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Research paper

Biological correlates of tinnitus-related distress: An exploratory study

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ABSTRACT

During the process of tinnitus diagnostics, various psychometric instruments are used to measure tinnitus-related distress. The aim of present work was to explore whether candidates for biological correlates of the tinnitus-related distress could be found in peripheral blood of patients and if so, whether there was association between them and psychometric scores that reflect tinnitus-related distress. The concentrations of interleukin-1β (IL1β), interleukin-6 (IL6), tumor necrosis factor-α (TNFα) and a brain-derived neutrotrophic factor (BDNF) were measured in serum of 30 patients diagnosed with chronic tinnitus and tested for correlation with psychometric scores collected on the same day. Spearman's correlation analyses detected significant positive association between the concentrations of tumor necrosis factor α and tinnitus loudness, total perceived stress, tension and depression and a negative association between tumor necrosis factor α and a psychometric score "joy". Concentrations of interleukin-1β correlated with the awareness grade of tinnitus. The correlation between visual analogue scale (VAS) "loudness" and tumor necrosis factor α as well as between "joy" and tumor necrosis factor α retained their significance ($p < 0.00167$) after the application of Bonferroni correction for multiple testing. Partial correlations removing the effects of age, hearing loss and the duration of tinnitus verified the results obtained using Spearman correlation. We conclude that measuring the concentrations of selected circulating cytokines could possibly become an additional objective element of tinnitus diagnostics in the future.

1. Introduction

Tinnitus – a phantom sound heard only by the affected person – is frequently a consequence of hearing loss (Mazurek et al., 2010), activation of the somatosensory system (Shore, 2011) or both. Tinnitus often accompanies various conditions that lead to hearing loss such as noise-induced hearing loss, presbyacusis, ototoxicity, a number of infectious diseases and autoimmune reactions, cardiovascular conditions, whiplash-associated disorders and many others. In some persons, tinnitus-related activation of auditory cortex may trigger a signal to the limbic and vegetative networks and cause so called "*tinnitus-related distress*" (Henry and Wilson, 1995; Seydel et al., 2013). Tinnitus-related distress is complex and manifests itself by the auditory attention focused on tinnitus sound, an increased irritability, insomnia, anxiety, depressive mood, difficulties with concentration (Tyler et al., 2014, 2007) and sometimes somatic complains (Hiller and Goebel, 1992). All of them can be measured with the use of psychometric instruments. Apart from the psychometric instruments, which provide subjective measures, no objective biological assessment of tinnitus-related distress is routinely used at present.

Cytokines are soluble peptides, proteins or glycoproteins produced and secreted by a variety of cells. Cytokines play an essential role in communication between cells, tissues and systems. They were first discovered as a part of the immune defense (Hanson et al., 1982) but soon it became clear that other systems can produce and react to them (Benton, 1991). During the process of inflammation, an array of so-called *pro-inflammatory cytokines* is produced. The pro-inflammatory cytokines comprise interleukin 1

Abbreviations: ADS, general depression scale; BDNF, brain-derived neutrotrophic factor; CI, confidence interval; HPA, hypothalamic-pituitary-adrenal; IL1 β, interleukin 1 beta; IL6, interleukin 6; PSQ, perceived stress questionnaire; TNFα, tumor necrosis factor alpha; TQ, tinnitus questionnaire; VAS, visual analogue scales

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beta (IL1β), interleukin 6 (IL6) and tumor necrosis factor alpha (TNFα). In the recent years, changes in concentration of circulating interleukin-1β, interleukin-6 and tumor necrosis factor α started to be associated not only with inflammatory or infectious diseases but also with the process of aging, exposure to stress and with some neurological disorders (Zhang et al., 2013). In fact, particular profiles of circulating proinflammatory cytokines were proposed as biomarkers for conditions such as post-traumatic stress disorder (Andrews and Neises, 2012), chronic stress (Hansel et al., 2010; Steptoe et al., 2007) or major depression (Bob et al., 2010). Under physiological and pathological conditions, proinflammatory cytokines can not only act on the immune system, but also on the nervous system, regulating synaptic strength and mediating synaptic plasticity (Eyre and Baune, 2012).

Brain-derived neurotrophic factor (BDNF) is also a secreted protein and belongs to the neurotrophin family of growth factors. Brain-derived neutrotrophic factor acts *via* specific receptors: TrkB and p75 and supports the neurogenesis and survival of already existing neurons plus it enhances the formation of synapses (Huang and Reichardt, 2001). Interestingly, brain-derived neutrotrophic factor is produced and present not only in the central nervous system but also in the periphery (e.g. platelets), where it stimulates growth of megakaryocytes *via* autocrine loop (Tamura et al., 2012). Fluctuation of circulating brain-derived neutrotrophic factor has been linked to neurodegenerative disorders and to mood disorders like depression, eating disorders, modulation of memory and cognitive functions (Pluchino et al., 2013). Furthermore, expression of brain-derived neutrotrophic factor was demonstrated to be modulated by cortisol released during stress exposure *via* hypothalamus-pituitary-adrenal axis HPA-axis (Pluchino et al., 2013), similarly to the expression of proinflammatory cytokines (Feuerecker et al., 2013). Interestingly, recent work of Goto and colleagues (Goto et al., 2012) pointed at possible diagnostic significance of brain-derived neutrotrophic factor in tinnitus. Severity of tinnitus has been demonstrated to correlate with the degree of stress and depressive mood perceived by the patients (Hebert et al., 2012; Savastano et al., 2007; Zirke et al., 2013). Thus, measuring stress-related biomarkers could be of relevance for the diagnosis and monitoring of tinnitus. In addition, our earlier work has demonstrated that following tinnitus treatment, not only a reduction in tinnitus-related distress but also a decrease of the tumor necrosis factor α concentration in blood (Weber et al., 2002).

The main aim of present work was to determine whether the profile and concentrations of circulating cytokines and neurokines could reflect tinnitus-related distress in hope of providing objective biological marker of tinnitus-related distress. We have evaluated the concentrations of tumor necrosis factor α, interleukin-1β, interleukin-6 and brain-derived neutrotrophic factor in the sera of patients with chronic tinnitus. In addition, using four relevant psychometric instruments, we have assessed tinnitus-induced distress. Lastly, we have analyzed the association between the concentration of circulating cytokines/brain-derived neutrotrophic factor and the psychometric scores.

2. Materials and methods

2.1. Patients

Thirty patients (14 women and 16 men) who were admitted to the Tinnitus Center at the Charité University Hospital between October 2007 and June 2008 were included in this study after signing written consent. The study was approved by a local Ethics Committee. The mean duration of tinnitus was 5 years, ranging from 9 months to 28 years. Twenty four patients (12 women and 12 men) had bilateral tinnitus and six patients (2 women, 4 men) had unilateral tinnitus. The tinnitus was described as a pure tone (8 women and 9 men) or a narrow-band noise (6 women and 7 men).

The patients were between 18 and 67 years old (mean age 47 years). The exclusion criteria included Ménière's disease, tumors of the middle/inner ear, severe forms of diabetes or circulatory diseases. No pharmacological or non-pharmacological intervention was used within four weeks prior to data collection.

2.2. Tinnitus parameters and hearing impairment

Tinnitus assessment included the determination of tinnitus pitch and loudness (Table 1). Hearing was measured by pure tone audiometry within the frequency range spanning between 0.25 and 8 kHz. The mean hearing loss was calculated separately for each ear as a mean value of hearing loss in 0.5 kHz, 1 kHz, 2 kHz and 4 kHz. Current WHO classification was used to describe the severity of hearing impairment. The individual superimposed and averaged audiogram data are presented in Fig. 1A and B, respectively.

2.3. Blood collection and serum processing

To minimize possible influence of the circadian rhythm, venous blood was collected between 7:30 and 8:30 AM. S-MONOVETTE 2.6 ml Z-GEL serum septum tubes (cat.# 04.1905.001, SARSTEDT, Nümbrecht, Germany) were used for this purpose. The blood was allowed to coagulate for 30 min at room temperature and was then centrifuged in the collection tubes, strictly according to the manufacturer's directions. After this, 200 μl aliquots of sera were labeled and frozen in aliquots at −80 °C. Each aliquot was used only once. All samples as well as the psychometric scores were coded and patient's identity blinded.

2.4. Cytokines and brain-derived neutrotrophic factor

To measure the concentration of interleukin-1β, interleukin-6, tumor necrosis factor α and brain-derived neutrotrophic factor in serum, human Quantikine kits specific for interleukin-1β (cat.# DLB50), interleukin-6 (cat.# D6050), tumor necrosis factor α (cat.# DTA00C) and brain-derived neutrotrophic factor (cat.# DBD00) were purchased from R and D Systems GmbH (Wiesbaden, Germany). All of the ELISA's were designed for the quantitative determination of human cytokines in the culture supernatant, serum and plasma, and were commercially optimized by detailed quality control procedures. The commercially tested minimum detectable doses reflecting assay's sensitivities are listed in Table 2.

Kits specifications provided by the quality control department of manufacturer:

Interleukin-1β (cat.# DLB50): the minimum detectable dose of interleukin-1β ELISA was less than 1 pg/ml; all samples from control individuals measured less than the lowest IL-1β standard, 3.9 pg/ml.

Interleukin-6 (cat.# D6050): the minimum detectable dose of interleukin-6 was less than 0.70 pg/ml; 40 samples from apparently healthy volunteers were evaluated for the presence of human IL-6;

Table 1
Mean tinnitus pitch and loudness measured on the left and right ear.

Parameter	Side	Mean ± standard deviation
Frequency kHz	Left	5.0 ± 2.2
Frequency kHz	Right	5.4 ± 2.14
Loudness dB SL	Left	3.3 ± 4.5
Loudness dB SL	Right	4.8 ± 5.3