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Research paper

# Plasma brain-derived neurotrophic factor levels are increased in patients with tinnitus and correlated with therapeutic effects

Hao Xiong[a,b,1], Haidi Yang[a,b,1], Maojin Liang[a,b,1], Yongkang Ou[a,b], Xiayin Huang[a,b], Yuexin Cai[a,b], Lan Lai[a,b], Jiaqi Pang[a,b], Yiqing Zheng[a,b,*]

[a] Department of Otolaryngology, Sun Yat-sen Memorial Hospital, Sun Yat-sen University, China
[b] Institute of Hearing and Speech-Language Science, Sun Yat-sen University, China

## HIGHLIGHTS

- Plasma BDNF levels are increased in patients with tinnitus.
- Plasma BDNF levels are declined in patients with tinnitus after effective TRT.
- Plasma BDNF may serve as a biomarker for evaluating tinnitus.

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## ABSTRACT

Tinnitus is the perception of sound without an external source and is known to be associated with altered neuronal excitability in the auditory system. Tinnitus severity can be assessed by various psychometric instruments and there is no objective measures developed to evaluate tinnitus severity and therapeutic effects so far. Brain-derived nerve growth factor (BDNF) is believed in playing a key role in regulating neuronal excitability in the brain. To determine whether BDNF correlates with tinnitus induction and severity, we described plasma BDNF levels in patients with tinnitus and healthy controls and evaluated the correlation between plasma BDNF levels and tinnitus severity measured by Tinnitus Handicap Inventory (THI) and Visual Analog Scale (VAS). Moreover, alteration of plasma BDNF levels before and after tinnitus retraining therapy (TRT) in patients with severe tinnitus was also analyzed. We found plasma BDNF levels were elevated in patients with tinnitus compared with healthy controls. In addition, plasma BDNF levels in patients with severe tinnitus were decreased significantly after effective TRT. However, plasma BDNF levels were not correlated with tinnitus loudness and tinnitus severity measured by THI and VAS. These findings support plasma BDNF as a marker for activity changes in the auditory system and could possibly evaluate therapeutic effects in patients with tinnitus.



## 1. Introduction

Tinnitus is the perception of sound within the ear in the absence of external sound source. Tinnitus is a very common condition, with an estimated prevalence of 10%–15% in adults [10] and about 20% of patients with tinnitus will require clinical intervention [11]. Tinnitus can impair the quality of life and lead to a variety of psychological disorders such as depression, anxiety, sleep disturbances and concentration difficulties [16,29,34]. These psychological symptoms also act as good predictors of the perception of tinnitus severity [29]. Currently, tinnitus severity can be measured with various psychometric tests. However, there is no objective clinical assessment developed for evaluation of tinnitus severity or therapeutic effects so far.

Brain-derived nerve growth factor (BDNF) is a member of the neurotrophin family, which is abundantly expressed in central nervous systems. BDNF has emerged as a key neurotrophin involved in brain plasticity after brain injury including tinnitus-inducing acoustic trauma [20,21,26]. Previous studies demonstrated BDNF expression was increased in the cochlea after tinnitus induction by application of salicylate or acoustic trauma [26,33]. Furthermore, midazolam, a GABAA receptor modulator, not only reversed salicylate-induced elevation of BDNF but also reduced tinnitus

* Corresponding author at: Department of Otolaryngology, Sun Yat-sen Memorial Hospital and Institute of Hearing and Speech-Language Science, Sun Yat-sen University, 107 West Yan Jiang Road, Guangzhou 510120, China.
*E-mail address:* yiqingzheng@hotmail.com (Y. Zheng).
[1] These authors contributed equally to this work.

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perception in a rat behavioral model [26]. These findings support BDNF can serve as a biomarker for auditory plasticity. Since BDNF can cross the blood-brain barrier in both directions [25], circulating levels of BDNF might be used for measurement of BDNF levels in the brain. It was reported that brain contributed to more than 70% of plasma BDNF in healthy humans, which implied that the changes in plasma BDNF might be considered to reflect its changes in the brain [19,30]. Interestingly, recent research revealed plasma BDNF levels differed between patients with mild tinnitus and healthy controls [8], suggesting plasma BDNF might be a novel biomarker for evaluation of tinnitus. However, whether plasma BDNF levels can evaluate therapeutic effects in patient with tinnitus remains unknown.

In the present study, we described plasma BDNF levels in patients with tinnitus and healthy controls and evaluated the correlation between BDNF levels and tinnitus severity measured by psychometric instruments. Moreover, alteration of plasma BDNF levels before and after tinnitus retraining therapy (TRT) in patients with severe tinnitus was also analyzed.

## 2. Material and methods

### 2.1. Participants

From April 2013 to December 2014, 82 patients (36 males and 46 females) with tinnitus were enrolled in this study at Sun Yat-sen memorial hospital of Sun Yat-sen University. During the same period, 32 health volunteers (17 males and 15 females) were recruited to serve as controls for the patients. The average age was $42.7 \pm 14.2$ years and $40.1 \pm 11.9$ years in patients and controls, respectively. The mean duration of tinnitus was 6 years, ranging from 1 to 15 years. Thirty-eight patients reported tinnitus in the right ear and 29 in the left ear. Fifteen patients had bilateral tinnitus. The study was approved by the ethical review board of Sun Yat-sen Memorial Hospital, Sun Yat-sen University and informed consent was obtained from all participants.

### 2.2. Audiological evaluation

All patients with tinnitus were evaluated by pure tone audiometry, tinnitus frequency match at first visit. Patients who received TRT were evaluated again at 3 months after first visit. The hearing threshold was calculated as the average of 0.5 kHz, 1 kHz, 2 kHz and 4 kHz. The health controls were also measured by pure tone audiometry for excluding possible hearing loss.

### 2.3. Questionnaires

We used the Tinnitus Handicap Inventory (THI) and Visual Analog Scale (VAS) to assess the tinnitus severity. The THI composed of 25 questions with scores ranging from 0 to 100, with higher scores indicating more severe symptoms [22]. The VAS regarding awareness, annoyance and loudness was measured from 1 to 10 scale with 10 most severe [1]. The THI and VAS were administered to all patients at first visit and also to patients who received TRT at 3 months after first visit.

### 2.4. TRT

We classified patients with tinnitus into two groups according to the scores of THI at first visit: mild tinnitus (less than 36) and severe tinnitus (more than 38) [8,23]. Patients with mild tinnitus only received counseling. Patients with severe tinnitus underwent TRT. The TRT program consisted of counseling and sound therapy. The counseling was conducted at patients' first visit and every month after the first visit. During the first counseling session, patients were taught about basic anatomy and physiology of the auditory system and other systems in the brain that are relevant to the source of tinnitus, the vicious circle of listening and reacting, explanation of habituation as the goal of TRT and the use of sound generators for TRT. Every month after the first visit, patients attended ongoing counseling to check therapeutic effect and repeated explanation of habituation as the goal. Sound therapy was conducted with a MP3 player. Patients were instructed to listen to the broadband noise for 4–6 h every day and to set the volume of noise generator at the mixing point.

### 2.5. Plasma BDNF measurement

Blood was collected in all patients at first visit and in healthy controls. Additional blood collection was also conducted in patients who received TRT at 3 months follow-up. Blood samples (6 ml) were drawn into tubes containing EDTA between 7:30 and 8:00 AM and centrifuged at 4000 g for 10 min. Plasma samples were stored at $-80\,^{\circ}C$ prior to use. The levels of human plasma BDNF were measured by ELISA according to the manufacturer's instructions (Boster, Wuhan, China). The minimum detectable dose of BDNF was less than 2 pg/ml. All samples were tested in triplicate.

### 2.6. Statistics

All data were analyzed using SPSS (ver. 18.0, SPSS, Chicago, IL, USA). Statistical analyses were performed using *t*-tests, ANOVA and Spearman correlation tests. Differences of $p < 0.05$ were considered to be significant.

## 3. Results

The clinical characteristic of all participants are presented in Supplementary Table S1 in the online version at DOI: 10.1016/j.neulet.2016.04.032. The average hearing threshold in patients with tinnitus was $40.1 \pm 27.4$ dB ($39.3 \pm 22.5$ dB in left ears and $40.9 \pm 33.1$ dB in right ears). All healthy controls have normal hearing. The average frequency of tinnitus was $4.0 \pm 3.2$ kHz, ranging from 0.25 kHz–8 kHz. THI scores ranged from 8–72 (average: $32.4 \pm 16.8$) in patients with tinnitus. The tinnitus VAS scores were $6.7 \pm 1.5$ (awareness), $6.3 \pm 1.0$ (annoyance) and $6.3 \pm 1.2$ (loudness). Plasma BDNF levels in patients with tinnitus (average: $1076.1 \pm 495.9$ pg/ml, ranged from 96–2475 pg/ml) was significantly higher than those in healthy controls (average: $810 \pm 566.9$ pg/ml, ranged from 123–2458 pg/ml, $p < 0.05$). Although plasma BDNF levels did not differ between mild tinnitus and severe tinnitus groups, they are both higher in comparison with controls ($p < 0.05$, Fig. 1). The age, the duration of tinnitus, site of tinnitus and hearing threshold had no correlation with the THI scores, VAS scores or plasma BDNF levels (See Supplementary Table S2 in the online version at DOI: 10.1016/j.neulet.2016.04.032). THI and VAS scores had no correlation with plasma BDNF levels in patients with tinnitus.

In total, 25 patients with THI scores above 38 were classified as having severe tinnitus and received TRT. None of them lost to follow-up. At 3 months after starting TRT program, the patients showed significant decrease in THI scores ($p < 0.05$, Fig. 2A), VAS awareness and annoyance scores ($p < 0.05$, Fig. 2B). Although VAS loudness scores showed a tendency for decline, it did not reach statistical significance. Additionally, plasma BDNF levels after TRT were significantly lower than those before TRT in 14 of 25 patients who agreed to receive a second blood collection ($p < 0.05$, Fig. 3). Of these patients, 10 exhibited simultaneous declines of BDNF levels and THI scores, 1 presented decline of BDNF levels with elevated THI scores, 2 displayed increased BDNF levels with decreased THI scores and 1 showed simultaneous increased BDNF levels and THI