

Comparative analysis of serum homocysteine, folic acid and Vitamin B₁₂ levels in patients with noise-induced hearing loss

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Received 7 July 2003; accepted 19 September 2003

Abstract

Objective: The aim of the present study was to determine the levels of homocysteine, folic acid, and Vitamin B₁₂ in subjects with noise-induced hearing loss. Furthermore, possible links between these parameters and noise-induced hearing loss were aimed to be evaluated. **Methods:** In the present study, blood samples were obtained from all subjects after overnight fasting for biochemical analysis. We examined the levels of homocysteine, Vitamin B₁₂ and folic acid levels in subjects with noise-induced hearing loss. Twenty-eight male patients with noise-induced hearing loss (mean age 37 ± 5 year) were included in the study group whereas the control group was composed of 32 healthy male volunteers (mean age 36 ± 4 year). **Results:** It was found that homocysteine levels of subjects with noise-induced hearing loss as significantly high compared to healthy controls ($P < 0.05$). On the other hand, Vitamin B₁₂ and folic acid levels of patients with noise-induced hearing loss were determined to be significantly low compared to the controls ($P < 0.05$ and < 0.01 , respectively). **Conclusion:** Our findings indicate that there might be a link between increased homocysteine levels and noise-induced hearing loss. Since increased homocysteine levels cause elevated levels of free radicals in addition to its atherogenic and thrombogenic effects. Further experimental studies are needed to decipher how this relationship is linked.

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Abbreviations: NIHL, noise-induced hearing loss; Hz, hertz; KHz, kilohertz; dB, decibel; SNHL, sensorineural hearing loss

Keywords: Noise-induced hearing loss; Homocysteine; Vitamin B₁₂; Folic acid

1. Introduction

Noise-induced hearing loss (NIHL) is a common occupational disease in the adult population. NIHL has been documented to occur by outcomes of metabolic effects. Patients exposed to noise for a long duration have hearing loss that especially maximal at 3–6 kHz and this hearing loss slightly improves at high frequencies, but it is not affected at low frequencies [1].

Reduction in blood flow of vertebral or basillar artery is shown in labirentin artery and than hypoxia in inner ear is developed. Changes in blood flow of inner ear result in pathologies such as acute hearing loss and hearing loss due to

aging [2]. When blood velocity, plasma viscosity and whole blood viscosity are taken together with recently used ball filtration parameters accordance between these parameters and hearing thresholds of patients were documented [3].

In the body, atherosclerosis causes damage in organs perfused especially by end-arterial system. Cochlea is also perfused from end-arterial system so labirentin dysfunction appeared according to the degree of arteriosclerosis [4].

Homocysteine is a sulphhydryl-containing amino acid derived from the metabolic demethylation of dietary methionine abundant in animal protein [5]. Mild to moderate elevations of homocysteine have been associated with vascular disease in retrospective case control studies. Association with peripheral vascular or cerebrovascular disease appears to be stronger than for coronary artery disease [6,7]. The prevalence of hyperhomocysteinemia has been estimated to be 5% in the general population whereas its prevalence

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among patients with symptomatic atherosclerotic vascular disease was reported to be 13–47% [8]. Homocysteine levels in adult patients with coronary artery disease or peripheral vascular disease are reported to be 30–50% higher than those in normal subjects [9]. Epidemiologic studies have consistently demonstrated that high plasma homocysteine level is an independent risk factor for atherosclerosis, as important as serum cholesterol level [10].

Humans can not synthesize folate and therefore they must obtain it in their diet. Major sources of folate are green vegetables, citrus fruits, liver and whole grains. Dietary supplementation with folate can normalize plasma homocysteine levels and may thereby reduce the risk of cardiovascular disease [11]. Folic acid therapy alone or combined with Vitamin B₆ and B₁₂ reduces homocysteine levels even in people who do not have vitamin deficiency. Dietary supplementation with folate at amount up to 2 g per day progressively decreases plasma homocysteine level, with significant decrease occurring with 200–400 µg per day. It seems likely that dietary supplementation with 0.4–1 mg of folate will reduce the risk of neurodegenerative disorders [12].

The aim of the present study was to determine the levels of homocysteine, folic acid, and Vitamin B₁₂ in subjects with NIHL. Furthermore, possible links between these parameters and NIHL were aimed to be evaluated.

2. Materials and methods

Workers in a local hydroelectric powerhouse were recruited to form the study group (*n*: 28; mean age: 37 ± 5 year). Control group was composed of 32 healthy volunteers (mean age 36 ± 4 year). All of the subjects in study and control groups were male.

A questionnaire form was applied to all subjects. Date of birth, age of initializing for work, duration of working, daily working and resting times were questioned and compiled. The patients who have the history of using ototoxic drugs, previous ear diseases, trauma to head and the ear, and familial history of hearing diseases were excluded from this study. Before clinical and laboratory investigations, consent of all subjects were taken. Complete physical examination of ear, nose and throat and audiometrical evaluation were performed in all subjects.

The audiometric tests were performed in a sound-proof chamber with an Interacoustics AC-40 audiometer that was calibrated according to ISO 1964 standards. Pure tone audiometry was applied to all patients and controls. Frequencies at octave intervals from 250 to 16 000 Hz were tested for air conduction and from 500 to 4000 Hz for bone conduction. Hearing was accepted as abnormal if the hearing threshold was 25 dB or more below than the age-corrected level at two or more test frequencies.

In our criterion for NIHL, hearing threshold is normally in 1 kHz but hearing loss is more than 25 dB in 4–6 kHz.

Noise levels were measured by a noise measurer (Bruel and Kjaer 2235, Copenhagen, Denmark).

Blood samples were obtained from all subjects after overnight fasting for biochemical analysis. Levels of folic acid and Vitamin B₁₂ were determined by ELECSYS-170 autoanalyzer using commercial kits (Roche Diagnostica GmbH, Monnkeim, Germany). Levels of homocysteine were measured by Shimadzu HPLC analyzer using Recipe commercial kits (Recipe Chemical and Instruments GmbH, Munich, Germany).

Statistical analyses were performed using SPSS version 10.0. All test used were two-tailed and *P* < 0.05 was considered as significant. The data are given as mean ± standard deviations (S.D.). Comparison between groups were performed using the Student's *t*-test. Pearson correlation coefficient was used to assess the relationship between variables.

3. Results and discussion

Determined levels of serum homocysteine, folate and Vitamin B₁₂ are summarized in Table 1. The audiograms of study and control groups are shown in Fig. 1. In study group the hearing loss was started at 4000 Hz and reached to maximum at 6000 Hz. Mean homocysteine levels were significantly higher in NIHL patients (*P* < 0.02; Fig. 2), but mean folate and Vitamin B₁₂ levels were significantly lower in NIHL patients (*P* < 0.05 and <0.001, respectively) compared to controls. There was no significant correlation between homocysteine levels and the other parameters.

Because of rapid industrialization, widespread increase of noise sources and increased population being exposed to noise, NIHL is the most important cause of sensorineural hearing loss (SNHL). To function normally, inner ear must be supplied by arterial system continuously and regularly. In addition to thrombus, emboli, bleeding in arterial system, spasm and hypercoagulation affecting vascular system also disrupts perfusion of inner ear [13].

Homocysteine is an atherogenic and thrombogenic risk factor. Homocysteine metabolism depends on the cofactors folate, Vitamin B₁₂ and B₆. Elevated homocysteine levels causes vascular complication(s) [14]. Hyperhomocysteinaemia has been associated with elevations in tissue iron stores and increased in vivo lipid peroxidation [15]. The

Table 1
The levels of homocysteine, folate and Vitamin B₁₂ in subjects with NIHL and control groups

Parameter	Control group (n=32)	NIHL group (n=28)	<i>p</i> value
Homocysteine (µmol/L)	11.67±2.54	14.015±5.10	<0.02
Folate (nmol/L)	12.69±3.61	10.71±4.16	<0.05
Vitamin B ₁₂ (pmol/L)	323.62±121.91	199.87±75.25	<0.001

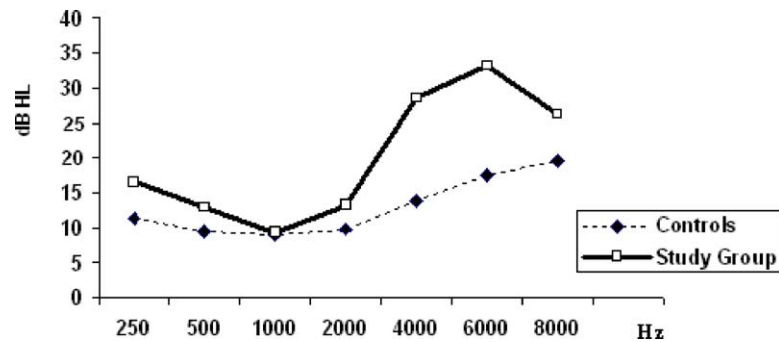


Fig. 1. The audiograms of study and control groups.

reason for this is the increasing evidence that homocysteine plays vital role(s) in developmental and adult neurological disorders, and the fact that homocysteine metabolism is affected by dietary factors, most notably folate. The amino acid methionine plays a pivotal role in one carbon metabolism, a series of biosynthetic pathways crucial for DNA synthesis and repair, and various methylation reactions [16].

Vitamins B₁₂ and B₆ are cofactors in the metabolic pathways that affect homocysteine levels. Vitamin B₁₂ deficiency can result in psychomotor regression, sensory neuropathy, severe hypotonia, seizures and apathy, which could result from impaired myelination [17].

Several findings suggest that elevated homocysteine levels might alter synaptic function. Overactivation of glutamate receptors is implicated in the pathogenesis of several neurodegenerative disorders, and homocysteine can activate synaptic glutamate receptors either directly or indirectly after metabolism into L-homocysteic acid [18]. Homocysteine might also render neurons vulnerable to excitotoxicity by inducing DNA damage [19]. Although it is not known if and how folate modifies synaptic plasticity, one study suggests that folate can enhance excitability of hippocampal circuits by presynaptic disinhibition of GABAergic neurons [20]. The available data suggest several possible mechanisms whereby homocysteine damages and kill neurons. Homocysteine induces DNA breakage in cultured neurons by a mechanism that may involve impaired transmethylation of DNA. Because folate and Vitamin B₁₂ deficient retard methionine regeneration, S-acetyl-methionine levels are also reduced as a consequence of folate or Vitamin B₁₂ deficiency [21]. Although the supplementation

with combination of folic acid 2–5 mg, Vitamin B₆ 25 mg, and Vitamin B₁₂ 250 µg per day reduces the progression of atherosclerosis, as measured by carotid plaque area, it remains to be confirmed that homocysteine-lowering therapy will prevent important atherosclerotic vascular events in patients with moderate hyperhomocysteinaemia [22].

Oxidative stress plays a substantial role in the genesis of noise-induced cochlear injury that causes permanent hearing loss [23]. It has been reported that relatively little oxidation occurs in plasma, due to high plasma concentration of antioxidants, but most of the oxidation may occur in the vascular intima [24].

Increased homocysteine levels may cause reductions in intracellular concentrations of glutathione, thus it increases lipid peroxidation [25].

In conclusion, present study demonstrates that the serum levels of homocysteine are increased and serum levels of folate and Vitamin B₁₂ are decreased in patients with NIHL. Measurement of homocysteine, folic acid and Vitamin B₁₂ levels during routine control of subjects working in a noisy environment might be useful. These data may give us some clues about how hearing loss is developed in these subjects. Experimental studies are needed to decipher exact mechanism and roles of homocysteine, folic acid and Vitamin B₁₂ in sensorineural hearing loss.

References

- [1] Ward WD. Noise induced hearing damage. In: Paperella MM, Shumrick DA, Gluckman JL, Meyerhoff WL, editors. Otolaryngology. London: Saunders; 1991. p. 1639–52.
- [2] Campbell JB, Pearman K, Nahl SS. Basilar artery ectasia: a rare cause of sensorineural deafness. *J Laryngol Otol* 1986;100:333–5.
- [3] Gatehouse S, Gallacher JE, Lowe GD, Yarnell JW, Hutton RD, Ising I. Blood viscosity and hearing levels in Caerphilly Collaborative Heart disease study. *Arch Otolaryngol Head Neck Surg* 1989;115:1227–30.
- [4] Donaldson JA, Ducker LG. Anatomy of the ear. In: Paperella MM, Shumrick DA, Gluckman JL, Meyerhoff WL, editors. Otolaryngology. London: Saunders; 1991. p. 23–59.
- [5] Ueland PM. Homocysteine species as components of plasma redox thiol status. *Clin Chem* 1995;41:340–2.
- [6] Genest J, Malinow MR. Homocysteine and coronary artery disease. *Curr Opin Lipidol* 1992;3:295–9.

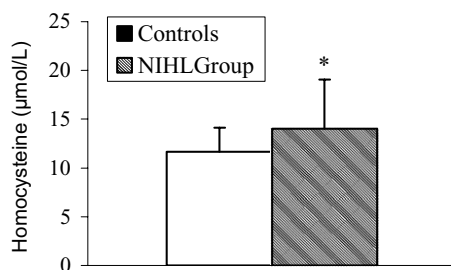


Fig. 2. Levels of homocysteine in controls and NIHL group, * $P < 0.02$.

- [7] Mattson MP, Kruman II, Duan W. Folic acid and homocysteine in age-related disease. *Ageing Res Rev* 2002;1:95–111.
- [8] McCully KS. Homocysteine and vascular disease. *Nat Med* 1996;2:386–9.
- [9] Kang SS, Wong PVK, Malinow R. Hyperhomocyst(e)inemia as a risk faktor for occlusive vascular disease. *Annu Rev Nut* 1992;12:279–98.
- [10] Clarke R, Daly L, Robinson K, Naughten E, Cahalane S, Fowler B, et al. Hyperhomocysteinemia: an independent risk faktor for vascular disease. *N Engl J Med* 1991;324:1149.
- [11] Graham IM, O Callaghan F. The role of folic acid in the prevention of cardiovascular disease. *Curr Opin Lipidol* 2000;11:577–87.
- [12] Brattstrom L. Lowering blood homocysteine with folic acid based supplements: meta analysis of randomized trials. *Br Med J* 1998;316:894–8.
- [13] Yamasoba T, Kikuchi S, Higo R, O'uchi T, Tokumaru A. Sudden sensorineural hearing loss associated with slow blood flow of the vertebrobasilar system. *Ann Otol Rhinol Laryngol* 1993;102:873–7.
- [14] Bosy-Westphal A, Peterson S, Hinrichsen H, Czech NJ, Muler M. Increased plasma homocysteine in liver cirrhosis. *Hepatol Res* 2001;20:28–38.
- [15] Veerkamp MJ, Graaf J, Heijer M, Blom HJ, Stalenhoef AFH. Plasma homocysteine in subjects with familial combined hyperlipidemia. *Atherosclerosis* 2003;166:111–7.
- [16] Mattson MP, Shea TB. Folate and homocysteine metabolism in neural plasticity and neurodegenerative disorders. *Trends Neurosci* 2003;26:137–46.
- [17] Hall CA. Function of Vitamin B₁₂ in the central nervous system as revealed by congenital defects. *Am J Hematol* 1990;34:121–7.
- [18] Float-Rahmel B, Schurmann M, Schullff P, Fingerhut R, Musshoff U, Fowler B. Homocysteic and homocysteine sulphinic acid exhibit excitotoxicity in organotypic cultures from rat brain. *Eur J Pediatr* 1998;157:112–7.
- [19] Kruman II, Culmsee C, Chan SL, Kruman Y, Guo Z, Penix L, et al. Homocysteine elicits a DNA damage response in neurons that promotes apoptosis and hypersensitivity to excitotoxicity. *J Neurosci* 2000;20:6920–6.
- [20] Otis LC, Madison DV, Nicoll RA. Folic acid has a disinhibitory action in the rat hippocampal slice preparation. *Brain Res* 1985;346:281–6.
- [21] Selhub J, Miller JW. The pathogenesis of homocysteinemia: interruption of the coordinate regulation by S-adenosylmethionine of the remethylation and transsulfuration of homocysteine. *Am J Clin Nutr* 1992;55:131–8.
- [22] Wilcken DE, Wilcken B. The natural history of vascular disease in homocystinuria and the effects of treatment. *J Inherit Metab Dis* 1997;2:295–300.
- [23] Kopke RD, Coleman JK, Liu J, Campbell KC, Riffenburgh RH. Candidate's thesis: enhancing intrinsic cochlear stress defenses to reduce noise-induced hearing loss. *Laryngoscope* 2002;112:1515–32.
- [24] Daugherty A, Zweifel BS, Sobel BE, Schonfeld G. Isolation of low density lipoprotein from atherosclerotic vascular tissue of Watanabe heritable hyperlipidemic rabbits. *Arteriosclerosis* 1988;8:768–877.
- [25] Moat SJ, Bonham JR, Cragg RA, Powers HJ. Elevated plasma homocysteine elicits an increase in antioxidant enzyme activity. *Free Radic Res* 2000;32:171–9.