Interleukin-1 Alpha Gene Polymorphism (IL-1α) and Susceptibility to Tinnitus in the Elderly

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Abstract

Aim: To investigate the association between the single nucleotide polymorphism in the position-889 (C/T) of the promoter region of the IL-1α gene and the susceptibility to tinnitus. Method: This was a case-control study with a sample of 108 independent elderly people over 60 years of age. Information on exposure to occupational noise and tinnitus was obtained by interviews. The genetic polymorphism was analyzed by polymerase chain reaction followed by cleavage with restriction enzyme NcoI. Data were analyzed using the Chi-square test, with the significance level set at 5%. For the statistical analysis all individuals with tinnitus on the right ear were eligible. Results: Among elderly with tinnitus, 42.9% had a history of exposure to occupational noise. There was statistically significant association between IL-1α gene polymorphism and tinnitus in subjects without a history of exposure to occupational noise (P = 0.006 and x² = 10.39). The elderly with the T allele were less likely to have tinnitus due to occupational noise exposure when compared to those carrying the C allele. Conclusion: This study suggests an association between the IL-1α gene polymorphism with susceptibility to tinnitus in individuals without a history of exposure to occupational noise. The present study demonstrated that allele T of IL-1α is a protective factor for presence and severity of tinnitus in the elderly and allele C contributes to the pathogenesis of the inflammatory response. The present observation implied the signaling IL-1α is involved in ear aging.

Keywords: Cytokines, noise, occupational, tinnitus

INTRODUCTION

Interleukin-1 alpha (IL-1α) is an essential cytokine that contributes to inflammatory responses.1 The discovery of a natural Interleukin-1 receptor antagonist (IL-1Ra) in human biological fluids has highlighted the importance of IL-1 in human diseases. At present, the IL-1 blockade as therapeutic approach is crucial for many hereditary autoimmune inflammatory diseases. There are many excellent reviews focusing on the IL-1 family in general or with regard to specific diseases or biological discoveries.2 More recently, it has become clear that the brain is the central regulator of fatigue perception. It has been suggested that pro-inflammatory cytokines, especially IL-1α and interleukin-1 beta (IL-1β), play a prominent role in the development of central fatigue, and several studies have been performed to elucidate the connection between inflammation and these central processes.3 As rupture of the blood-labyrinth barrier is associated with increased permeability of the blood vessels in the inner ear, inflammation may be related to the etiology of these inner ear diseases. Although the mechanism and function of these cytokines are still obscure, it is known that the structure and expression of a cytokine can be influenced by genetic variation, which results in obvious pathological consequences. It is an important subject in association studies of disease and is related to avoiding disease in the end of life. It is important to have a view of the pleiotropic and hematopoietic action of this cytokine. Because of the important role of IL-1 in the innate immune system and
other physiological systems, it has become a field of great interest. Whereas IL-1α and IL-1β activate an inflammatory signal upon binding to the IL-1R1, IL-1Ra binds to the same receptor but does not activate a signal. [2,3] The protein encoded by this gene is a member of the interleukin 1 cytokine family. This cytokine is a pleiotropic cytokine involved in various immune responses, inflammatory processes, and hematopoiesis. This cytokine is produced by monocytes and macrophages as a proprotein, which is proteolytically processed and released in response to cell injury, and thus induces apoptosis. [4]

While viral infection of the stria vascularis, organ of Corti or spiral ganglion cells, which can cause hearing loss and hearing symptoms such as tinnitus, is discussed in the American literature, a vascular genesis with resulting impaired perfusion of the inner ear is favored by European investigators. Although both hypotheses are supported by different therapeutic strategies to regain normal hearing, the influence of spontaneous remission remains unclear. Other organs during viral vasculitis circulating immunoglobulins are deposited perivascularly, which leads to a local decrease in perfusion and tissue hypoxia. Also, in autoimmune diseases, perivasculitis is common with the endothelium playing a major role at the initial stages of the disease. These endothelial cells promote vasculitis by secreting pro-inflammatory cytokines like IL-1, IL-6 or TNF-alpha in addition to the expression of adhesion molecules. Due to the persistence of these immunopathological mechanisms, stenosis or atresia with ischemic necrosis results. [5]

Age and exposure to noise are among the factors that may cause damage to the hair cells of the organ of Corti. [6] The etiology of damage to the hair cells of the organ of Corti is multifactorial, with overexposure to sound, the use of certain drugs, and presence of infection or immune-induced inflammation being common causes. These factors lead to damage and death of the sensory receptor cells in the cochlea and the neurons that relay auditory information from the inner ear to the central auditory circuitry, and then process information as it ascends the central auditory pathway; and they may also be accelerating factors of presbycusis. [7]

Whereas tinnitus is a symptom usually related to cochlear alterations that may arise from noise exposure, this study aimed to evaluate the association between polymorphism in IL-1α and tinnitus related to the history of work in a noisy environment of physically independent Brazilian elderly.

**METHODS**

This was a case-control study, conducted in partnership with the Unopar University. It is part of a wider research, “ELLO project—Aging and Longevity Study,” which was conducted in Londrina since 2009. The city of Londrina (about 500,000 inhabitants) is located in the state of Parana, Brazil. All patients were previously informed about the objectives of the research and procedures to be performed and signed the Informed Consent Form.

From a population of 43,610 elderly enrolled in 38 basic health units in the urban area of the city, the sample size was set at 343 individuals, considering a 95% confidence interval and a margin of error of 5%. Aiming to sample representativeness, random stratification considering gender and regions of the city was carried out. The study included individuals aged 60 years or over, of both genders, who were living independently and were classified at level 3 or 4, as proposed by Spiriduso. [8]

This rating assesses the level of independence of the elderly, level 1 indicating a lack of self-mobility and level 5 indicating athletes. Elderly people who had an illness or limitation preventing the performance of the tests, such as physical or mental disability, were excluded from the sample. All participants signed an informed consent form. The patients included had tinnitus and a history of exposure to occupational noise. For the control group, individuals without history of exposure to occupational noise and matched to the case group by gender and age were included.

In order to carry out the audiological assessment research, the patients’ clinical information was collected from routine care at the Speech-language-hearing Clinic (Audiology department)(Unopar University), based on the Miller protocol for history, including questions about age, gender and tinnitus. [9] It was used specifically for tinnitus, to investigate whether the sensation of tinnitus is present or not, in which ear, how often, when the symptom began, and what type of tinnitus the patient presented.

The assessment of occupational noise exposure was obtained through interviews with the elderly participants, using a semi-structured questionnaire. Information was collected about the work, whether it was done in a noisy environment, how many years they worked in a noisy environment and whether they wore hearing aids. In addition, demographic characteristics were collected.

The genetic polymorphism was analyzed by polymerase chain reaction followed by cleavage with restriction enzyme NcoI. DNA sample from each elderly was matched to the case group by gender and age were included. For the control group, individuals included had tinnitus and a history of exposure to occupational noise. For the control group, individuals without history of exposure to occupational noise and matched to the case group by gender and age were included.

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For blood samples and DNA extraction from each patient, 5 ml of peripheral blood were collected by venule puncture. Blood samples were stored at −80°C. Deoxyribonucleic Acid (DNA) was extracted with the PureLink—Invitrogen kit, according to the manufacturer’s instructions.

The evaluation of the purity and concentration of DNA was performed by the analysis of absorbance in a spectrophotometer (NanoDrop ND-2000–Thermo Scientific) at 260 and 280 nm. Subsequently, the DNA dilution was made in ultrapure Milli-Q® water to a final concentration of 30 ng/μL. The polymerase chain reaction (PCR) mixture consisted of a final volume of 15 ml composed of: buffer pH 8 1x, 1.5 mM
MgCl₂, 0.2 mM dNTPs, 20 M each primer, 1U of Taq DNA polymerase (Invitrogen, São Paulo, Brazil) and 1 ml of the DNA solution (100 ng / ml). The amplification conditions were: denaturation at 95°C for four minutes, followed by annealing of the primers at 55°C for one minute and extension at 70°C for one minute, repeated for 35 cycles amplification, 6 ml of each sample of the PCR product were analyzed by agarose gel electrophoresis (1%), stained by Syber-safe (Invitrogen, São Paulo, Brazil), and the newly synthesized fragments were visualized under ultraviolet light.

The size of the product amplified by PCR was estimated from the electrophoretic migration of the product relative to the molecular weight marker 100 pb DNA Ladder (Invitrogen, São Paulo, Brazil). For the C-889T IL-1 heterozygous allele, the digestion products obtained were: C allele (83 +16 bp) and T allele (99 bp). The visualization of the digestion product was done in 2% agarose gel electrophoresis stained by Syber-safe (Invitrogen, São Paulo, Brazil).

### Statistical analysis

The Statistical Package for Social Sciences version 20.0 software (SPSS, UK) was used for statistical data analysis, with a 95% CI and a significance level of 5% (P < 0.05) established for all tests used.

The chi-square test was used to verify that the genotype frequencies were in Hardy–Weinberg equilibrium, as well as to assess possible associations between the presence of polymorphisms in the IL-1α gene and the occurrence of tinnitus. For the statistical analysis all individuals with tinnitus on the right ear were eligible.

### RESULTS

Data were analyzed using descriptive statistics [Table 1]. Of the total (n = 343), molecular genetic procedures were conducted in 108 elderly. Of these, 38% reported a history of noise exposure with 25.9% presenting tinnitus. Of the 62% without a history of exposure to noise, 57.1% presented tinnitus.

There was a statistically significant association between the genotypic frequency of IL-1α in the groups with and without tinnitus (P = 0.006; χ² = 10.39). There was also another correlation between data by Cramer’s V that although weak was statistically significant (V = 0.252; P = 0.026). This means that 6.35% of the classification variation is shared and can be explained by the association between tinnitus and IL-1α. [Table 2]. Likewise, an association was found for allelic frequency of IL-1α and tinnitus (P = 0.025; χ² = 5.395). There was also a correlation between the data that although considered very weak was statistically significant (V = 0.158; P = 0.025) [Table 3].

The adjusted analyzes for the groups with and without exposure to noise [Table 4] showed that there was an association between the genotypic frequency of IL-1α and tinnitus for the group without exposure to noise (P = 0.047; χ² = 5.641). There was a weak correlation between the variables, though statistically significant (V = 0.303; P = 0.037). This means that 9.18% of the classification variation is shared and can be explained by the association between tinnitus and IL-1α for the group without exposure to noise.

The elderly with the T allele were less likely to present associated tinnitus without a history of exposure to occupational noise (OR = 0.703, 95% CI: 0.469–1.056) when compared to those with the C allele. However, participants with the C allele were more likely to present associated tinnitus without a history of occupational noise exposure (OR = 1.585, 95% CI: 1.031–2.436) when compared to those with the T allele. No associations were found for the other variables according to Table 4.

### DISCUSSION

There was a statistically significant association between IL-1α gene polymorphism and tinnitus. This finding suggests that this polymorphism may be a genetic factor that leads to the development of tinnitus in the elderly with no history of noise exposure. This association is...
important to prevent future damage to the auditory pathways such as hearing loss, since tinnitus may be the first symptom of auditory pathway damage in these patients.

In addition, the IL-1α T allele has previously been associated with an enhanced promoter activity resulting in increased gene expression, both at mRNA and protein level, compared with the C allele. It seems likely that patients with the IL-1α T genotype have overall enhanced levels of IL-1α, resulting in an increased activation of IL-1 receptors and a more pronounced inflammatory response. Actually, the IL-1α is a very strong inducer of inflammation and causes a broad spectrum of systemic changes in neurological, metabolic, hematological, and endocrinological systems.

Functionality of single nucleotide polymorphism (SNP) with respect to gene expression is an important subject in studies of association with diseases such as SNP, which results in pathological consequences. The structure has been observed as the expression of cytokines that may be affected by genetic variation, although the mechanism and role of proinflammatory cytokines in noise-induced damage to the hair cells of the organ of Corti are not yet well understood.

The aging process is accompanied by a chronic sub-clinical systemic inflammation. Inflammatory responses occur in the inner ear under various damaging conditions and can cause tinnitus including overstimulation with noise and cisplatin-induced ototoxicity. The mechanism of tinnitus is postulated to involve inhibition of cyclooxygenase (COX) that could potentiate N-methyl D-aspartate receptor (NR) currents at synapses between inner hair cells and dendrites of the cochlear spiral ganglion neuron. However, an association between proinflammatory cytokines and tinnitus has been rarely reported.

| Table 2: Association between genotypic frequency of polymorphism of the interleukin 1 alpha (IL-1α) position −889 and tinnitus in elderly (N = 108) |
|---|---|---|
| Tinnitus Genotyping IL-1α | Genotypic frequency | P-value |
| Genotypic frequency | CTN (%) | TTN (%) | CCN (%) |
| With tinnitus | 7 (26) | 5 (18) | 15 (56) | 0.006*<sup>χ^2 = 10.39</sup>; P = 0.252 |
| Without tinnitus | 4 (5) | 27 (34) | 50 (61) | *Statistically significant association |

| Table 3: Association between allelic frequency of polymorphism of the interleukin 1 alpha (IL-1α) position-889 and tinnitus in elderly (N = 108) |
|---|---|---|
| Tinnitus Allelic frequency IL-1α | P-value |
| Genotypic frequency | CTN (%) | TTN (%) | CNT (%) |
| With tinnitus | 29 (53.7) | 25 (46.3) | 0.025*<sup>χ^2 = 5.395</sup> |
| Without tinnitus | 58 (35.8) | 104 (64.2) | * Statistically significant association. |

| Table 4: Association between genotypic frequency of polymorphism of the interleukin 1 alpha (IL-1α) position-889 and tinnitus in elderly |
|---|---|---|---|
| Occupational noise exposure Genotyping | With tinnitus | No tinnitus |
| | N (%) | N (%) | Chi-square test |
| Genotypic frequency | | | |
| No history | CC 3 (20.0) | 2 (3.8) | 0.047*<sup>χ^2 = 5.641</sup> |
| TT 2 (13.3) | 19 (36.5) |
| CT 10 (66.7) | 31 (59.6) |
| Allelic frequency | | | |
| C 16 (53.3) | 35 (33.7) | 0.580<sup>χ^2 = 3.825</sup> |
| T 14 (46.7) | 69 (66.3) |
| With history | C 13 (54.2) | 23 (39.7) | 0.328<sup>χ^2 = 1.451</sup> |
| Genotypic frequency | T 11 (45.8) | 35 (60.3) |
| TT 3 (25.0) | 8 (27.6) |
| CT 5 (41.7) | 19 (65.5) |
| Allelic frequency | | | |
| C 13 (54.2) | 23 (39.7) |
| T 11 (45.8) | 35 (60.3) |

*Statistically significant association.
and tinnitus disturbance, paralleled by a reduction of TNF-α, but not IL-6 or IL-10.\[^{20}\]

A study aimed to assess the association between hearing impairment and polymorphisms of genes encoding cytokines deeply committed to the inflammatory response and immune homeostasis in an elderly Japanese population. Two hearing impairment criteria were taken into account as pure-tone average in the better ear (PTABE) greater than 25 dB and greater than 40 dB. They analyzed cumulative data using generalized estimating equations to investigate the effect of polymorphisms in interleukin IL-1\(\alpha\), interleukin IL-1\(\beta\), tumor necrosis factor (TNF) \(\alpha\), rs1800630; TNF receptor super family (TNFRSF) 1B, rs1061624 and others. Only TNF-\(\alpha\) rs1800630 and TNFRSF1B rs1061624 demonstrated association.\[^{115}\]

Another study found a significant association of risks for both sudden sensorineural hearing loss and Ménière’s disease with IL-1\(\alpha\) rs 1800587 polymorphism.\[^{21}\]

A paper with Brazilian elderly found significant association between the genotype and allele frequencies of the IL6 gene (rs1800795) and tinnitus among the elderly with history of occupational noise exposure. The elderly with the C allele were less likely to have tinnitus associated with history of exposure to occupational noise, when compared to those carrying the T allele with mechanism of tinnitus, including induction of the expression of these proinflammatory cytokines.\[^{122}\] Thus, it is noted that there are few studies in the literature related to IL-1\(\alpha\) and tinnitus.

After this one, it is believed that more studies based on large populations and with different ethnicities should be conducted to confirm these findings, and the determination of IL-1\(\alpha\) in tinnitus patients related to the history of occupational noise exposure. It clinically suggests that this proinflammatory cytokine is a possible prognostic factor for the SNHL and points out to new perspectives regarding the development of new therapeutic approaches. More studies may also help understand the individual variability of inflammation resulting in this symptomatology, in order to determine the individual susceptibility, providing a new strategy for the prevention of cochlear symptoms related to exposure to occupational noise.

Furthermore, tinnitus is multifactorial. Some studies now seek to better understand the involvement of proinflammatory cytokines, such as IL1\(\alpha\), which plays a central role in the immune response through inflammation of the inner ear against noise. The identification of the gene susceptibility to tinnitus will allow the early identification of this symptom, reflecting in individualized forms of treatments and therapies against this comorbidity that interferes greatly in the quality of life of elderly people. Several inflammatory mediators such as interleukin IL-1\(\alpha\) have the potential to induce/aggravate risk factors in age-related pathology. Thus, it is possible that inflammatory mediators are a link between lifestyle factors, infections and physiological changes in the aging process.

**CONCLUSION**

The present study demonstrated that IL-1\(\alpha\) contributes to the incremental risk of tinnitus in the elderly and to the pathogenesis of the inflammatory response. Moreover, it suggests an association between the IL-1\(\alpha\) with susceptibility to tinnitus in individuals without a history of exposure to occupational noise. This points out to new perspectives regarding the development of therapeutic approaches.

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**Conflicts of interest**

There are no conflicts of interest.

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