Association Between Polymorphism of Tumor Necrosis Factor Alpha (TNFα) in the Region −308 G/A With Tinnitus in the Elderly With a History of Occupational Noise Exposure

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Abstract

Context: Tinnitus is a common disorder that occurs frequently across all strata of population and has an important health concern and is often associated with different forms of the hearing loss of varying severity. Aims: To investigate the association between the polymorphism of tumor necrosis factor alpha (TNFα) in the region −308 G/A with the susceptibility to tinnitus in individuals with the history of exposure to occupational noise. Settings and Design: This was a cross-sectional study with a sample of 179 independent elderly people above 60 years of age. Materials and Methods: Information on exposure to occupational noise was obtained by interviews. Audiological evaluation was performed using pure tone audiometry and genotyped through polymerase chain reaction by restriction fragment length polymorphism. Statistical Analysis Used: Data were analyzed using the chi-square test and the odds ratio (OR), with the significance level set at 5%. Results: Among elderly with tinnitus (43.01%), 33.76% had a history of exposure to occupational noise. A statistically significant association was found between genotype frequencies of the TNFα gene in the −308 G/A region and the complaint of tinnitus (P = 0.04 and χ² = 4.19). The elderly with the G allele were less likely to have tinnitus due to occupational noise exposure when compared to those carrying the A allele (OR = 2.74; 95% CI: 1.56–4.81; P < 0.0005). Conclusion: This study suggests an association between the TNFα with susceptibility to tinnitus in individuals with a history of exposure to occupational noise.

Keywords: Cytokines, noise-induced hearing loss, occupational, tinnitus, TNFα

INTRODUCTION

There is a larger aging population, both in developed countries as in developing countries.¹ It is estimated that by 2040 developing countries would have a billion people aged 60 or more.² Given the great speed and extent of this growth, care with this specific group is essential, so that they can age in a healthy way and with quality of life.³

The prevalence of chronic tinnitus is estimated between 10.1 and 14.5% in adult population and increased with age.⁴ In a study developed by Nondahl et al.,⁵ the 5-year incidence of tinnitus among the 2513 participants at risk was 5.7%. In another study, the same authors found that the incidence had more than doubled after 10 years, reaching 12.7%.⁶

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The presence of inflammatory cells in the stationary state and its increase after lesions in the inner ear have been reported by several researchers.⁷ Noise exposure induces the expression of proinflammatory cytokines including tumor necrosis factor alpha (TNFα), interleukin-1β (IL-1β) and interleukin-6 (IL-6).⁹ So et al. observed a transitory upregulation of IL-6 in animal models treated with cisplatin.¹⁰ Wakabayashi et al. investigated the effect of inhibition of IL-6 using an anti-IL-6 (MR16-1) antibody in mice. These authors found that MR16-1 showed a protective effect against cell damage induced by noise, mainly due to the suppression of neuronal loss and presumably through the relief of the inflammatory response. Similar data were found by Nakamoto et al.¹¹ These authors suggested that the
suppression of the proinflammatory cytokine HSF-1 in the cochlea by the administration of geranylgeranylation (GGG) may be an important way of protecting the inner ear.

Cytokine expression may be influenced by genetic variation, resulting in pathogenic conditions[12] and several studies have investigated single nucleotide polymorphisms (SNPs) as risk factors for inflammatory diseases.[13] These SNPs may affect the expression, secretion and cellular transport of interleukins[14,15] and suggest that the IL-1 beta/IL-1Ra allelic cluster may participate in defining the biological basis of predisposition to chronic inflammatory bowel diseases.[16]

The TNFα gene is located within the class III region of the major histocompatibility complex on the small arm of chromosome 6 (6p21.1–21.3). The production of TNFα is under control and is influenced by a promoter polymorphism, –G308A.[17] This is a well-defined biallelic base exchange polymorphism, which includes a common variant with a guanine (G) at position −308 and an uncommon variant with an adenine (A) at −308. The A allele has been significantly associated with higher TNFα production and, in some cases, with increased morbidity and mortality in many infectious, autoimmune, and other immune disorders.[18]

Whereas tinnitus is a symptom usually related to cochlear alterations that may be arising from noise exposure, this study aimed to evaluate the association between the gene polymorphism TNFα with tinnitus associated with the work history in a noisy environment of physically independent elderly Brazilians.

Materials and Methods

This cross-sectional study was approved by the Ethics Committee on Human Research of the University of North Paraná (0070/09). It is part of a wider research, “Study on aging and longevity,” which was conducted in Londrina since 2009. The city of Londrina (about 500,000 inhabitants) is located in the northern state of Paraná, Brazil.

From a population of 43,610 elders 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 (95% CI) and a margin of error of 5%.[19] With the aim of sampling representativeness, random stratification of regions of the city and gender was done. The study included individuals aged 60 years or more, of both sexes, who were living independently and classified at level 3 or 4, as proposed by Spirduso.[20] This rating assesses the level of independence of the elderly, and the level 1 indicates a lack of self-mobility and the five-level indicates 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 a free and informed consent term.

Audiological assessment

In order to accomplish research in relation to the audiologic data, routine audiological evaluation from Audiology Department of the Clinic of Speech, University of Northern Paraná (UNOPAR) based on the protocol for anamnesis by Miller, including question about age, gender and tinnitus.[21] Specifically for tinnitus, it was used to investigate whether the sensation of tinnitus is present or not, which ear, how often, when the symptom began, and what type of tinnitus the patient has presented.[20] A visual analog scale was also used to assess the level of intensity of tinnitus, consisting of a visual graphic tool to determine the volume level or intensity or discomfort caused by the tinnitus, on a scale of 0–10. The patient is asked to give a score from 0 to 10 to the intensity generated by the tinnitus, where 0 represents the total absence of tinnitus symptom and 10 indicates a maximum intensity of tinnitus symptom, requiring going to a hospital to receive care.[19]

Evaluation of occupational noise exposure

The assessment of occupational noise exposure was obtained through interviews with the elderly participants, using a semistructured questionnaire. We collected information whether the work was conducted in noisy environment or not, how many years of work in a noisy environment, and wore hearing aids. In addition, demographic characteristics were collected.

Analysis of genetic polymorphism of TNFα

Blood samples collect and DNA extraction

From each patient, 5 ml of peripheral blood were collected by venule puncture. Blood samples were stored at −80°C. Deoxyribo Nucleic Acid (DNA) extraction was performed by using the kit PureLink – Invitrogen 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.

Analysis of the polymorphism in the TNFα gene by polymerase chain reaction in real time

To analyze the SNP of genes TNFα, the amplification technique of DNA fragments was performed by polymerase chain reaction (PCR) in real time by TaqMan system (Applied Biosystems, Foster City, USA). Polymorphism at position −308 G/A of TNFα was checked.

The standard reaction contained 20 µL final volume of 10 µL with Genotyping TaqMan Master Mix (1x), 0.5 µL probe (1x) (Applied Biosystems, Foster City, USA) 7.5 µL ultrapure Milli-Q® and 1 µL of DNA (30 ng/µL). StepOnePlus Thermocycler™ Real-Time PCR System (Applied Biosystems, Foster City, USA) with the following cycling used: 50 cycles of 60°C for 30 s, 95°C (pre denaturation), 95°C for 10 min for initial denaturation for 15 s (denaturation) and 60°C for 1 min and 30 s (primer annealing) and a final
extension cycle of 30 s at 60°C. The evaluation of the results was performed by the StepOne version 2.3 software.

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.

For quantitative variables, the Kolmogorov–Smirnov test was used and data with normal distribution were presented by mean and standard deviation, as the data without normal distribution were presented as median and interquartile range.

The chi-square test or Fisher–Freeman–Halton test was used as 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 TNF\( \alpha \) gene with the occurrence and severity of tinnitus.

RESULTS

Of the total of 343, molecular genetic procedures were conducted in 179 elderlies. Of these, 34.07% reported a history of noise exposure with 39.34% presenting tinnitus. Of the 66.48% without a history of exposure to noise, 42.85% presented tinnitus. The mean age was \( 67.76 \pm 5.55 \) years, with a higher proportion (65.4%) of the female elderly [Table 1].

Of the elderly evaluated, 65.4% were homozygous for the G allele, 14% were homozygous for the A allele and 20.7% were heterozygous and 75.7% of elderly patients presented the G allele [Table 1]. The genotypic distribution for the TNF\( \alpha \) gene agreed with the Hardy–Weinberg equilibrium \( (P > 0.05) \).

A statistically significant association was found between the genotypic and allelic frequencies of the TNF\( \alpha \) gene in the \(-308\) G/A region and the complaint of tinnitus \( (P = 0.001) \) [Table 2]. Elderly patients with the A allele presented 3.18 more likely to have tinnitus than elderly individuals with G allele (OR = 3.1813; 95% CI: 1.82–5.53; \( P < 0.0001 \)).

DISCUSSION

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 PAIR are not yet well understood such as SNP, which results in obvious pathological consequences.\(^{[12]}\) The functionality of SNPs with respect to gene expression is an important subject in the studies of association to diseases.\(^{[13]}\)

An association was found between the GG genotype of TNF\( \alpha \) and tinnitus with the history of exposure to occupational noise [Table 2].

Table 1: General characteristics and allele/genotypes frequencies among elderly (\( n = 179 \))

| Characteristics | \( n \) | % |
|-----------------|-------|---|
| Gender          |       |   |
| Male            | 62    | 34.6 |
| Female          | 117   | 65.4 |
| Age (years)     |       |   |
| 60–64           | 55    | 30.7 |
| 65–74           | 101   | 56.5 |
| 75 ou +         | 23    | 12.8 |
| Mean age        | 67.76 (SD = 5.55) |
| Groups          |       |   |
| Tinnitus with history of occupational noise-exposure | 60 | 33.5 |
| Tinnitus without history of occupational noise-exposure | 119 | 66.5 |
| Genotypic frequency |       |   |
| GG              | 117   | 65.4 |
| AA              | 25    | 14.0 |
| GA              | 37    | 20.7 |
| Allelic frequency |     |   |
| G               | 98    | 72.1 |
| A               | 38    | 27.9 |

\( n = \) number of participants, SD = standard deviation, GG = mutant homozygotes, AA = wild-type homozygotes, GA = heterozygotes.

Table 2: Association between genotype frequency for the TNFA \(-308\) G/A gene polymorphism and tinnitus related to history of occupational noise exposure (\( n = 179 \))

| Occupational noise exposure | Genotyping | No tinnitus \( n \) (%) | With tinnitus \( n \) (%) | \( P \)-value and chi-square test |
|-----------------------------|------------|------------------------|--------------------------|---------------------------------|
| No history                  | Genotypic frequency | 42 (61.7) | 40 (78.4) | \( P = 0.02 \) |
|                             | AA         | 12 (17.6) | 1 (01.9) | \( \chi^2 = 7.75 \) |
|                             | GA         | 14 (20.7) | 10 (19.7) | \( \chi^2 = 9.19 \) |
|                             | G          | 98 (72.1) | 90 (88.2) | \( P = 0.00 \) |
|                             | A          | 38 (27.9) | 12 (11.8) | \( \chi^2 = 6.01 \) |
| With history                | Genotypic frequency | 16 (45.7) | 19 (76) | \( P = 0.04 \) |
|                             | AA         | 10 (28.5) | 2 (08) | \( \chi^2 = 3.61 \) |
|                             | GA         | 9 (25.8) | 4 (16) | \( \chi^2 = 3.61 \) |
|                             | G          | 41 (68.3) | 42 (84) | \( P = 0.09 \) |
|                             | A          | 19 (31.7) | 8 (16) | \( \chi^2 = 3.61 \) |

\( n = \) number of participants, \% = percentage, GG = mutant homozygotes, AA = wild-type homozygotes, AG = heterozygotes, \( P = \) \( P \)-value; \( \chi^2 = \) chi-square test.
The tinnitus levels and TNF have been poorly reported in the literature. A study that evaluated releasing soluble TNF also exerts anti-inflammatory properties during injury by neuronal injury, contributing to its regeneration. However, it microglia and regulates the expression of neuropeptides after and natural killer cells. In addition, it activates astrocytes and differentiation/maintenance of cytotoxic T-lymphocytes, neutrophil maturation and activation, macrophages maturation and noise-induced hearing loss (NIHL) has recently been evidence in humans regarding proinflammatory interleukins. These strong evidences of IL-6 involvement with noise related cochlear alterations have been observed through research in animal models.8-11 Evidence in humans regarding proinflammatory interleukins and noise-induced hearing loss (NIHL) has recently been reported by our group investigating the association of the polymorphism in the IL-1β gene with NIHL, where the polymorphism in this gene has not been shown to be associated with NIHL in the assessed elderly. The subsequent findings showed that the polymorphism in the IL-6 gene should contribute to the risk to NIHL in Brazilian elderly.23 IL-6 is a cytokine that has a proinflammatory effect produced and secreted by endothelial, muscle, adipose, and leukocyte tissues.24 IL-6 is a proinflammatory cytokine that promotes neutrophil maturation and activation, macrophages maturation and differentiation/maintenance of cytotoxic T-lymphocytes, and natural killer cells. In addition, it activates astrocytes and microglia and regulates the expression of neuropeptides after neuronal injury, contributing to its regeneration. However, it also exerts anti-inflammatory properties during injury by releasing soluble TNFα (sFNTRs) and IL-1AR receptors.25 Changes in gene expression for TNFα during tinnitus have been poorly reported in the literature. A study that evaluated the tinnitus levels and TNFα mRNA expression after the intraperitoneal injections of salicylate in mice found that tinnitus increased in response to daily treatments salicylate. The levels of TNFα mRNA expression were significantly increased in the group treated with salicylate compared with the control group in both ears (1.89 ± 0.22 vs. 0.87 ± 0.07, P < 0.0001) and CI (2.12 ± 0.23 vs. 1.73 ± 0.22, P = 0.0040). Linear regression analysis showed a significant positive association between tinnitus scores and levels of TNFα expression in the cochlea, concluding that salicylate treatment increases the expression of TNFα in the cochlea of mice, and it is suggested that these proinflammatory cytokines may cause tinnitus directly or through the modulation of the N-methyl-d-aspartate (NMDA) receptor.14 A recent study cites that inflammatory responses occur in the inner ear under various adverse conditions, including overstimulation with noise, although an association between the proinflammatory cytokines are rarely reported.9 Furthermore, the study indicates that in individuals with chronic tinnitus symptoms, relaxation training program can result in significant reduction of stress, depression, anger, and perception of tinnitus, in parallel with a TNFα reduction.26 In this study, it can be verified that the frequency of the A allele in the tinnitus group was higher than in the no tinnitus group. TNFα, one of the most important proinflammatory cytokines, plays an essential role in the pathogenesis of acute inflammatory response and is involved in systemic inflammation.27 The A allele has been significantly associated with higher TNFα production and, in some cases, with increased morbidity and mortality in many infectious, autoimmune, and other immune disorders.18 In summary, this study demonstrated an association between TNFα polymorphism and the risk and tinnitus in elderly patients with a history of exposure to occupational noise. Other disorders have also been investigating the association to proinflammatory cytokines, such as IL6 and TNFα. A study developed by Braga et al.23 found an association between the presence of IL-6 gene polymorphism and hearing loss associated with occupational noise exposure in elderly Brazilians. The identification of the polymorphism in the IL-6 gene in patients with a history of hearing loss related to occupational exposure to noise may help us to understand the individual variability of inflammation resulting in hearing loss as well as in the future, to suggest genotyping individuals for this particular polymorphism (rs1800795) to determine individual susceptibility, providing a new strategy for the prevention of hearing loss related to noise exposure.

Another study, a systematic review, conducted by Gan et al.28 reported the relationship between chronic obstructive pulmonary disease (COPD) and levels of various systemic inflammatory markers, such as TNFα and interleukins 6 and 8. They found a large body of evidence to indicate that systemic inflammation is present in patients with stable COPD, and circulating leukocytes were also higher in COPD than in control individuals as were serum TNFα levels; so reduced lung function is associated with
increased levels of systemic inflammatory markers, which may have important pathophysiological and therapeutic implications for individuals with stable COPD.

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In this context, it is believed that more studies based on large populations and with different ethnicities should be conducted to confirm our findings, and that the determination of TNFα in tinnitus patients related to the history of occupational noise exposure may also help us to understand the individual variability of inflammation resulting in this symptomatology to determine the individual susceptibility, providing a new strategy for the prevention of cochlear symptoms related to exposure to noise.

CONCLUSION

A statistically significant association was found between TNFα gene polymorphism in the −308 G/A region and tinnitus in the elderly with a history of occupational noise exposure.

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Conflicts of interest

There are no conflicts of interest.

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