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Endothelial progenitor cells in patients with age-related hearing loss

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ABSTRACT

Objective: This study was conducted to determine the concentration of EPCs in patients with ARHL.

Methods: Twenty patients with ARHL were evaluated. The number of EPCs was analyzed by flow cytometry analysis of peripheral blood $CD34^+/CD133^+$ cells.

Results: The concentration of circulating EPCs, both for $CD34^+/CD133^+$ cells, was significantly lower in ARHL patients compared to controls ($P < 0.05$). No statistically significant differences were found between these two groups in terms of the level of total cholesterol, LDL, HDL, triglycerides and GLU.

Conclusions: The possible role of circulating epithelial progenitor cells in the pathogenesis of age related hearing loss should be considered based on their significant reduction in patients with ARHL, although the association alone does not prove causality. Further studies were warranted to confirm the role of circulating EPCs in the pathogenesis of ARHL.

1. Introduction

Age-related hearing loss (ARHL), also known as presbycusis, presents as progressive loss of hearing with age [1]. ARHL is typically defined as a progressive, bilateral, high-frequency hearing loss, which is associated with difficulty in speech discrimination and central auditory message processing for ARHL patients [2,3]. ARHL is a disabling condition which is extremely common among older adults in China. In 2004, epidemiological studies were largely carried out in China, which showed that the number of ARHL patients accounted for 51.61 % of the total hearing loss patients. Furthermore, with the increase of ARHL incidence, more than 50% of the individuals in the population older than 65 years suffered from ARHL in China [4].

ARHL has been recognized for centuries; however, it is only recently that we have begun to understand the etiology of ARHL [5]. The process of aging is associated with many molecular, biochemical and physiological changes [6]. Altered vascular characteristics, such as reduced red blood cell velocity and vascular plasticity [7], increased vascular permeability [8] are proposed as the factors that contribute to this aging process. Thus vascular occlusion or ischemia associated with aging process may induce ARHL. Moreover, the blood supply of the inner ear is provided by the labyrinthine artery, and then shunting from the periphery cannot compensate for disturbances of regional blood flow [9]. In addition, sufficient evidence suggests that this microvascular hypothesis is the mechanism of sudden sensorineural hearing loss (SSHL) [10–12]. The essential roles of endothelium in vascular metastasis suggested a large probability of its involvement in ARHL etiology. Endothelial progenitor cells (EPCs) are a circulating, bone marrow-derived cells that seems to involve in both vasculogenesis and vascular homeostasis [13]. These cells have the potential to proliferate and to differentiate into mature endothelial cells [14]. The healthy subjects have lower number

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of EPCs and circulating endothelial cells [15]. Other studies showed that circulating EPCs increased when acute vascular damage occurred and reduced when cardiovascular risk factors existed [14,16].

This study was conducted to determine the concentration of circulating EPCs in patients of ARHL, and to evaluate the potential association between EPCs and ARHL.

2. Materials and methods

Twenty patients with age-related hearing loss (ARHL) admitted to hospital from January 2009 to October 2010 were enrolled in our study. All patients were 65–75 years old, had Hearing loss of >30 dB hearing level (HL) affecting at least three contiguous frequencies in both ears, and the duration of hearing loss should be more than 6 months. The patients with a noise damage induced hearing loss or with a middle ear hearing loss were excluded. Those patients who have been treated with ototoxic medications, who had serious medical health problems or impairment of cranial nerves, and with Meniere's disease or herpes zoster oticus, who were diagnosed with Meniere's disease or labyrinthitis were excluded. The subjects who failed cognitive screening tests (Mini-Mental Test) or had poor speech discrimination scores of 80% or less were excluded. The current/heavy smokers also were excluded. A control group of twenty normal participants recruited from clinical age- and gender-matched healthy population were established to obtain baseline measurements served as normal reference values. In addition, all subjects taking statins were excluded, since these drugs can affect the circulating levels of EPCs [16,17].

A comprehensive clinical history was obtained and the standard audio vestibular investigation was carried out according to the report of Quaranta et al. [17]. Air conduction pure tone average (PTA) was defined as the mean of the air conduction thresholds at 0.25, 0.5, 1, 2, 3, 4, and 8 kHz. Pure tone and speech audiometry were tested in outpatient. A mild hearing loss (HL) was determined by PTA between 20 and 40 dB; a moderate HL by PTA between 40 and 70 dB; and the severe HL by PTA between 70 and 90 dB; and profound HL by PTA >90 dB [17].

Morning fasting venous blood samples were collected with EDTA-coated tubes (0.5 mM final concentration) in the morning between 7 and 9 AM. The first 3 ml of blood was discarded to avoid contamination with circulating endothelial cells, and then 10 ml of blood was collected from each patient. Blood samples were also collected from age- and gender-matched healthy participants to obtain baseline normal reference values. A full set of clinical chemistry and hematological tests were performed to measure the concentration of total and fractionated cholesterol (LDL and HDL), triglycerides, and blood glucose (GLU). Blood samples were processed within 2 h after collection.

Peripheral mononuclear cells were extracted from blood samples to quantify EPCs in circulation as previous researches described [18]. Briefly, 2 ml of blood was subjected to a density gradient centrifugation at 300×*g* for 20 min at room temperature. The isolated cells were washed three times with phosphate-buffered saline (pH 7.2) and then resuspended in 200 μL of phosphate-buffered saline supplemented with 0.5% bovine serum albumin and 2 mM EDTA. The cells were then labeled with an R-phycoerythrin-conjugated monoclonal CD133 antibody (Miltenyi Biotech, Gladbach, Germany) or fluorescein isothiocyanate-conjugated CD34 monoclonal antibody (BD Pharmingen, San Jose, CA) or both for 20 min at room temperature. Two cellular markers were used to identify circulating EPCs. CD34 can recognize stem cells of endothelial lineage, whereas CD133 primarily detects immature EPCs that are highly capable of differentiating into endothelial cells. The double staining will allow us not only to identify endothelial cells in circulation, but also determine the ratio of mature to immature cell populations, that can potentially be used to gauge the patient's ability to repair the vasculature. Stained cells were washed with phosphate-buffered saline/bovine serum albumin and then analyzed by flow cytometry (BD FACS Calibur, BD Biosciences, San Jose, CA). Two isotype controls of R-phycoerythrin and fluorescein isothiocyanate-conjugated mouse immunoglobulin G were used for background nonspecific binding. Cells were first run on forward and side scatter to select mononuclear cells to reduce signal noises from cell aggregates, platelets, and cellular debris. Three populations of cells, $CD34^+$, $CD133^+$ and double-stained cells, were analyzed and expressed as numbers of positive cells in each population. For double fluorescence detection, cells were first gated for their CD34 positivity and then for CD133 staining. EPCs were then detected as gated cells that were stained for fluorescein isothiocyanate–CD34 and R-phycoerythrin–CD133 and quantified as the number of EPCs per 2×10^6 mononuclear cells [18].

All data shown in Results were presented as mean ± standard error. The differences of all parameters between case and control group were analyzed using two-tailed Student's t-tests. A *P*-value of <0.05 was considered to be statistically significant.

3. Results

3.1. *Comparison of clinical characteristics of ARHL and normal participants*

The clinical characteristics of patients with ARHL and normal participants are shown in Table 1. Among the 20 ARHL patients, there were 1, 16, 2 and 1 patient suffered from to mild (5%), moderate (80%), severe (10%) and profound (5%) hearing loss respectively. The average PTA of ARHL group was 56.1 ± 11.7 dB HL, which was significantly worse ($P < 0.01$) than the control

Table 1 – Demographics of the ARHL patients and controls.

Parameter	ARHL patients (n = 20)	Controls (n = 20)
Age (years)	68.7 (65–74)	69.5 (66–75)
Sex	9F/11M	12F/8M
Side of HL	Both sides	–
Tinnitus	25%	10%
Ear fullness	20%	NA
Vestibular disturbances	NA	NA
Hypertension	15%	10%
Diabetes	20%	25%

HL, hearing loss; NA, not available; ARHL, aged related hearing loss.