Age-related hearing loss, vitamin B12, and folate in the elderly

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ABSTRACT

OBJECTIVE: Determine the correlation between the hearing threshold and the serum levels of vitamin B12 (cobalamin) and folic acid among elderly subjects (> 60 years) with age-related hearing loss (ARHL).

STUDY DESIGN: Cross-sectional.

SETTING: Community.

SUBJECTS AND METHODS: Subjects included elderly who were found apparently healthy following repeated examination by physicians. The pure tone average (PTA) for the speech and high frequencies, and the serum folate and cobalamin were determined and the correlation found.

RESULTS: The mean ± SD values of serum folate among the subjects with normal PTA in the speech frequencies (0-30 dB) was 412.3 nmol/L ± 17.6 nmol/L, while among those with hearing loss (HL), it was 279.1 nmol/L ± 17.2 nmol/L (P = 0.01). In the high frequencies, the mean ± SD values among the subjects with normal PTA was 426.3 nmol/L ± 17.6 nmol/L, while among those with HL, it was 279.14 nmol/L ± 171.2 nmol/L (P = 0.01). The serum cobalamin among the subjects with normal PTA within the speech frequencies was 49.7 pmol/L ± 9.4 pmol/L, while among those with speech-frequency HL, it was 42.6 pmol/L ± 10.2 pmol/L. However, for high frequencies, the mean ± SD values among the subjects with normal PTA was 47.4 pmol/L ± 7.3 pmol/L, while among those with HL, it was 41.3 pmol/L ± 9.2 pmol/L. Spearman’s correlation revealed that low folate (correlation coefficient = -0.27, P = 0.01) and cyanocobalamin (correlation coefficient = -0.35, P = 0.02) were significantly associated with increasing hearing threshold in the high frequencies. After adjusting for age, serum folate (correlation coefficient = -0.01, P = 0.01) was significant, while vitamin B12 (correlation coefficient = -0.01, P = 0.74) was not.

CONCLUSION: Serum folate was significantly lower among elderly with ARHL. Trials on nutritional supplementation may substantiate the role of serum folate in ARHL.

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while the exclusion criteria involved those with history of diabetes, stroke, hypertension, ear diseases, exposure to noise and ototoxic drugs such as aminoglycoside antibiotics or diuretics, ear infections, ear trauma, or ear surgery.

**Participants Recruitment**
Participants included elderly subjects who have been examined repeatedly by physicians in the outreach program; the blood pressure and random blood sugar were tested, and urinalysis was done. The subjects were repeatedly examined in order to recruit them among those elderly with no known medical illness. Consecutive eligible participants who were found to be free of any medical conditions were counseled for consent and recruitment into the study. Each participant was taken through an already prepared questionnaire for that purpose. Specifically, questions aimed at eliciting otological and general medical conditions were asked and these included: otorrhea, tinnitus, vertigo, otalgia, polyuria, polydipsia, significant weight loss, chronic cough, and palpitation. Participants were also asked about history suggesting of allergy and use of such medications as: aminoglycosides, diuretics, and 4-aminoquinolines antimalarial drugs. History of living near noise, as in, for example, a blacksmith shop, radio room/disco room, or welding shop for at least two hours per day for at least five days a week was also obtained.

This was followed by ENT examination and hearing test using pure tone audiometry. Specifically, subjects were examined for evidence of arteriosclerosis by palpating the walls of the radial artery or presence of locomotor brachialis—observing the pulsation of the brachial artery at the elbow. After the examination, collection of blood for estimation of serum levels of folate and vitamin B12, and pure tone audiometry were done. The criteria for the diagnosis of the medical condition were based on simple definitions, and all the subjects with medical conditions were excluded.

The study was approved by the Oyo State Research Ethical Review Committee.

**Blood Sample Collection and Storage**
Approximately 5 mL of whole blood was collected using the antecubital vein under aseptic conditions. The samples were stored at −80°C in batches for quantitative assay of folate and vitamin B12.

**Pure Tone Audiometry**
The pure tone audiometry was done using a computer audiometer BA 20 Kamplex (Interacoustic A/S, DK 5610, Assens, Denmark) with the subjects in a sitting position in the soundproof (acoustic) booth in the ENT clinic. The subjects were instructed to raise their hand if the tone presented to the ears was heard. The hearing acuity was measured in decibels (dB) at the frequencies 250 to 8000 Hz. The average for the four frequencies, 250 Hz, 500 Hz, 1000 Hz, and 2000 Hz was recorded as pure tone average (PTA) for speech frequency, while the average for the 3000 Hz, 4000 Hz, 6000 Hz, and 8000 Hz was recorded as the PTA for the high frequencies.

**Quantitative Assay of Folate**
A sample was prepared by pipetting 1.5 mL of the sample into a set of centrifuge tubes, and then 20.0 mL of ascorbic acid and 10.0 mL of sodium hydroxide (NaOH) were added and mixed properly. To this was added 5.0 mL of hydrogen chloride (HCl), and the mixture was shaken for 30 minutes and then centrifuged at 1500 revolutions per minute (rpm) for 30 minutes. The supernatant was collected and transferred onto a set of clean vials, and determination of the folate was done with high-performance liquid chromatography (HPLC).

**Quantitative Assay of Vitamin B12**
Into a clean beaker, 1.0 mL of the sample and 25 mL of 0.2 N HCl was added and warmed in a water bath for 30 minutes, then cooled and the pH adjusted to 6.0 using NaOH. This was followed by adding 1N HCl to lower the pH to 4.5, then transferred into a set of 50.0-mL centrifuge tubes, shaken for 30 minutes, and centrifuged for a period of 20.0 minutes at 2000 rpm. The supernatant was collected, and vitamin B12 determined by HPLC.

As a quality control measure, control and standard sera were included in the analysis at every sera assay to ensure reliability and quality of the procedure. An initial pilot study was conducted to test all instruments, and this was followed by a preliminary statistical analysis to detect outliers and correct factors.

**Statistics**
The main outcome variables were the serum levels of folate and vitamin B12 in elderly subjects with audiometric evidence of HL in the speech and high frequencies and those with normal PTA. In this study, HL was defined as PTA > 30 dB, and the control subjects were selected among elderly who have normal PTA (0-30 dB).

Data were initially explored using Stata software (StataCorp LP, College Station, TX), and Spearman’s correlation was utilized to determine the correlation between ranked and continuous variables. In order to adjust for the effect of age on the hearing threshold and the plasma levels of vitamin B12 and folate, a linear regression model was used. Level of statistical significance was at $P < 0.05$ for all the analyses.

**Results**
The subjects included 126 elderly subjects (males and females) and the ages ranged from 60 to 98 years (mean ± SD = 66.9 ± 7.77). Among the 126 subjects who had audiometry, the mean ± SD of the PTA for the air conduction was 29.4 dB ± 1.6 dB, while for the bone conduction (BC) it was 36.5 dB ± 1.8 dB. The mean ± SD of the PTA for the early frequency was 30.1 dB ± 1.5 dB, while the late frequency was 50.8 dB ± 2.0 dB.
The prevalence of speech-frequency HL increased with increasing age; Spearman’s correlation revealed that the association was significant \( (P < 0.03) \). Similarly, the prevalence of high-frequency HL increased with increasing age, although Spearman’s correlation was not significant \( (P = 0.09) \). In addition, increasing age was significantly associated with decreasing levels of serum cyanocobalamin \( (P = 0.04) \), while it was not significant for serum folate \( (P = 0.2) \).

Tables 1 and 2 show the frequency distribution of the serum levels of folate and cobalamin, respectively, according to the range of the PTA among the elderly subjects. Table 1 shows that the mean serum levels of folate among the subjects with normal hearing threshold (0-30 dB) was 412.3 nmol/L, while among those with hearing threshold above 30 dB, the mean was between 195.1 nmol/L and 380.0 nmol/L. Table 2 shows that the mean serum levels of cyanocobalamin among the subjects with a normal hearing threshold was 49.7 pmol/L, while among those with hearing threshold above 30 dB, the mean was between 39.9 pmol/L and 46.4 pmol/L.

The mean ± SD values of serum levels of folate among the elderly subjects with normal hearing threshold in the speech frequencies (0-30 dB) was 412.3 nmol/L ± 17.6 nmol/L, while among the elderly subjects with HL, it was 279.1 nmol/L ± 17.2 nmol/L. In the high frequencies, the mean ± SD values of serum levels of folate among the elderly subjects with normal hearing threshold was 426.3 nmol/L ± 17.6 nmol/L, while among those with HL, it was 279.14 nmol/L ± 17.12 nmol/L.

For the serum cobalamin, the serum levels among the elderly subjects with normal hearing threshold within the speech frequencies was 49.7 pmol/L ± 9.4 pmol/L, while among those elderly with speech-frequency HL, it was 42.6 pmol/L ± 10.2 pmol/L. However, for high frequencies, the mean ± SD values of serum levels of cobalamin among the elderly subjects with normal hearing threshold was 47.4 pmol/L ± 7.3 pmol/L, while among those with HL, it was 41.3 pmol/L ± 9.2 pmol/L.

Spearman’s correlation revealed that decreasing serum folate \( (P = 0.01) \) and cyanocobalamin \( (P = 0.02) \) were significantly associated with increasing hearing threshold among subjects with high-frequency HL (Table 3). However, after adjusting for age, linear regression revealed significant correlation between the levels of serum folate \( (P = 0.01) \) and hearing threshold in the high frequencies (Table 4).

On the other hand, the serum levels of folate and cyanocobalamin did not show any significant correlation with

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**Table 1**

<table>
<thead>
<tr>
<th>Mean hearing threshold (PTA), in decibels</th>
<th>Number of subjects</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-30</td>
<td>38</td>
<td>135.0</td>
<td>589.0</td>
<td>412.3</td>
<td>17.6</td>
</tr>
<tr>
<td>31-40</td>
<td>20</td>
<td>134.3</td>
<td>587.3</td>
<td>287.9</td>
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<td>41-50</td>
<td>15</td>
<td>100.1</td>
<td>499.3</td>
<td>195.2</td>
<td>9.7</td>
</tr>
<tr>
<td>51-60</td>
<td>24</td>
<td>126.0</td>
<td>581.5</td>
<td>284.5</td>
<td>16.5</td>
</tr>
<tr>
<td>61-70</td>
<td>14</td>
<td>134.0</td>
<td>581.5</td>
<td>273.5</td>
<td>16.2</td>
</tr>
<tr>
<td>71-80</td>
<td>15</td>
<td>121.1</td>
<td>581.5</td>
<td>380.0</td>
<td>12.4</td>
</tr>
</tbody>
</table>

*PTA, pure tone average.*

**Table 2**

<table>
<thead>
<tr>
<th>Mean hearing threshold (PTA), in decibels</th>
<th>Number of subjects</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-30</td>
<td>38</td>
<td>29.0</td>
<td>62.1</td>
<td>49.7</td>
<td>9.2</td>
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<tr>
<td>31-40</td>
<td>20</td>
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<td>11.8</td>
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<tr>
<td>41-50</td>
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<td>26.0</td>
<td>66.7</td>
<td>39.9</td>
<td>10.9</td>
</tr>
<tr>
<td>51-60</td>
<td>24</td>
<td>29.0</td>
<td>58.1</td>
<td>42.0</td>
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<tr>
<td>61-70</td>
<td>14</td>
<td>26.2</td>
<td>70.2</td>
<td>42.9</td>
<td>13.5</td>
</tr>
<tr>
<td>71-80</td>
<td>15</td>
<td>28.1</td>
<td>65.0</td>
<td>46.4</td>
<td>10.6</td>
</tr>
</tbody>
</table>

*PTA, pure tone average.*
Discussion

The main finding in this study is that low serum levels of folic acid are significantly associated with HL in high frequencies among apparently healthy elderly people. Also, we found correlation between high-frequency HL and low-serum vitamin B12, although serum vitamin B12 seemed to be affected by increasing age, hence the correlation with vitamin B12 was not found to be significant after adjusting for age. These findings suggest that the low levels of these nutritional markers, particularly folate, may be significant for the development of HL among these elderly subjects. This is similar to the findings of Roman, who reported sensorineural hearing impairment in the high frequencies in Cuba. In that study on an epidemic of peripheral neuropathy, he found association between sensorineural HL and low intakes of vitamin B12, folate, thiamine, and sulfur amino acids. It is also supported by an animal experiment, which found impairment in the cellular mechanism involving both the nervous and vascular systems, hence, it was concluded that B12 deficiency is responsible for the neuropathological effect. The fact that our selected subjects were apparently healthy elderly people suggests to us that the low-serum folate and cobalamin might be the factor responsible for the HL. This could be due to the eighth-nerve neuropathy or cochleopathy, or both. A controlled study by Houston et al examined 55 healthy elderly women and found that PTAs over the 500- to 4000-Hz range were inversely correlated with serum vitamin B12 and red cell folate, and that women with impaired hearing had a 38 percent lower B12 vitamin level and a 31 percent lower red cell folate level. In contrast, Berner et al did not find any association between hearing levels and either B12 vitamin, folic acid, or homocysteine levels in elderly subjects.

In our study, the serum levels of folate were between 100.1 nmol/L and 589.0 nmol/L, while the levels of vitamin B12 were between 26.2 pmol/L and 70.2 pmol/L. This was low compared to the work of Berner et al, which reported folate between 295 nmol/L and 1160 nmol/L and vitamin B12 between 90 pmol/L and 737 pmol/L. But it is comparable to the report of Houston et al on elderly women, which reported folate to be between 79 nmol/L and 380 nmol/L and vitamin B12 between 28 pmol/L and 502 pmol/L. These two studies cited are from developed countries of the West; however, in medically underserved populations like ours, these relatively low levels of vitamins are expected. Although there is no documented figure for the country as of now, these figures may be a reflection of the low levels of the vitamins in the general population. It may be due to poor nutrition and may also be due to problems with storage of specimens and other stages in the assay procedure.

In addition, our findings revealed that increasing age had significant effect on hearing threshold in the speech frequencies and the serum levels of vitamin B12, but not folate. Thus, it is suggested that low serum folate could account for the increased hearing threshold observed among those with high-frequency HL. Similar to the question raised for reduction in the other medical conditions, one main issue arising from this study is whether elderly people should, in general, receive folate and vitamin B12 supplements in order to reduce the risk of hearing impairment. It has been proposed that low levels of vitamin B12 and folate are associated with destruction of the microvasculature of the stria vascularis, which might result in

<table>
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<tr>
<th>Variables</th>
<th>Spearman’s coefficient</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Folate</td>
<td>-0.27</td>
<td>0.01</td>
</tr>
<tr>
<td>Cyanocobalamin</td>
<td>-0.35</td>
<td>0.01</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Variables</th>
<th>Coefficient</th>
<th>Significance</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Folate*</td>
<td>-0.01</td>
<td>0.01</td>
<td>-0.01–0.01</td>
</tr>
<tr>
<td>Cyanocobalamin*</td>
<td>-0.01</td>
<td>0.74</td>
<td>-0.01–0.01</td>
</tr>
</tbody>
</table>

*Adjusted for age.
decreased endocochlear potential and hence, hearing impairment. Vitamin B12 has been used in conjunction with other agents, such as clari-thromycin, prednisolone, and immunoglobulin G, to treat auditory dysfunction. In addition, cognitive indexes and peripheral and central nervous system symptoms indicating deficiencies of vitamin B12, folate, or both, have been sometimes reversed following repletion. This may change our present belief about the irreversibility of age-related hearing impairment, thus improving the outcome of the disease and quality of life of the affected elderly people.

Conclusion
Serum folate was significantly lower among elderly people with ARHL. Trials on nutritional supplementation may substantiate the role of serum folate in ARHL.

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References