

Association between Serum Vitamin B12 and Sensorineural Hearing Loss: A Case-Control Study

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Abstract

Introduction:

The latest evidence from systematic reviews and meta-analyses indicates that nutritional intake and dietary habits are not only associated with an individual's health but also influence hearing function through complex biological mechanisms. Vitamin B12 plays a role in neuronal function, myelin synthesis, and cellular metabolism. Previous studies have suggested a possible association between vitamin B12 deficiency and hearing impairment; however, the evidence remains inconclusive. This study aimed to compare serum vitamin B12 levels in adults with sensorineural hearing loss (SNHL) and individuals with normal hearing and to evaluate the association between vitamin B12 levels and audiometric parameters.

Materials and Methods:

A case-control study was conducted among 102 participants, comprising 51 patients with SNHL and 51 healthy controls. Serum Vitamin B12 levels and audiometric parameters were compared between the two groups. An additional exploratory Receiver Operating Characteristic (ROC) curve analysis was performed to assess the discriminative ability of serum B12 levels in the study sample.

Results:

The mean serum vitamin B12 was 6465.16 ± 5493.20 pg/ml in the SNHL group and 9154.98 ± 6151.63 pg/ml in the control group. The serum Vitamin B12 level in patients with sensorineural hearing loss was significantly lower than that in the control group ($p=0.022$). Exploratory ROC analysis demonstrated an area under the curve of 0.64 (95% CI: 0.531-0.748).

Conclusion:

Participants with sensorineural hearing loss (SNHL) had lower serum B12 levels compared to the control group. These findings indicate an association between serum vitamin B12 levels and sensorineural hearing loss (SNHL). However, the modest discriminatory performance and the observational case-control design do not warrant the use of serum vitamin B12 as a reliable diagnostic or predictive marker. Larger prospective studies incorporating relevant clinical and metabolic confounders are required to clarify this association.

Keywords: Vitamin B12, Hearing Loss, SNHL, ROC curve, pure tone audiometry.

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Introduction

Hearing loss (HL) is the third-leading cause of disability according to the Global Burden of Disease (1,2). It is the most common chronic condition, resulting from several factors such as noise exposure, viral infections, trauma, ototoxic medications, ageing, genetic factors, and chronic diseases, which affects approximately 466 million people worldwide (3)—a global condition affecting 5% of the global population, most importantly, 34 million preadolescent children. According to the World Health Organization, by 2050, nearly 2.5 billion people will be affected by hearing loss (3). About 80% of the people with disabling hearing loss live in low-and middle-income countries. Hearing loss becomes more common with age. Over 25% of people over 60 years of age have disabling hearing loss (4). Environmental factors, maternal infections, or birth complications account for 30% of hearing loss. Genetic factors account for 50-60% of childhood hearing loss. Hearing loss has long-term implications. Among young adults who experienced hearing loss in childhood, 40% report at least one limitation in daily activities. (5). Depending on the affected area, HL is typically sensorineural, conductive, or mixed. Conductive hearing loss (HL) occurs when sound waves are obstructed in their path to the cochlea. This type of hearing loss results from diseases of the external and middle ear. In contrast, sensorineural hearing loss results from diseases of the inner ear and is caused by disorders of the eighth cranial nerve and hair cell dysfunction (6–8). Presbycusis, or age-related hearing loss (ARHL), is a bilateral, symmetric sensorineural impairment due to the effects of ageing on the auditory system. It affects about 50% of people in their seventh decade and is the most common type of hearing loss in older adults (2). Sensorineural hearing loss (SNHL) is the most common type of hearing loss. It occurs due to dysfunction in the sensory structures of the auditory nerve or inner ear. The causes of SNHL are multifactorial, involving both non-modifiable factors, such as age, genetics, and congenital abnormalities, as well as modifiable factors. Modifiable factors include excessive noise exposure, smoking, obesity, diabetes mellitus, hypertension, chronic kidney disease, ototoxic medications, and nutritional deficiencies.(9,10).

Damage to the hair cells of the inner ear is the main pathological basis of SNHL. It plays an important role in converting sound waves into neural signals. Ototoxic drugs such as platinum-based chemotherapeutics, loop diuretics, and aminoglycosides, along with mitochondrial dysfunction, age-related degeneration, and noise exposure, can lead to hair cell damage. The transmission of the auditory signal is compromised due to loss of spiral ganglion neurons and hair cells, which contributes to age-related hearing loss (presbycusis). Pathologically, SNHL is usually due to degenerative changes in synaptic connections and in cochlear structures, including hair cells and spiral ganglion neurons (SGNs), which connect the ear to the brain (6,11,12). In recent years, the role of nutrition and diet in hearing health has received increasing attention. A recent meta-analysis found that nutritional intake and dietary habits are not only associated with an individual's health but also influence hearing function through complex biological mechanisms (13). A systematic review by Rodrigues et al. found that cochlear dysfunction, a higher prevalence of hearing impairment, and poorer hearing thresholds may be linked to low serum vitamin B12 levels.(14).

Vitamin B12, also known as cobalamin, is an essential micronutrient primarily found in animal-based foods, with only small amounts present in a few plant foods. Higher levels of vitamin B12 are associated with the consumption of milk, meat, and fish. Rich sources of vitamin B12 include veal, beef, lamb, and mutton from sheep and cattle. As it is mainly involved in red blood cell formation, DNA synthesis, maintenance of the myelin sheath, and nerve function, its deficiency causes several metabolic and neurologic complications. Its deficiency is associated with decreased intake or absorption, as measured by plasma or serum vitamin B12 levels. The formation of vitamin B12 in cells from dietary sources is a complex process. As a vitamin, it moves through the blood and intestine by binding to proteins. These proteins involve intrinsic factor, haptocorrin, and transcobalamin II. This becomes part of the holotranscobalamin complex, which is absorbed by target cells after binding to the transcobalamin receptor (14,15).

Vitamin B12 contributes to the normal functioning of the vascular and nervous systems. Low levels of Vitamin B12 and folate are associated with elevated homocysteine levels, which can affect cochlear blood flow and contribute to vascular disease. Vitamin B12 deficiency has been linked to vascular dysfunction, altered cellular metabolism, and impaired myelination of the cochlear nerve, which may contribute to hearing loss (16).

Several studies have investigated the association between vitamin B12 status and hearing impairment, with many reporting lower serum vitamin B12 concentrations among individuals with SNHL, tinnitus, age-related hearing loss, and high-frequency auditory dysfunction (17). A recent systematic review suggested that vitamin B12 deficiency may increase susceptibility to hearing impairment, particularly at higher frequencies. However, the available evidence remains inconclusive because of differences in study design, sample size, participant characteristics, and audiological assessment methods (18).

Furthermore, data from the Indian population remains limited despite the high prevalence of vitamin B12 deficiency and the growing burden of hearing disorders (19). The current study aimed to compare serum vitamin B12 levels between adults with sensorineural hearing loss and those with normal hearing, and to examine the relationship between serum vitamin B12 levels and audiometric parameters.

Materials and Methods

Study Design, Setting, and Sample Size Calculation

A case-control study was conducted in the Department of Otorhinolaryngology at a tertiary care hospital to investigate the diagnostic ability of serum vitamin B12 levels in sensorineural hearing loss (SNHL). A total of 102 participants were enrolled in the study: 51 patients with SNHL (case group) and 51 individuals without hearing loss (control group), after approval from the Institutional Ethics Committee (Protocol No: YEC-1/2023/105) and written informed consent. The required sample size was determined using G*Power software, yielding an effect size of 0.56 with 80% power and a 5% significance. *This is equal to*
$$= \frac{(Z\alpha/2 + Z\beta)^2}{d^2}$$

where Effect size (d) = 0.56, α =0.05, Power = 80% (β =0.20)

Eligibility Criteria

The study population consisted of adults aged 18 years and above attending the Otorhinolaryngology outpatient department. Participants with audiometrically confirmed sensorineural hearing loss were included in the case group, and subjects with normal hearing were included in the control group. Sensorineural hearing loss was determined by pure-tone audiometry with elevated air and bone conduction thresholds without a clinically significant air-bone gap.

The cases represented varying degrees and laterality of sensorineural hearing loss. Specific etiological subtypes, including presbycusis and noise-induced hearing loss, were not systematically classified in the present study. Individuals with conductive hearing loss, congenital hearing loss, syndromic hearing disorders, traumatic injuries to the eighth cranial nerve, vestibular schwannoma, vascular disorders, or those who had vitamin B12 supplementation within the last three months were excluded from the study.

Data Collection and Clinical Assessment

Participants in the SNHL group underwent a comprehensive audiological evaluation to determine hearing thresholds. Data on Age, gender, Pure-Tone Average, and Bone-Conduction thresholds were collected from participants. A complete otorhinolaryngological examination, including otoscopic assessment, was performed to evaluate the condition of the tympanic membrane and external auditory canal. Pure-tone audiometry was conducted using the Interacoustics AC40 audiometer in a sound-treated room. Air and bone conduction thresholds were measured at 0.25, 0.5, 1, 2, 4, and 8 kHz. Pure-tone average was calculated as the average air-conduction threshold at 0.5, 1, and 2 kHz.

Participants with confirmed SNHL on audiometric testing were included in the case group, that is, elevated air- and bone-conduction thresholds without an AB gap. Based on the pure-tone average (PTA), the severity of hearing loss was categorized as follows: mild (26–40 dB), moderate (41–55 dB), moderately severe (56–70 dB), severe (71–90 dB), profound (91–120 dB), and complete deafness in the absence of measurable hearing.

Blood Sample Collection and Estimation of Serum Vitamin B12.

Approximately 3 mL of venous blood was collected from each participant by a trained staff nurse or laboratory technician. Before the procedure, the process was explained to the participants, and written consent was obtained. The blood samples were then transferred into red-top vacutainers, wrapped in aluminum foil to protect them from light, and transported to the Research Centre for vitamin B12 estimation. Serum methyl cobalamin (Vitamin B12) concentrations were quantified using liquid chromatography–tandem mass spectrometry (LC-MS/MS). Analyses were performed on a QTRAP 6500 Triple Quadrupole Mass Spectrometer (SCIEX, USA) coupled with an Agilent 1290 Infinity II Liquid Chromatography system. Serum samples were centrifuged and extracted with methanol and other organic solvents. Quantification was carried out using a targeted Multiple Reaction Monitoring (MRM) approach. Mass spectrometry (MS)- grade vitamin B12 was used to observe transition (fragment) ions.

Human serum samples were analyzed to detect Methylcobalamin concentrations. An LC-MS/MS Multiple Reaction Monitoring (MRM) method was used to quantify methyl cobalamin. A QTRAP 6500 coupled to an Agilent 1290 Infinity II LC system was used to develop the MRM method.

A working solution of 1microg/ml of Methyl cobalamin was used to optimize and develop the MRM method for detection and quantification.

Statistical Analysis

The data were described using descriptive statistics such as mean and standard deviation for continuous variables, and frequency and percentage for categorical variables.

Further, the data were analyzed using inferential statistics (Unpaired t-test and One-way ANOVA) to draw inferences from the study. An exploratory receiver operating characteristic (ROC) analysis was additionally performed to examine the ability of serum vitamin B12 levels to discriminate between participants with SNHL and controls in the study sample. Statistical significance was considered at $p < 0.05$.

Results

Sociodemographic Details of Study Participants

A total of 102 participants (51 cases and 51 controls) were enrolled in the present study, of whom 53.9% were female, and 46.1% were male, with a mean age of 43.79 ± 15.73 years. The majority of the study participants were in the 40-60 years age group (49%), followed by the 18-40 years age group (38.2%) and the 60+ years age group (12.7%) (Table 1).

Table 1: The sociodemographic details of the study participants

Gender	Group		Total	Percentage
	Control	Case		
Gender				
Male	20	27	47	46.1
Female	31	24	55	53.9
Age Groups (43.79±15.73)				
≤40 years	24	15	39	38.24
40-60	25	25	50	49.0
>60 years	2	11	13	12.7

Serum Vitamin B12 Level and Sensorineural Hearing Loss (SNHL)

Vitamin B12 was slightly higher in females than in males. It was relatively constant across

age groups, with no significant differences in average vitamin B12 levels by gender and age among the study participants (Table 2).

Table 2: Comparison of Vitamin B12 based on the age groups

Parameter	N	Vitamin B12 (pg/ml) Mean $\pm$ SD	SE	p-value
Gender				
Male	47	7441.11 $\pm$ 5109.729	745.331	0.566
Female	55	8125.36 $\pm$ 6628.349	893.766	
Age Groups				
18-40 yrs	39	8114.79 $\pm$ 6116.40	979.408	0.862
40-60 yrs	50	7479.80 $\pm$ 5918.64	837.022	
>60 yrs	13	8166.15 $\pm$ 6031.76	1672.910	

However, the Serum Vitamin B12 level in patients with sensorineural hearing loss (6465.16 pg/ml) was significantly lower (p-

value: 0.022) than that in the control group (9154.98 pg/ml) (Table 3).

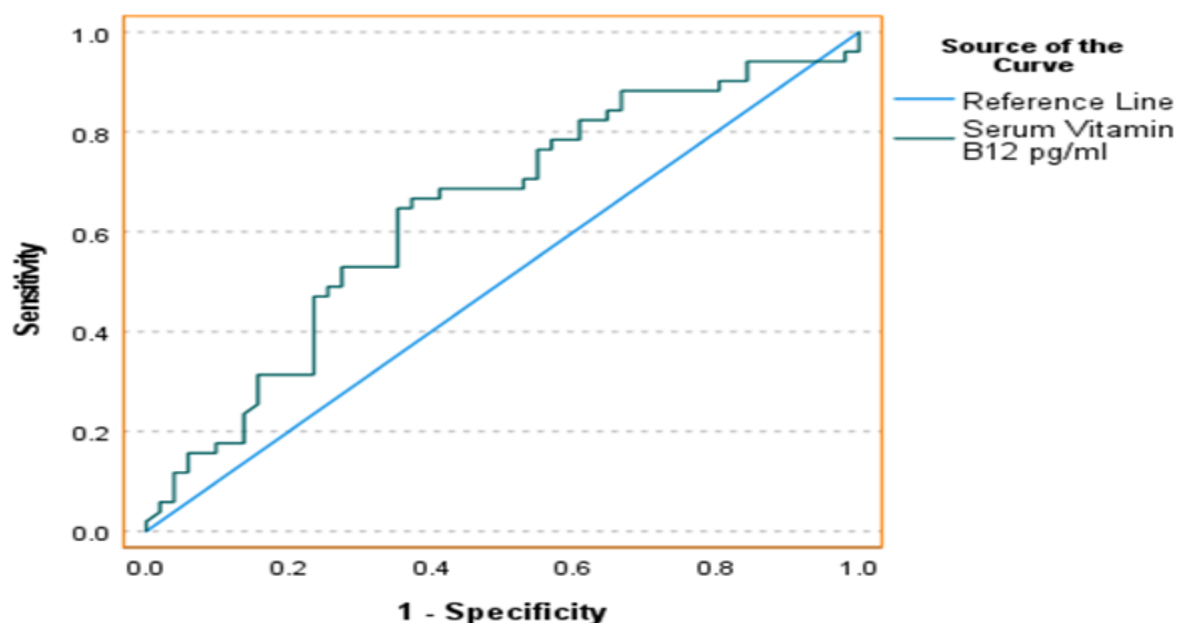
Table 3: Serum vitamin-B12, Pure Tone Average (PTA), and Bone Conduction (BC) for left and right ears.

Parameters	Group	N	Mean	SD	SE	MD	p-value
Serum Vitamin B12 (pg/ml)	Control	51	9154.98	6151.630	861.401	2689.824	0.022*
	Case	51	6465.16	5493.199	769.202		
Average PTA (Right Ear)	Case	51	39.8794	18.05960	2.52885	22.60241	P<0.001**
	Control	51	17.2770	5.00833	0.70829		
Average PTA (Left Ear)	Case	51	39.8873	18.87898	2.64359	22.03225	P<0.001**
	Control	51	17.8550	5.89030	0.83301		

Note: PTA: Pure-Tone Average, BC: Bone Conduction, MD: Mean Difference

These findings suggest an association between lower serum vitamin B12 levels and sensorineural hearing loss. Serum Vitamin B12

Level and Receiver Operating Characteristics (ROC) Analysis

**Fig 1:** ROC (Receiver Operating Characteristic) curve and Serum Vitamin B12 for SNHL.

AUC: 0.64 (Lower bound: 0.531, Upper bound: 0.748), Standard error: 0.055 ($p = 0.012$)

An exploratory receiver operating characteristic (ROC) analysis was performed to examine the ability of serum vitamin B12 levels to discriminate between participants with SNHL and controls in the study sample. The exploratory ROC analysis demonstrated an area under the curve (AUC) of 0.64 (95% CI: 0.531-0.748; $p=0.012$), indicating modest discriminatory ability. At the identified cut-off of 5995 pg/ml, the sensitivity and specificity were both 64.7%. (Figure1) Given the case control design, relatively small sample size, potential spectrum and selection bias, these findings should not be interpreted as evidence of clinical diagnostic accuracy. The ROC analysis was therefore considered exploratory and hypothesis-generating.

Discussion

The association between vitamin B12 deficiency and sensorineural hearing loss (SNHL) is complex and multifactorial. These include impairment in myelination of auditory neurons, vascular compromise due to elevated homocysteine levels, and hair cell damage due to decreased cochlear perfusion (20). This study aims to explore the relationship between Vitamin B12 deficiency and sensorineural hearing loss SNHL.

In the present study, we demonstrated that serum Vitamin B12 levels are significantly lower in patients with SNHL compared to healthy controls. Our study findings are consistent with an Egyptian study by Taha et al., where they observed that the healthy controls have significantly higher ($P < 0.001$) median vitamin-B12 and folic acid levels compared to the patients with severe-to-profound SNHL (planned for cochlear implantation) and moderate-severe SNHL (hearing aid users), suggesting that measuring Vitamin B12 and folic acid can be useful (21). Similarly, a cross-sectional study among the elderly population by Lasisi et al. reported that Vitamin B12 levels were higher (49.7 ± 9.4 pmol/L) in subjects with normal PTA than in those with speech-frequency HL (42.6 ± 10.2 pmol/L). Further, they found that cyanocobalamin levels negatively correlate with increasing high-frequency hearing thresholds (correlation coefficient = -0.35 , $P = 0.02$) (22). Further, Akyay et al. observed better

pure-tone hearing thresholds (0.25-4 kHz) and PTA in healthy controls than in vitamin B12-deficient children ($P < 0.05$), which corresponds with the PTA pattern seen among controls and cases in our study (23). Although few studies have examined the association between Vitamin B12 and hearing loss, none have quantified its diagnostic potential in SNHL. Some reports indicate that vitamin B12 deficiency, when combined with folate deficiency and elevated homocysteine levels, may contribute to hearing loss (14). A study by Huang et al. reported that elevated serum homocysteine and low serum folate levels are associated with sudden-onset total-frequency deafness (24).

The findings of this study are consistent with previous reports suggesting an association between Vitamin B12 status and auditory dysfunction. However, because the present study was observational and case-control in design, the observed association should not be interpreted as evidence that Vitamin B 12 deficiency causes sensorineural hearing loss. Several biological mechanisms link Vitamin B12 deficiency to auditory dysfunction. Vitamin B12 is involved in myelin synthesis and neuronal function, and its deficiency may contribute to elevated homocysteine concentrations and vascular dysfunction. These mechanisms could affect cochlear or auditory neuronal function. Nevertheless, the present study did not assess folate, homocysteine, or other metabolic pathways; therefore, these mechanisms remain hypothetical in the context of our findings.

Additionally, a prospective study by Karaer et al. reported that Transiently Evoked Otoacoustic Emissions (TEOAEs) and Distortion Product Otoacoustic Emissions (DPOAEs) were significantly ($p<0.01$) lower in women with low serum vitamin B12 levels, suggesting a role for vitamin B12 in cochlear function. (25). Similarly, another study by Karli et al. also found that TEOAEs and spontaneous OAEs (SOAEs) were lower among the Vitamin B12-deficient group than in the control group, with a significant association between Vitamin B12 deficiency and cochlear dysfunction (17).

Several studies have investigated the potential impact of Vitamin B12 supplementation on hearing loss. A Japanese study by Kurioka et al. reported that hearing recovery was better among Idiopathic Sudden Sensory Neural

Hearing Loss (ISSNHL) patients after 16-24 weeks of administration of Vitamin B12 with ATP compared to a short-term regimen (<8 weeks), suggesting Vitamin B12 supplementation might slow down the progression toward hearing loss among ISSNHL patients (20). Similarly, another study by Houston et al. found that women with hearing loss had 48% lower serum vitamin B12 levels ($P = 0.0007$) than women with normal hearing (26).

However, the association between vitamin B12 and hearing loss has not been consistently demonstrated. Kabagambe et al., using data from 1149 participants in the 2003-2004 National Health and Nutrition Examination Survey, found that serum Vitamin B12 was not significantly associated with hearing loss after adjustment for demographic, lifestyle and other potential confounding factors. Their findings contrast with the lower Vitamin B12 levels observed among participants with SNHL in the present study. Differences in study populations, age distributions, hearing-loss definitions, nutritional status, confounder adjustment, and study designs may partly explain these discrepancies.

The available literature on this topic is not completely consistent. Factors such as age differences, the causes of hearing loss, nutritional status, comorbidities, and the methods used for audiological and biochemical assessments may lead to variations between studies. Therefore, the findings of this study should be viewed in the context of existing evidence rather than as proof of a causal relationship. Diabetes mellitus and other metabolic disorders may represent important confounding factors because they can independently influence auditory function. The present study did not systematically collect or adjust for diabetes and other relevant metabolic and lifestyle factors. Consequently, residual confounding cannot be excluded.

The specific etiology of SNHL was not systematically recorded or classified in this study and has therefore been acknowledged as a limitation rather than inferred retrospectively.

The exploratory ROC analysis demonstrated an AUC of 0.64, suggesting only modest discrimination between the case and control groups. Importantly, this result should not be interpreted as demonstrating clinically useful

diagnostic accuracy. Case-control studies may introduce spectrum and selection bias when evaluating diagnostic performance, and the present sample was relatively small and recruited from a single centre.

Validation in an appropriately designed prospective cohort representing the clinical spectrum of patients with hearing loss would therefore be necessary before the diagnostic utility of vitamin B12 can be assessed. Consequently, future studies should be conducted at multiple centers, enroll a larger cohort, address confounding factors, such as medical and medication history, and include multiple vitamin B12 measurements at different time points.

Conclusion

In this case-control study, lower serum Vitamin B12 levels were associated with sensorineural hearing loss. However, the observational design does not establish a causal relationship between vitamin B12 status and hearing loss.

Larger, multicentric studies with appropriate age matching or multivariable adjustment, systematic assessment of SNHL etiology, and comprehensive evaluation of potential confounding factors are required to clarify this association.

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