Int. Adv. Otol. 2009; 5:(3) 340-344

ORIGINAL ARTICLE

Homocysteine, Folate and Vitamin B12 Concentrations in Middle Aged Adults Presenting with Sensorineural Hearing Impairment

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Objective: Aim of this study was to investigate a possible association between hearing impairment and homocysteine, folate and vitamin B12 concentrations in subjects presenting with sensorineural hearing loss before the geriatric age, namely before the age of 60.

Materials and Methods: Pure tone audiometry was performed and the age expected deviations were calculated according the ISO 7029. Two groups of patients were studied, one (n=78) with hearing impairment, and a control group (n=53), matched in age, without hearing impairment.

Results: In blood, mean concentration of vitamin B12 was lower in the hearing impairment group (251.7 +/- 158.6 pg/ml, mean +/- SD) than in the control group (304.9 +/- 152.0 pg/ml, p=0.004); folate concentration was also lower in patients with hearing impairment (7.26 +/- 3.25 ng/ml) compared to controls (8.29 +/- 3.11 ng/ml, p=0.029), whereas homocystein was higher (hearing impairment: 16.59 +/- 5.15 µmol/ml; control: 13.69 +/- 4.61 µmol/ml, p=0.001).

Conclusion: In both groups, no correlation between the age and the blood concentrations of vitamin B12, folate and homocysteine could be found.

Our findings suggest that elevated homocystein and decreased folate and vitamin B12 concentrations accompany to hearing loss before the geriatric age.

Submitted : 14 April 2009 *Revised : 30 June 2009* *Accepted : 9 July 2009*

Presbycusis, characterized by age-related symmetrical high frequency sensorineural hearing loss, is a major health issue in the elderly. The prevalence is high, but the biological basis of presbycusis is not clear yet. Presbycusis can also be described as aging of inner ear and/or cochlear nerve, and several factors may have a role in etiopathogenesis such as vascular, biochemical and genetic disorders. Defining relevant factors objectively may help to take precautions.

Ultrastructurally, age-relatedhearing loss is associated with loss of hair cells and other cellular elements of the cochlea. The cochlea is a highly vascularized organ and is supported by a single artery. Ischemic disorders may result in serious hearing loss at any age. High homocysteine concentrations associated with low vitamin B12 and folate concentrations were shown to be risk factors for cerebral, coronary and peripheral vascular diseases. [1] This condition may also adversely affect the blood supply of the cochlea.

Homocysteine is a sulphydryl-containing amino acid derived from the metabolic demethylation of dietary methionine abundant in animal protein. Folate therapy alone or combined with vitamin B6 and B12 reduces homocysteine concentrations even in people who do not have vitamin deficiency.[2]

In this study, we aimed to evaluate homocysteine, vitamin B12 and folate blood concentrations in middle aged adults (40-60 years) with sensorineural hearing loss. Since the geriatric age was accepted as beginning at the age of 60, this type of sensorineural hearing loss may be called early presbycusis or sensorineural hearing loss before the geriatric age. We thought that this study would contribute useful data for promoting preventive strategies if higher homocysteine and

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Acknowledgement
Special thanks to S. Kenan Köse, PhD for statistical analysis

decreased vitamin B_{12} and folate blood concentrations were found in patients presenting with sensorineural hearing loss before the geriatric age.

Materials and Methods

One hundred and ten patients between 40 and 60 years of age were retrospectively identified from clinical records, and invited to participate in the study. Eighty-three patients (75.5%) accepted to participate in the study, but 5 of them were excluded due to recent consumption of vitamin B12 and/or folate supplements. Other exclusion criteria were ototoxic drug usage, noise exposure, ear surgery, perforated tympanic membrane, Méniere's disease, cranial trauma, metabolic and systemic diseases, a family history of ischaemic heart disease and a personal history of cardiovascular disease.

The control group comprised of age matched 58 persons who did not complain about hearing loss. This group was chosen from the hospital employees and patients who were planned for nasal surgery. Informed consent was obtained from all participants. The study was approved by the ethics committee.

Clinical investigation

Hearing levels were analyzed as follows: Pure tone average (PTA1): Hearing threshold at 250 Hz ; PTA2: Average hearing threshold at 500, 1,000 and 2,000 Hz and PTA3: Average hearing threshold at 4,000, 6,000 and 8,000 Hz. Hearing threshold deviations expected with age according to ISO 7029 were calculated with the equation: $\Delta H_{md,,Y} = ks_u$[3]. Hearing thresholds were adjusted in terms of age according to ISO 7029, and deviation of the hearing thresholds *(ΔH)* was calculated (Figure 1). Discrimination levels were not compared since just cochlear hearing losses were taken into account in this study.

Audiometry

The initial hearing examination included otoscopy, tympanometry and a complete audiologic evaluation, including pure tone air and bone conduction audiometry and speech audiometry. Pure tone audiometry was performed at the frequencies of 250, 500, 1,000, 2,000, 4,000, 6,000 and 8,000 Hz using an

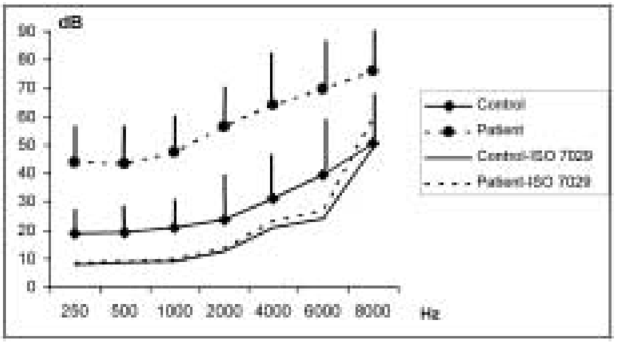

Figure 1. Comparison of median hearing thresholds of patient and control groups. Thresholds have been corrected to the age according to ISO 7029 standards.

AC-40 diagnostic audiometer in a sound-treated cabin (Interacoustic Company, Denmark). Normal middle-ear function was defined by immittance and acoustic reflex results using an Interacoustic AZ 26 clinical impedence meter (Interacoustic Company, Denmark). The patients and controls who had normal peak compliance, peak pressure, gradient and ear canal volume obtained by immittance measures (as defined by the American Speech Language and Hearing Association) were included in the study.[4]

Laboratory analysis

Seven ml of blood were taken from antecubital vein by BD Vacutainer(tm) after overnight fasting, and transported to the laboratory in a cool box. Samples were centrifuged and seperated into two portions. The first portion was processed within an hour for vitamin B12 and folate analysis, and second portion was frozen at -20ºC for subsequent homocysteine analysis.

Vitamin B12 and folate concentrations were determined in serum by microparticle enzyme immunoassay method (MEIA), using Abbott commercial kits (Abbott Laboratories, Abbott Park, Illinois/USA) on Abbott Architect I2000 (Abbott Inc, IL, USA) system. The reference ranges for vitamin B12 was 145- 980 pg/ml and >2.7 ng/ml for folate.

Concentrations of homocysteine were measured in plasma in ethylene diamine tetraacetic acid (EDTA) by Shimadzu HPLC analyzer (Shimadzu Corporation, Kyoto/Japan) using Recipe commercial kits (Recipe Chemical and Instruments GMBH, Munich, Germany) with a reference range of 5-14 µmol/l.

Statistical analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences version 11.5 software for Windows. A "p" value less than 0.05 was considered significant. The relationship among blood concentrations, age and hearing loss were investigated by Mann-Whitney U test. Chi-square testing was used to compare the gender of patients and controls. Independent t- test was used to compare age of the patients and control groups. Spearman Rank Correlation analysis was used to investigate the relationship among the quantitative variables.

Results

The mean age of patients and controls were 49.3 ± 6.29 years (male/female = 33/45) and 47.5 ± 6.43 years (male/female = 31/22) respectively.

Pure tone averages in the hearing impaired group and control group are summarized in Table 1. Mean concentrations of vitamin B12, folate and homocysteine are summarized in Table 2.

In the hearing impaired group, mean homocysteine concentrations were significantly higher compared to control group (p= 0.001). Vitamin B_{12} and folate levelss were significantly lower in the hearing impaired group (p=0.004 and p=0.029, respectively). In the hearing impaired group, comparison of blood parameters and hearing levels showed that there was only a weak positive correlation (r=0.228; p=0.045) between hearing loss at 250 Hz and homocysteine levels. There was a weak negative correlation between hearing loss at 250 Hz and vitamin B12 level (r=0.246; p=0.030).

When ΔH values were taken into consideration, there was a positive correlation (r= 0.255; p=0.024) between ΔH at 250 Hz and homocysteine concentrations. At 250 and 500 Hz f, there was a negative correlation between ΔH and vitamin B12 levels (r=-0.359; p=0.007). There was no correlation at any frequency between folate and ΔH values.

Both in the hearing impaired and control groups, no correlation could be found between both sex and age with blood concentrations of vitamin B12, folate and homocysteine.

Discussion

Deterioration in hearing thresholds is an expected

Table 1. Descriptive statistics for PTA in hearing impaired and control groups.

Frequency (Hz)	Hearing impaired Mean±SD	Control Mean±SD	p
250	40.48±14.32	18.66±6.81	<0.001
500	40.26±12.04	19.09±6.81	<0.001
1,000	44.96±10.76	20.60±7.32	<0.001
2,000	55.86±12.99	23.36±7.26	<0.001
4,000	63.36±14.95	30.95±9.31	<0.001
6,000	69.20±12.49	39.22±10.92	<0.001
8,000	75.35±10.18	50.34±11.04	<0.001

Table 2. Descriptive statistics of homocysteine, folic acid and vitamin B12 levels in study and control groups.

	Patient (n=78)			**Control (n=58)**			**P**
	Mean ± SD	**Median**	**Min- Max.**	**Mean ± SD**	**Median**	**Min- Max**	
Vitamin B12 (pg/ml)	251.7 ± 158.6	211.5	85-1239	304.9 ± 152.0	279	140-965	0.004
Folic acid (ng/ml)	7.26 ± 3.25	6.275	3.12-15.60	8.29 ± 3.11	7.68	3.34-15.12	0.029
Homocysteine (μmol/l)	16.59 ± 5.15	16.7	3.68-28.92	13.69 ± 4.61	13.22	5.70-23.60	0.001

Normality test was made by Shapiro-Wilk test. Since data did not show a normal distribution, Mann-Whitney–U test was applied for comparing control and patient groups.

outcome with aging, whereas individuals having greater hearing loss corrected with their age might possess some risk factors. Association of homocysteine and sensorineural hearing loss before the geriatric age has not been studied before. Hyperhomocysteinaemia as a risk factor for age-related hearing loss and sudden sensorineural hearing loss has been investigated by some authors with divergent results. In one study comprising of a patient group with a median age of 78 years, comparison of the hearing levels between those with increased and normal homocysteine concentrations failed to show any significant differences. However, there was no control group in that research.[5] In a recent study, results of the effects of three-year folate supplementation on hearing loss have been reported. Folate supplementation with its lowering effect on homocysteine concentrations slowed the decline in hearing at the low frequencies by 0.7 dB annualy in 728 older adults.[6] Beneficial effect of folate supplementation on low frequency hearing sensitivity in that study supports our findings. Age-related hearing loss at the low frequencies (250, 500, and 1,000 Hz) has been correlated with cardiovascular disease in white men and women.[7] High homocysteine concentrations appear to be a common risk factor both in low frequency hearing loss and cardiovascular diseases.

Other studies have shown an association between low folate and elevated total homocysteine levels[8,9] and sudden sensorineural hearing loss and noise induced hearing loss.[10] An association between age-related hearing loss, vitamin B12 and folate concentrations have been found in a female population aged between 60-71 by Houston et al.[7]

Hyperhomocysteinaemia may contribute to vascular pathology through its effects on atherosclerosis, endothelial dysfunction, impaired arterial compliance, chronic inflammation, oxidative stress and dysregulation of lipid metabolism.[11] In atherosclerotic lesions of animals and humans, folate deficiency has been shown to induce DNA hypomethylation, a process closely linked to hyperhomocysteinaemia.[12,13]

The Hordaland Homocysteine Study (HHS) screened a large population and investigated the association between homocysteine concentrations and many diseases.[14] They found that raised homocysteine levels were associated with increased risk for cardiovascular morbidity, cardiovascular and noncardiovascular mortality, depression and cognitive deficiency, osteoporosis and pregnancy complications. An association between homocysteine and hearing status was not investigated in the HHS.

In this study, mean homocysteine concentrations were significantly higher whereas vitamin B12 and folate concentrations were lower in the hearing impaired group compared to controls. Although correlation analysis between PTA and homocysteine, folate and vitamin B12 concentrations yielded a statistical significance at 250 Hz only, the mean concentrations between the hearing impaired and control groups exhibited statistically significant differences.

According to our results, high homocysteine with low folate and vitamin B12 concentrations may be risk factors leading to early decline of hearing levels. Presbycusis initially affects high frequencies and slowly extends to lower frequencies by aging. Our finding of low frequency (250 Hz) hearing loss may be due to the possibility that hearing loss has already passed the initial phase. The apex and base of the cochlea are responsible for transducing low-frequency sounds and high-frequency sounds, respectively. It has been postulated that cochlear sensory cells of the apex may be the most susceptible to aberrations in microcirculation in the stria vascularis because the apex is the farthest point that blood supply of the cochlea reaches.[15] Affects of high homocysteine and low folate and vitamin B12 concentrations on vascular supply may be the cause of decline in hearing sensivity at 250 Hz frequency. Low vitamin B12 and folate concentrations may also cause an auditory dysfunction by altering neuronal functions.[7]

The hearing threshold deviations expected with age according to ISO 7029 were calculated. Pure tone averages in control group were found to be higher especially at lower frequencies then expected values according to their ages. This finding must be evaluated to find the real levels of hearing in this age group in the Turkish population within an outpatient population based study.

As with all association studies, the proof of causality for early presbycusis requires intervention trials with homocysteine lowering treatment, to see if progression of sensorineural hearing loss is slowed.

In conclusion: (1) As in vascular and some chronic disorders, high homocysteine, low vitamin B12 and folate levels were found in patients presenting with sensorineural hearing loss before geriatric ages, (2) populations with sensorineural hearing loss before geriatric age may be screened for cardiovascular and some chronic disorders and (3) homocysteine lowering supplementation may be needed for prevention of presbycusis..

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