and written informed consent was obtained from all participants prior to participation in the study. All data were publicly available and de-identified and therefore determined exempt from institutional ethical review board review.

Assessment of LTBI and vitamin B-12 deficiency

The primary outcome of interest in the present study is the presence of LTBI in the population. In the 2011-2012 NHANES, LTBI was tested by tuberculin skin test (TST) and IGRA, the QuantiFERON-TB Gold In-Tube (QFT-GIT). In 2017, the US Centers for Disease Control and Prevention (CDC), American Thoracic Society, and Infectious Diseases Society of America jointly published the clinical practice guidelines for LTBI testing, recommending IFN-γ release assays over TST in people aged 5 years and older.[15] Therefore, we defined LTBI based on the result of QFT-GIT in the present study, and QFT-GIF was performed and interpreted in accordance with CDC guidelines for the use of IGRA.[16]

MMA is one of the metabolites accumulating in vitamin B12-deficient cells, and blood concentrations of MMA are functional markers of vitamin B-12 status.[17] Hence, MMA concentration was used to evaluate vitamin B-12 status of the individuals in the present study. Also, the results of serum vitamin B-12 levels were included. MMA was analyzed using LC-MS/MS, and Vitamin B-12 was measured using an electrochemiluminescence immunoassay.[18] Vitamin B-12 deficiency was defined as serum vitamin B-12 concentration <150 pmol/L or serum MMA concentration >271 nmol/L.[19]

Covariates

The following variables were assessed for confounders: age, sex, marital status, country of birth, educational levels, body mass index (BMI), renal function, smoking status and poverty income ratio (PIR). BMI was calculated as weight in kilograms divided by height in meters squared, based on body measurements data collected in the Mobile Examination Center (MEC). The participants were classified as underweight or normal weight (BMI <25.0 kg/m^2), overweight (BMI 25.0- 29.9 kg/m^2), class 1 obesity (30.0-34.9 kg/m^2), and class 2+ obesity (≥35 kg/m^2).[20] We defined categories of normal renal function based on previously published guidelines: Estimated Glomerular Filtration Rate (eGFR) ≥60 mL/min/1.73m^2 and Albumin-to-Creatinine Ratio (ACR) <30 mg/g.[21] Smoking status was evaluated based on serum cotinine concentration, measured using an isotope dilution-high performance liquid chromatography/atmospheric pressure chemical ionization tandem mass spectrometry (ID HPLC-APCI MS/MS). We classified participants with cotinine levels of <0.05, 0.05-3.00, and ≥3.00 ng/mL based on the data distribution. Also, serum cotinine level of 0.05 ng/mL was the lower limit value of secondhand smoking. Those with a serum cotinine level of <0.05 ng/mL were considered as non-smokers.[22] PIR represented the annual earnings relative to poverty

index based on family size and was classified as <1 and ≥1, poverty group (PIR <1) and non-poverty group (PIR ≥1).[23]

Statistical analysis

We treated responses coded as "don't know", "refused", or "missing" in the original NHANES data as missing. Kolmogorov-Smirnov normality tests were used to test the normality of continuous variables and mean ± standard deviation was used to describe normal distributed variables, median (P25, P75) for non-normal distributed variables. For the current study, the participants were categorized into two groups (LTBI or Non-LTBI) according to the results of QFT-GIT. Student's t-test was used to compare the mean levels between the two groups if the variable was normally distributed. Otherwise, the Mann-Whitney U test was adopted. Chi-square tests were used to compare the percentages of categorical variables between the groups.

The exposure of vitamin B12 deficiency was defined as serum vitamin B-12 concentration <150 pmol/L or serum MMA concentration >271 nmol/L, and the others were set as the reference category. Main confounders were treated as follows: age (<35 years and ≥35 years); sex (male and female); marital status (married/living with partner and widowed/divorced/separated/never married); country of birth (US born and Non-US born); educational levels (junior high school or below, high school, and college or above); BMI (<25 kg/m^2, 25 to <30 kg/m^2, 30 to <35 kg/m^2 and ≥35 kg/m^2), renal function (impaired and normal); smoking status (cotinine concentration <0.05, 0.05-3.00 and ≥3.00 ng/mL)； PIR (calculated by dividing family (or individual) income by the poverty guidelines specific to the survey year. < 1, poverty; ≥ 1, non-poverty). We summarized the demographic characteristics as well as vitamin B-12 status of the study population and performed unadjusted analysis of the associations between these indexes and LTBI to screen for risk factors. Logistic regression models were performed to analyze the association between low vitamin B-12 status and the prevalence of LTBI. Odds ratio [OR, with 95% confidence intervals (CI)] was used to evaluate the effect size. In multivariate logistic regressions, Model 1 adjusted for impaired renal function, age, and sex, Model 2 further adjusted for born country and Model 3 further adjusted for smoking status (indicated by cotinine concentration), BMI, educational levels, marital status and PIR. In order to further clarify the association, stratified analysis by age and sex was performed to elucidate the effect of vitamin B-12 deficiency on LTBI in different age group (age ≥35, age <35) and sex group (male, female).

All statistical analyses were performed by using SPSS 22.0 software, and $p<0.05$ was considered statistically significant.

RESULTS

Participant characteristics

Table 1 summarizes the demographic characteristics of the study population. Overall, 479 (10.0%) of the participants had positive QFT-GIT. Individuals in the LTBI group were older and more predominantly male than the Non-LTBI group (both $p<0.001$). In addition, they were significantly more likely to be born in foreign countries, Mexican American, other Hispanic, and other races than those in the Non-LTBI group (both $p<0.001$). Persons in the LTBI group had a lower education level than the Non-LTBI group ($p<0.001$). A significantly higher proportion of vitamin B-12 deficiency was observed in subjects with LTBI than Non-LTBI (13.2% vs 9.2%, $p<0.01$). No significant differences were found in BMI, serum cotinine level, prevalence of impaired renal function and PIR between the two groups ($p>0.05$).

Association between vitamin B-12 deficiency and LTBI

Figure 2 showed the distribution of vitamin B12 deficiency in total, as well as stratified by age (≥35, <35 years) and gender. We found that the percentage of adults with low serum vitamin B12 was actually low, about 2.0% in the total and different age and sex groups, and there was no significant difference between age or sex subgroups. A significantly higher proportion (8.7%) of increased serum MMA was observed, and subjects aged ≥35 years (10.7%) had higher MMA than those <35 years (3.2%). Totally, 9.6% of the adults had vitamin B-12 deficiency, similarly, the percentage in subjects age ≥35 years (11.5%) was higher than those <35 years (4.4%). We did not find the difference between male and female ($p>0.05$).

In Table 2, the logistic regression results for the association between vitamin B-12 deficiency and LTBI are presented. Crude binary logistic regression models revealed a significantly higher odds ratio (OR) of LTBI among participants with vitamin B-12 deficiency (crude OR: 1.50; 95% CI: 1.12-1.99). To get rid of the possible confounding factors, further multivariable analysis was implemented. After adjusted impaired renal function, age and sex in Model 1, there remained a marginally significant correlation between low vitamin B-12 and LTBI (aOR: 1.33; 95% CI: 0.99-1.78, $p=0.058$), indicating this correlation was not influenced by impaired renal function, age and sex of the participants. After further adjusting for born country in Model 2 (aOR: 1.36; 95% CI: 1.01-1.85) and further adjusting for smoking status,

BMI, educational levels, marital status, PIR in Model 3 (aOR: 1.37; 95% CI: 1.01-1.86) vitamin B-12 deficiency remained significantly associated with a higher odds ratio of LTBI with similar size effects. Our results suggest that vitamin B-12 deficiency is independently correlated with a nearly 37% increased risk of LTBI in NHANES adults. In addition, in the final model, we found that born in countries other than the United States (aOR: 5.12; 95% CI: 4.10-6.39), age ≥35 years (aOR: 3.13; 95% CI: 2.29-4.29), male (aOR: 1.50; 95% CI: 1.22-1.85) was also independently associated with a higher risk of LTBI, while education level higher than college showed a protective effect compared with those lower than high school (aOR: 0.61, 95% CI: 0.48-0.77).

The results of the stratified analysis by age and sex in the final multivariable model were shown in Figure 3. In each subgroup, Non-US born was found to be independently correlated with LTBI of about 4-6 fold increased odds ratios, while educational levels ≥college showed protective effects of about 50% lowered risk of LTBI. In subjects age ≥35 years, vitamin B12 deficiency was related with LTBI (aOR: 1.46; 95% CI: 1.07-2.01), however, no such correlation remained in those aged <35 years. Sex stratified analysis showed that low vitamin B-12 status is independently correlated with nearly 70% increased risk of LTBI (aOR: 1.70; 95% CI: 1.06-2.73) in female subjects, while not in male. In addition, serum cotinine ≥3 ng/mL (aOR: 1.62; 95% CI: 1.05-2.50) was associated with LTBI in the female.

DISCUSSION

In this study, we found vitamin B-12 deficiency was significantly associated with higher odds ratio of LTBI in a nationally representative sample of US adults. Participants with LTBI had a higher frequency of vitamin B-12 deficiency than the Non-LTBI subjects, and the adjusted ORs of LTBI prevalence were nearly 37% higher in the participants with vitamin B-12 deficiency.

Both serum vitamin B-12 and MMA were detected in NHANES 2011-2012. Data on serum vitamin B-12 directly reflect the range of circulating vitamin B-12 concentrations in individuals. Also, MMA, the levels of which are markedly elevated in the vast majority (>98%) of participants with clinical vitamin B-12 deficiency was detected. Vitamin B-12 is a crucial cofactor in the conversion of L-methylmalonyl-CoA to succinyl-CoA, with MMA being an intermediate in this metabolic pathway. Due to vitamin B-12 deficiency，this conversion is impaired, leading to an accumulation of MMA in the body.[24] As reported, an elevated level of MMA was a sensitive and specific index for the diagnosis of vitamin B-12

deficiency.[25] Therefore, vitamin B-12 deficiency in the present study was operationally defined as having a low serum vitamin B-12 (serum vitamin B-12 <150 pmol/L], an elevated MMA (serum MMA >271 nmol/L), or both, as reported by Bailey RL.[19] The use of these two markers in combination provides a more comprehensive assessment of vitamin B-12 status compared to relying solely on serum vitamin B-12 concentration. Vitamin B-12 deficiency induced immune impairment and MMA associated metabolic changes might jointly disrupt the normal immune surveillance and clearance mechanisms against MTB, increasing the risk of LTBI.[26]

To our best knowledge, this is the first large population-based study to provide comprehensive estimates of the association between the nutritional status of vitamin B-12 and LTBI. In agreement, vitamin B-12 deficiency was found in tuberculosis patients in a Chinese Han population;[9] Oh et al. conducted a study to measure the concentration of MMA in Korean patients with tuberculosis and found that MMA levels were significantly elevated in these patients compared to the control, indicating a potential deficiency of vitamin B-12 among the tuberculosis patients. Also, a significantly higher incidence of tubercle infection has long been found in those taking a vegetarian diet.[27, 28] Therefore, these evidences suggest that low vitamin B-12 status is associated with MTB infection in the general population. The role of vitamin B-12, also named cobalamin, in the killing of phagocytosed organisms is of significant mechanistic concerns. An impairment in bacterial killing by phagocytes among patients with vitamin B-12 deficiency was first noted by Kaplan and Basford.[29] Consistently, in vitamin B-12-deficient patients whose red cell count was below $2*10^{12}$/L, it was reported that intracellular killing of ingested bacterial was impaired, although the phagocytic activity was normal,[30] and it worth noting that the treatment with vitamin B-12 was followed by a return to normal phagocytic function 1.5-2.5 weeks later. Therefore, vitamin B-12 deficiency may contribute to LTBI via impaired bacterial killing of phagocytosed bacilli. In summary, our findings demonstrated that vitamin B-12 deficiency was associated with LTBI in the general US adults.

Besides possible impaired phagocytic activity against *MTB*, vitamin B-12 deficiency may also increase the risk of LTBI via other immunity-related mechanisms. In patients with pernicious anemia, vitamin B-12 replacement therapy restored the abnormalities in immune system, indicated by increased CD4+/CD8+ ratio, restored NK cell activity, and elevated levels of C3, C4, and immunoglobulins.[31, 32] It is well known that many types of immune cells including monocytes, macrophages, and T-lymphocytes, play an important role against *MTB*. Therefore, vitamin B-12 may have important immunomodulatory effects on anti-*MTB* cellular

immunity. To be noticed, in other types of Mycobacterium infection, vitamin B-12 was also found to be related to antibacterial immunity. Vitamin B-12, together with folate, affected cell survival and inflammation during avium subsp. Paratuberculosis infection (MAP). In those afflicted with Crohn's disease, MAP-positive patients have significantly lower plasma B-12 in comparison to MAP-negative patients. In addition, pro-inflammatory cytokines IL-1β and TNF-α were significantly upregulated during vitamin B-12 and folate deprivation after MAP infection, while the supplementation significantly restored their expression.[33] *In vitro*, vitamin B-12 deficiency in the media led to the decreased macrophages apoptosis during MAP infection, suggesting this key mechanism to counteract intracellular pathogens was inhibited during the vitamin deficiency.[34] In addition, approximately 10% of the variability in the immune response was attributed to the microbiome according to an estimate that compared the relative contributions from genetics.[35] Vitamin B-12 is supposed to affect the gut microbiome ecology because the presence of its analog (corrinoid) transporters in Bacteroides thetaiotaomicron, a gut commensal, was demonstrated to improve microbial fitness.[36] Also, vitamin B-12 possibly affects lung microbiota, followed by lung immunity via the gut–lung axis. It has been hypothesized that perturbations of microbial communities result in alterations of inflammatory cytokines that could affect immunological responses against MTB.[37] Taken together, these findings suggest that vitamin B-12 may play a key role in anti-MTB immunity.

In addition, the central cobalt moiety in the vitamin B-12 corrin ring scavenges cyanide ions and reactive oxygen species (ROS).[38] *In vitro* studies, vitamin B-12 and its derivatives were found to regulate inflammatory processes and protect against oxidative stress associated pathologies, playing important roles on regulating NOS activity in normal and pathological conditions.[39] In a randomized, placebo-controlled clinical study, a complex multivitamin comprising vitamin B-12 decreased low density lipoprotein (LDL) oxidation and protected against coronary artery disease.[40] So, we speculate that vitamin B-12 deficiency may also increase the odds ratio of LTBI due to the impaired ability in eliminating ROS, as well as inflammation produced by *MTB* infection, which needs to be verified in future studies.

Furthermore, vitamin B-12 may be of notable importance in the metabolism of *MTB*. *MTB* encodes three functional vitamin B-12-dependent enzymes, methionine synthase (MetH), methylmalonyl CoA mutase (MutAB), and ribonucleotide reductase (NrdZ). Vitamin B-12 may regulate the gene expression by binding to riboswitches in mRNA.[7, 41] Interestingly, *MTB* has the capacity to regulate core metabolic functions according to vitamin B-12 availability, i.e., whether vitamin B-12 is acquired via endogenous synthesis or through uptake from the

host environment. This finding implies that vitamin B-12 has a role in the pathogenesis of MTB infection.[8]

Tuberculosis remains a global health burden, and LTBI is a key factor in the transmission and recurrence of TB. Our finding that vitamin B-12 deficiency is associated with an increased odds ratio of LTBI has important implications for preventive strategies. While the data utilized in this study were collected over a decade ago (2011-2012), the biological relationship between vitamin B-12 status and immune response to *MTB* infection remains mechanistically relevant. This study provides foundational evidence to justify prospective investigations and targeted interventions in current populations.

This study has several strengths. Firstly, the NHANES is a representative sample of the US population that rigorously follows well-designed study protocols with extensive quality assurance and quality control. Therefore, the large study population in this analysis was representative of the US population. Moreover, our findings are robust, reliable and generalizable. Secondly, besides serum vitamin B-12 levels, we used serum MMA levels to evaluate vitamin B-12 deficiency, which is a much more accurate determination of vitamin B-12 state than using only serum vitamin B-12 or using dietary vitamin B-12 intake through dietary review, and is widely used.[19] Lastly, many variables, including age, born country, smoke exposure, and education level status were assessed as potential confounders and adjusted for in the analysis.

Also, there are several limitations in the present study. Firstly, causal relationships could not be ruled out due to the cross-sectional design. Especially in participants with *MTB* infection, the nutritional status may be interfered with the infection. Therefore, future prospective studies are needed to clarify the causality. Secondly, elevated plasma MMA and/or total homocysteine, as well as low serum vitamin B-12 and/or holotranscobalamin, are indicative of vitamin B-12 deficiency. For various investigative reasons, we were only able to use MMA and serum vitamin B-12 to determine individual vitamin B-12 status in NHANES cycle 2011-2012, and this may introduce bias. Finally, although we adjusted for many potential confounders, we cannot rule out residual confounding or the effect of unmeasured confounders. Despite the limitations, this study is valuable in light of the association between low vitamin B-12 states and LTBI.

Conclusions

In summary, in this large national sample, vitamin B-12 deficiency was independently associated with higher odds ratio of LTBI in US adults, which indicates the potential benefits

of maintaining optimal vitamin B-12 levels to prevent LTBI, and vitamin B-12 may be optional to help eradicate TB. More studies in other population and prospective cohort studies are warranted to verify our findings. Also, future studies are needed to assess the roles and clinical implications of vitamin B-12 in *MTB* infection.

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CONFLICT OF INTEREST AND FUNDING DISCLOSURE

The authors declare no conflict of interest.

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Table 1. Demographic characteristics of the subjects with and without LTBI

	Total	LTBI	Non-LTBI	*p*
	n=4773	n=479	n=4294	
Age (year)	48 (33, 63)	58 (45, 67)	47 (32, 62)	<0.001*
<35, n (%)	1295 (27.1)	53 (11.1)	1242 (28.9)	<0.001
≥35, n (%)	3478 (72.9)	426 (88.9)	3052 (71.1)	
Male, n (%)	2365 (49.5)	280 (58.5)	2085 (48.6)	<0.001
BMI	27.7	27.6	27.7	0.751*
	(24.0, 32.3)	(24.2, 32.1)	(24.0, 32.3)	
Country of birth, n (%)				<0.001
US born	3304 (69.3)	168 (35.1)	3136 (73.1)	
Non-US born	1466 (30.7)	311 (64.9)	1155 (26.9)	
Serum vitamin B12 (pmol/L)	391	399	391	0.382*
	(287, 545)	(283, 577)	(287, 541)	
Serum MMA (nmol/L)	135	136	134	0.166*
	(104, 181)	(104, 197)	(104, 179)	
Vitamin B-12 deficiency, n (%)	458 (9.6)	63 (13.2)	395 (9.2)	0.006
Cotinine (ng/mL)	0.037	0.038	0.037	0.726*
	(0.011, 4.338)	(0.016, 0.552)	(0.011, 5.438)	
<0.05, n (%)	2622 (55.0)	277 (57.9)	2345 (54.6)	0.194
0.05~2.99, n (%)	918 (19.2)	94 (19.7)	824 (19.2)	
≥3, n (%)	1230 (25.8)	107 (22.4)	1123 (26.2)	
Impaired renal function, n (%)	900 (18.9)	100 (20.9)	800 (18.6)	0.234
Race, n (%)				<0.001
Mexican American	478 (10.0)	72 (15.0)	406 (9.4)	
Other Hispanic	488 (10.2)	86 (18.0)	402 (9.4)	
Non-Hispanic white	1806 (37.8)	67 (14.0)	1739 (40.5)	
Non-Hispanic black	1204 (25.2)	114 (23.8)	1090 (25.4)	
Others	797 (16.7)	140 (29.2)	657 (15.3)	
Educational levels, n (%)				<0.001
<High school	1084 (22.7)	180 (37.5)	904 (21.1)	
High school	997 (20.9)	98 (20.5)	899 (20.9)	
≥College	2690 (56.4)	201 (42.0)	2489 (58.0)	
Marital status, n (%)				0.003
Married/living with partner	2712 (56.8)	303 (63.3)	2409 (56.1)	
Widowed/ divorced/ separated/ never married	2059 (43.2)	176 (36.7)	1883 (43.9)	
Poverty income ratio, n (%)				0.222
< 1 (poverty)	1080 (22.6)	119 (24.8)	961 (22.4)	
≥ 1 (non-poverty)	3693 (77.4)	360 (75.2)	3333 (77.6)	

BMI: body mass index.
†Mann-Whitney U test
Data are presented as median (25th, 75th) for continuous variables because the distribution was non-normal or participants (percentage) for categorical variables.

Table 2. Multivariate regression analysis of the association between vitamin B-12 deficiency and LTBI

	OR	95% CI	*p*
Crude			
Vitamin B-12 deficiency	1.50	1.12~1.99	0.006
Model 1[†]			
Vitamin B-12 deficiency	1.33	0.99~1.78	0.058
Age≥35 years	3.27	2.43~4.40	<0.001
Male	1.51	1.24~1.83	<0.001
Model 2[‡]			
Vitamin B-12 deficiency	1.36	1.01~1.85	0.044
Age≥35 years	3.11	2.30~4.20	<0.001
Male	1.50	1.22~1.83	<0.001
Non-US born	5.02	4.08~6.12	<0.001
Model 3[§]			
Vitamin B-12 deficiency	1.37	1.01~1.86	0.043
Age≥35 years	3.13	2.29~4.29	<0.001
Male	1.50	1.22~1.85	<0.001
Non-US born	5.12	4.10~6.39	<0.001
Educational levels			<0.001
<High school	ref		
High school	0.82	0.62~1.08	0.163
≥College	0.61	0.48~0.77	<0.001
PIR＜1	1.02	0.80~1.30	0.872

OR: odds ratio; CI: confidence interval; Ref: reference; PIR: poverty income ratio
[†]Model 1: adjusted for impaired renal function, age, sex
[‡]Model 2: Model 1 + born country
[§]Model 3: Model 2 + cotinine, BMI, educational levels, marital, PIR.

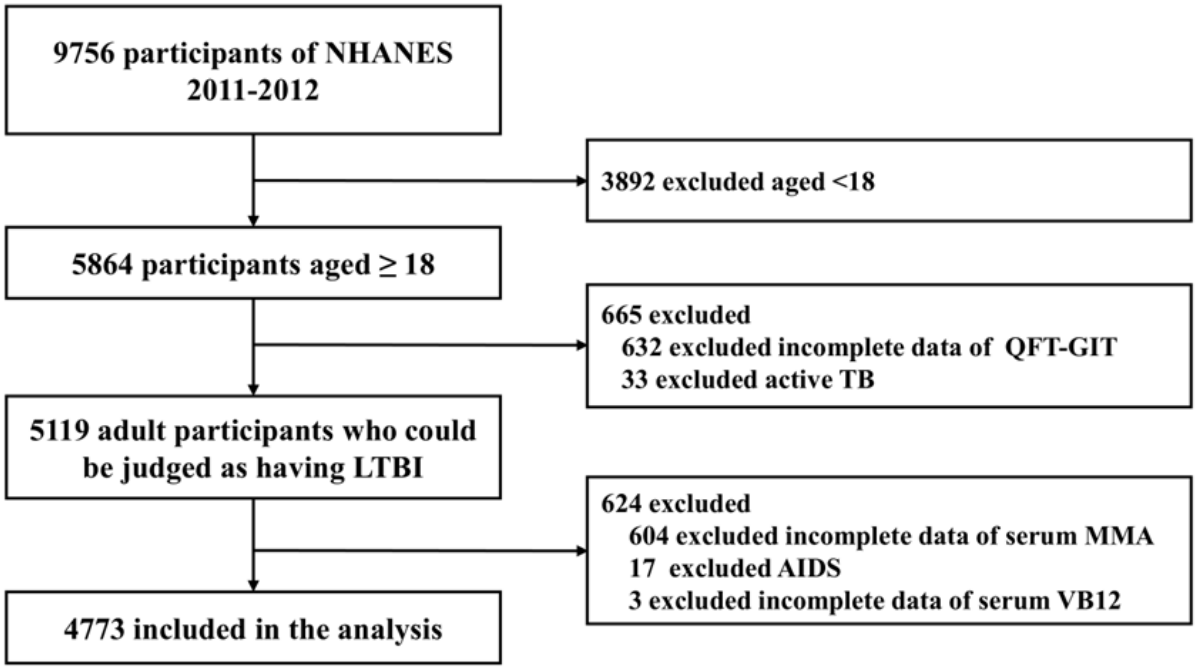

Figure 1. Flow chart of the screening process for the eligible participants.

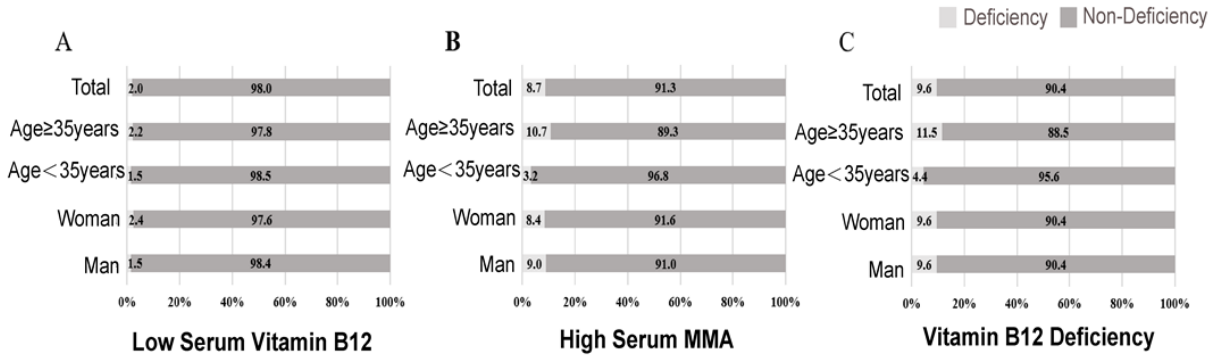

Figure 2. The actual percentage of participants with different low vitamin B-12 status based on age and gender. Low vitamin B-12 status was defined as serum vitamin B-12 <150 pmol/L or serum MMA >271 nmol/L.

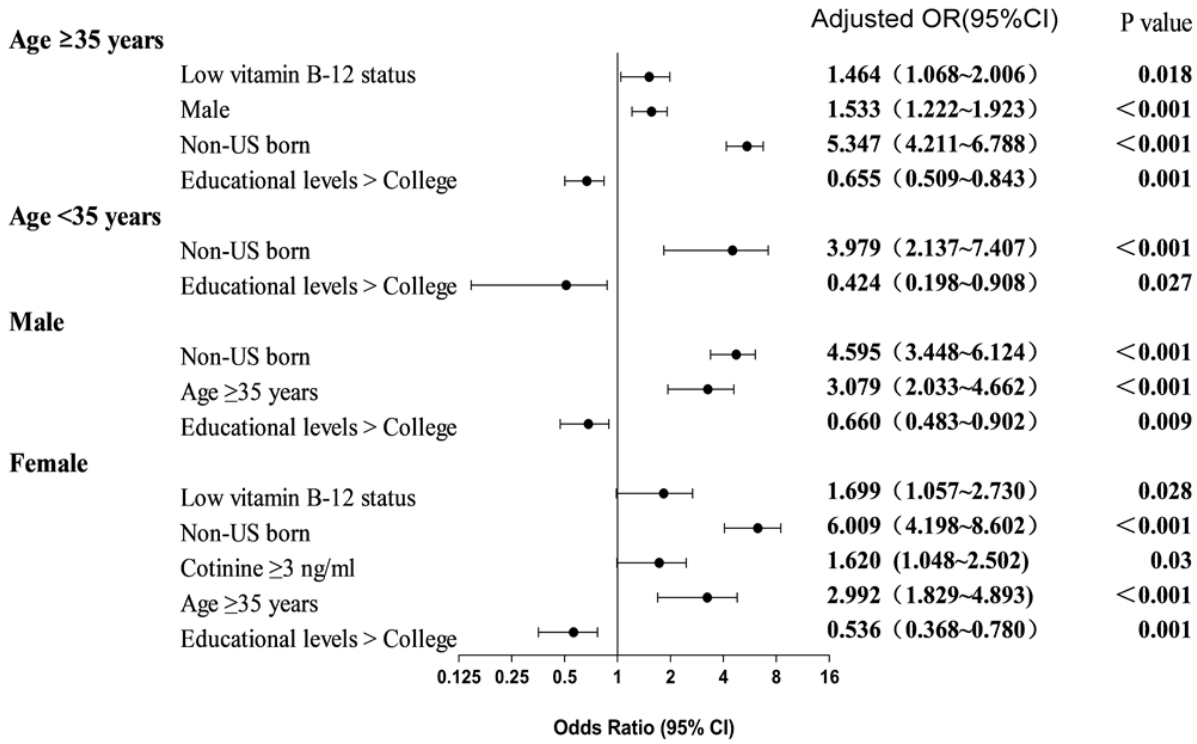

Figure 3. The stratified analysis between vitamin B-12 deficiency and LTBI of the final multivariable model.