

# Folic acid and vitamin-B<sub>12</sub> in idiopathic sensorineural hearing loss in children

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## Aim

The aim of the study was to evaluate the role of vitamin-B<sub>12</sub> and folate blood concentrations in children suffering from moderate, severe, and severe-to-profound sensorineural hearing loss (SNHL).

## Materials and methods

This study was conducted on 95 children: 30 children with severe-to-profound SNHL who were scheduled for cochlear implantation, 25 children who are hearing aid users for moderate and severe SNHL, and another 40 healthy volunteers considered as the control group. Full audiological examination was performed to all children, as well as computed tomographic scan and MRI to temporal bone were performed for the SNHL groups.

## Results

Median vitamin-B<sub>12</sub> and folic acid levels were significantly higher in the control group 3 than in the SNHL groups 1 and 2 patients ( $P < 0.001$ ). There was no significant correlation between vitamin-B<sub>12</sub> and folic acid levels and the other parameters. There was no significant difference between groups 1 and 2 with respect to median vitamin-B<sub>12</sub> and folic acid level.

## Conclusion

Study demonstrated that the serum levels of folate and vitamin-B<sub>12</sub> are decreased in patients with SNHL. Measurement of folic acid and vitamin-B<sub>12</sub> in patients suffering SNHL might be useful. These data may give us some clues about how hearing loss is developed in these patients.

## Keywords:

folic acid deficiency/complications, hearing loss/blood, vitamin-B<sub>12</sub> deficiency/complications

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## Introduction

Hearing loss (HL) is one of the most common birth defects; it is the third-leading chronic disability after arthritis and hypertension [1]. HL has become a common problem in industrial societies due to combined effects of noise, aging, and hereditary factors. Infection is an added factor contributing to HL in developing countries [2].

There is mounting evidence that essential but often overlooked vitamins play a crucial role in preventing HL. In fact, having low levels of these vitamins has been linked to a form of HL often accompanied with tinnitus [3].

Some studies have reported that HL, tinnitus, and noise-induced HL are associated with vitamin-B<sub>12</sub> and folic acid deficiency, with a possible relationship between vitamin-B<sub>12</sub> and folic acid deficiency and dysfunction of the auditory pathway [4–6]. However, other investigators have failed to find any strong evidence to support such an association [7–10].

Homocysteine is a sulphhydryl-containing amino acid derived from the metabolic demethylation of dietary

methionine abundant in animal protein [11]. Mild-to-moderate elevations of homocysteine have been associated with vascular diseases [12,13]. Increase in plasma total homocysteine concentrations may play an etiologic role in sensorineural hearing loss (SNHL) [14,15]. Folic acid therapy alone or combined with vitamin-B<sub>6</sub> and vitamin-B<sub>12</sub> reduces homocysteine levels even in people who do not have vitamin deficiency [16].

The development of cochlea and hair cells is dependent on a genetic pathway called planar cell polarity pathway. This pathway is involved in the formation of the polarized structure of the auditory sensory organ and regulates the embryonic development. It is postulated that genetic abnormalities disturb this pathway leading to severe-to-profound congenital SNHL [17]. Permanent congenital SNHL may result in serious disability including delayed speech and language development, emotional disturbance, and scholastic underachievement. Fortunately, hearing aids and cochlear implant can help to restore some of functional disability resulting from SNHL [18–20].

Accordingly, this study was designed to evaluate the role of vitamin-B<sub>12</sub> and folate blood concentrations in children suffering from moderate, severe, and severe-to-profound SNHL who are either hearing aid users or scheduled for cochlear implantation.

## Materials and methods

Thirty children (group 1) scheduled for cochlear implantation due to idiopathic severe-to-profound SNHL in Otorhinolaryngology Department, Ain Shams University Hospital were enrolled in this study. The second group consisted of another 25 children who are hearing aid users suffering from moderate and severe SNHL. Their age ranged from 2 to 18 years. Groups 1 and 2 were considered as the SNHL group.

The control group (group 3) comprised age-matched and sex-matched 40 children with normal peripheral hearing sensitivity. This group was chosen from the hospital patients who were planned for adenotonsillectomy surgery. Informed consent was obtained from all participants. The study was approved by the ethical committee in the university.

Exclusion criteria included syndromic HL, history of intake of ototoxic drugs, noise-induced HL, ear surgery, perforated tympanic membrane, Ménière's disease, cranial trauma, metabolic and systemic diseases, a family history of ischemic heart disease, and a personal history of cardiovascular disease or history of vitamin-B<sub>12</sub> or folic acid intake.

The study groups were subjected to the following: history, clinical examination, and full audiological evaluation were performed to all children by play audiometry or voluntary threshold estimation according to their age using air conduction thresholds (0.25–8 kHz), bone conduction thresholds (0.5–4 kHz), and speech audiometry including speech reception thresholds and word discrimination scores. Aided sound field hearing threshold in double-walled sound-treated booth IAC 1602, using two-channel audiometer Orbiter 922 (GN Otometrics, Taastrup, Denmark), warble tones was used to estimate aided thresholds. Immittanceometry including tympanometry and acoustic reflex thresholds was also performed using Zodiac immittanceometry. Finally, auditory brainstem response was performed to infants who were unable to perform play audiometry using biologic navigator. The level of hearing impairment was classified according to the criteria recommended by the WHO [21].

Radiological studies including computed tomographic scan and MRI of the temporal bone were performed for the SNHL group.

After an overnight fast, 3 ml of venous blood was collected in plain test tubes and left to clot completely. Serum was separated by centrifugation at 1500g for 15 min and collected in aliquots and stored at -20°C for the estimation of vitamin-B<sub>12</sub> and folic acid, which were performed by Magic Lite assay system (Ciba Corning Diagnostic Corp., Medfield, Massachusetts, USA) using chemiluminescent technique where the production of light is by a chemical reaction, which produces an electronically excited state in one of the reactants or products. The de-excitation process is accompanied by the emission of photons [22]. The normal level of vitamin-B<sub>12</sub> ranges from 240 to 1100 pg/ml, whereas that for folic acid is at least 3.6 ng/ml.

## Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences version 17 (SPSS; SPSS Inc., Chicago, Illinois, USA). The Shapiro–Wilk test was used to examine the normality of numerical variables. As these variables were skewed, they were presented as median and interquartile range. The Kruskal–Wallis test was used to compare differences among multiple groups with application of the Mann–Whitney *U*-test for post-hoc comparisons whenever the Kruskal–Wallis test revealed a statistically significant difference. The Mann–Whitney *U*-test was used to compare cases and controls with respect to numerical data. Nominal data were presented as ratio and intergroup differences were compared using the Pearson  $\chi^2$ -test.

Multivariable linear regression was used to identify determinants of serum vitamin-B<sub>12</sub> and folate levels. Variables not fulfilling the assumptions of linear regression were subjected to logarithmic transformation to eliminate skewness and to stabilize the variance of the sample.

The Bonferroni method was used to correct for multiple pairwise comparisons. This indicated that the level of significance for post-hoc comparisons with the Mann–Whitney *U*-test should be set at a two-tailed *P*-value of less than 0.0024 to keep the type I error at 0.05. Otherwise, a two-sided *P*-value less than 0.05 was considered statistically significant.

## Results

The study included 95 children, divided into three groups. Group 1 consisted of 30 children with severe-to-profound SNHL; their median age was 4.5 years, male-to-female ratio was 14 : 16, median vitamin-B<sub>12</sub> level was 124.5 pg/ml, and the median

folic acid level was 1.4 ng/ml. The second group (group 2) consisted of 25 children with moderate and severe SNHL using hearing aid; their median age was 5 years, male-to-female ratio was 12 : 13, median vitamin-B<sub>12</sub> level was 110 pg/ml, and median folic acid level was 1.3 ng/ml. The third group (group 3) consisted of 40 healthy volunteers; their median age was 5 years, male-to-female ratio was 21 : 19, vitamin-B<sub>12</sub> level was 686 pg/ml, and folic acid level was 3.9 ng/ml (Table 1; Figs 1 and 2).

Median vitamin-B<sub>12</sub> and folic acid levels were significantly higher in group 3 than in groups 1 and 2 patients ( $P < 0.001$ ). Multivariable linear regression model for determinants of serum vitamin-B<sub>12</sub> and folic acid level as adjusted for age, sex, and degree of SNHL was performed, showing that there was no significant correlation between vitamin-B<sub>12</sub> and folic acid level with other parameters such as age and sex and confirming the relationship between SNHL and their deficiency (Tables 2 and 3). In addition, there was no significant difference between groups 1 and 2 with respect to median vitamin-B<sub>12</sub> and folic acid level.

## Discussion

The cochlea is a highly vascularized organ and is supported by a single artery. Ischemic disorders may

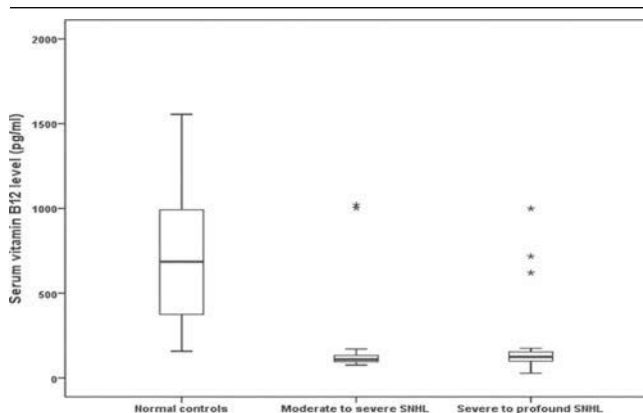
result in serious HL at any age. High homocysteine concentrations associated with low vitamin-B<sub>12</sub> and folate concentrations were shown to be risk factors for cerebral, coronary, and peripheral vascular diseases [23]. This condition may also adversely affect the blood supply of the cochlea resulting in SNHL.

HL has been documented as one of the neuropathological effects of vitamin deficiency [24,25]. However, the potential influence of the deficiencies of the B group of vitamins on auditory function has received little attention, and direct evidence linking SNHL with vitamin-B<sub>12</sub> (cobalamin) or folate deficiency is scarce in the literature.

In this study, we found that the median vitamin-B<sub>12</sub> and folic acid levels were significantly higher in group 3 than in groups 1 and 2 patients ( $P < 0.001$ ). These results suggest an effect of folate and vitamin-B<sub>12</sub> status on hearing threshold.

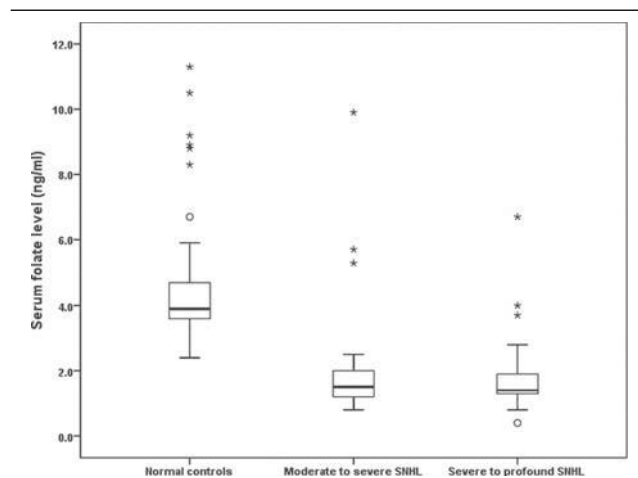
Roman [26] found high-frequency SNHL among elderly patients in Cuba and ascribed it to cobalamin and folate deficiency. Similarly, in a study on elderly female patients, poor cobalamin and folic acid status were found to be associated with age-related auditory dysfunction. In addition, some improvement was found

Figure 1



Box plot showing serum vitamin-B<sub>12</sub> level in the three study groups. It represents interquartile range. Line across box represents median. Error bars represent minimum and maximum values excluding extreme observations (asterisks). SNHL, sensorineural hearing loss.

Figure 2



Box plot showing serum folate level in the three study groups. It represents interquartile range. Line across box represents median. Error bars represent minimum and maximum values excluding outliers (rounded markers) and extreme observations (asterisks). SNHL, sensorineural hearing loss.

Table 1 Comparison of the three study groups

Variables	Group 3 (n = 40)	Group 2 (n = 25)	Group 1 (n = 30)	P-value
Age (years)	5.0 (4.0–7.0)	5.0 (3.5–5.0)	4.5 (3.75–6.0)	0.047
Male : female	21 : 19	12 : 13	14 : 16	0.877
Serum vitamin-B <sub>12</sub> level (pg/ml)	686.0 (345.25–995.0)	110.0 (92.5–135.5)*	124.5 (97.5–154.5)*	<0.001
Serum folate level (ng/ml)	3.9 (3.6–4.85)	1.5 (1.2–2.05)*	1.4 (1.275–1.9)*	<0.001

Data are represented as median (interquartile range) or ratio; \* $P < 0.001$  versus control group.

**Table 2 Multivariable linear regression model for determinants of serum vitamin-B<sub>12</sub> level as adjusted for age, sex, and degree of sensorineural hearing loss<sup>a</sup>**

Independent variables	Regression coefficient	SE	$r_{\text{partial}}$	$t$	$P$ -value
Constant	2.752				
Log (age) (years) <sup>a</sup>	0.059	0.160	0.038	0.365	0.716
Female sex <sup>b</sup>	-0.052	0.064	-0.086	-0.816	0.417
Moderate-to-severe SNHL <sup>c</sup>	-0.647	0.077	-0.661	-8.363	<0.0001
Severe-to-profound SNHL <sup>c</sup>	-0.638	0.073	-0.679	-8.772	<0.0001
Analysis of variance					
Source	$d.f.$	Sum of squares	Mean square	$F$ -ratio	Significance level
Regression	4	9.837	2.459	28.004	$P < 0.001$
Residual	90	7.904	0.088		

$d.f.$ , degrees of freedom;  $r_{\text{partial}}$ , coefficient of correlation between independent variable and outcome variable as adjusted for other variables in the model; SNHL, sensorineural hearing loss;  $t$ ,  $t$ -statistic; <sup>a</sup>Serum vitamin-B<sub>12</sub> level and age were subjected to logarithmic transformation owing to marked positive skewness; <sup>b</sup>Referenced to male sex; <sup>c</sup>Referenced to normal controls.

**Table 3 Multivariable linear regression model for determinants of serum folate level as adjusted for age, sex, and degree of sensorineural hearing loss<sup>a</sup>**

Independent variables	Regression coefficient	SE	$r_{\text{partial}}$	$t$	$P$ -value
Constant	0.629				
Log (age) (years) <sup>a</sup>	0.048	0.115	0.044	0.415	0.679
Female sex <sup>b</sup>	-0.058	0.046	-0.132	-1.262	0.210
Moderate-to-severe SNHL <sup>c</sup>	-0.397	0.056	-0.601	-7.132	<0.0001
Severe-to-profound SNHL <sup>c</sup>	-0.435	0.052	-0.659	-8.301	<0.0001
Analysis of variance					
Source	$d.f.$	Sum of squares	Mean square	$F$ -ratio	Significance level
Regression	4	4.260	1.065	23.444	$P < 0.001$
Residual	90	4.088	0.045		

$d.f.$ , degree of freedom;  $r_{\text{partial}}$ , coefficient of correlation between independent variable and outcome variable as adjusted for other variables in the model; SNHL, sensorineural hearing loss;  $t$ ,  $t$ -statistic; <sup>a</sup>Serum folate level and age were subjected to logarithmic transformation owing to marked positive skewness; <sup>b</sup>Referenced to male sex; <sup>c</sup>Referenced to normal controls.

in some of these patients following replacement therapy, suggesting a relationship between the deficiencies of these vitamins and auditory dysfunction. In addition, they statistically analyzed their findings and found a significant correlation between folate status and hearing threshold in their impaired hearing group [5]. In another study, Durga *et al.* found, interestingly, that low levels of homocysteine and high levels of B vitamins were associated with elevated hearing thresholds [27]. In the same way, Park *et al.* found that patients with impaired hearing had significantly higher prevalence of B<sub>12</sub> deficiency than those with normal hearing [28]. Now, a growing body of research suggests that folic acid can

lower homocysteine levels in both children and adults, and thereby decrease their likelihood of experiencing thrombotic events, including sudden HL [3].

Our results can explain that poor vascular health can affect the cochlea as it is heavily vascularized, thereby affecting hearing [5,26]. In addition, it was noted that there are links between low folate or B vitamin levels and elevated homocysteine as being risk factors for vascular disease. Many authors hypothesized that elevated homocysteine levels have been associated with many changes in microvessel flow mechanics, microvessel permeability, cochlear blood flow, and stria vascularis atrophy, thus resulting in HL [23,27,29,30]. In addition, Houston *et al.* [5] hypothesized that B<sub>12</sub> deficiency may impair myelination of neurons in the cochlear nerve, thus affecting hearing.

A person's blood concentration of folate and vitamin-B<sub>12</sub> can be affected by many factors such as their deficiency during pregnancy, which may lead to the risk for neural tube birth defects, including cleft palate, HL, spina bifida, and brain damage [31,32]. In addition, diet has been shown to affect folate and B<sub>12</sub> levels [31]. Medical conditions such as atrophic gastritis have been shown to cause malabsorption of folate and vitamin-B<sub>12</sub> [33].

In contrast, many studies have found no association between nutritional biology and auditory function [34,35]. Berner *et al.* [7] examined folate and B<sub>12</sub> status versus hearing thresholds in a correlational single-subject design study but did not find any relationship. This suggests the need for continued research into the role of vitamins in auditory function, particularly in developing countries where malnutrition is still rife.

## Conclusion

Study demonstrated that the serum levels of folate and vitamin-B<sub>12</sub> are decreased in patients with SNHL. Measurement of folic acid and vitamin-B<sub>12</sub> in patients suffering SNHL might be useful. These data may give us some clues about how HL is developed in these patients.

## Acknowledgements

### Conflicts of interest

There are no conflicts of interest.

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