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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. 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.

Age-related hearing loss (ARHL) has been associated with environmental factors such as noise, malnutrition, ototoxicity, infections, and genetics.[1-6] Hearing loss (HL) has been documented as one of the neuropathological effects of some vitamin deficiencies;[7] however, the potential influence of the deficiencies of the B group of vitamins on auditory function has received little attention,[3] and direct evidence linking ARHL with vitamin B12 (cobalamin) or folate deficiency is scarce in the literature. Roman[5] found high-frequency sensorineural HL among elderly patients in Cuba and ascribed it to cobalamin and folate deficiency. Similarly, in a recent study of elderly female subjects, poor cobalamin and folic acid status were found to be associated with age-related auditory dysfunction.[8] In addition, some improvements were found in some of these patients following replacement therapy, suggesting a relationship between the deficiencies of these vitamins and auditory dysfunction.[8] In contrast, many works have found no association between nutritional biology and auditory function.[1-4,9] This suggests the need for continued research into the role of vitamins in auditory function, particularly in developing countries where malnutrition is still rife. Our hypothesis is that multiple vitamin deficiencies may be a significant contributor to the severity of ARHL. The objective of this study was to determine, in a population of apparently healthy elderly subjects with ARHL, the association, if any, between the hearing threshold and the serum levels of cobalamin and folic acid.

Method

Study Design

This is a cross-sectional study of the immunobiology of HL in apparently healthy elderly subjects. The participants were drawn from the community tagged to an outreach program organized for the detection and prevention of illnesses among the elderly.

Included in the study were all elderly men and women above 60 years of age who had no known medical condition,

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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 suggestive 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.[10,11] 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).[12]

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.[12]

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 $\pm$ SD = 66.9 $\pm$ 0.77). Among the 126 subjects who had audiometry, the mean $\pm$ SD of the PTA for the air conduction was 29.4 dB $\pm$ 1.6 dB, while for the bone conduction (BC) it was 36.5 dB $\pm$ 1.8 dB. The mean $\pm$ SD of the PTA for the early frequency was 30.1 dB $\pm$ 1.5 dB, while the late frequency was 50.8 dB $\pm$ 2.0 dB.