Original Article

Audiometric notch for the prediction of early occupational hearing loss and its association with the interleukin-1beta genotype

Nagat M. Amer, MD, Mona M. Taha, PhD, Khadiga S. Ibrahim, PhD, Heba M. Abdallah, MD* and Eman M. El Tahlawy, MD

Environmental & Occupational Medicine Department, Environmental Research Division, National Research Centre, Giza, Egypt

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Abstract

Objectives: Noise-induced hearing loss (NIHL) is a frequent and irreversible industrial-health problem, the early diagnosis of which can prevent hearing deterioration, especially of speech frequencies. This study aimed to assess hearing impairment in workers occupationally exposed to noise. Audiometric notches and IL-1β gene polymorphisms were evaluated.

Methods: The study included 98 workers employed in a textile factory. Pure-tone audiometric testing was conducted for all workers, using a manual pure-tone diagnostic audiometer, and the noise level was measured at four different work sites in a spinning section. IL-1β gene polymorphism was determined using PCR-RFLP methods.

Results: Workers were exposed to a mean noise level of 105.5 dB. As many as 27.6% of them complained of tinnitus and the majority suffered from some degree of hearing loss in either ear, but none of them exceeded 60 dB. Audiometric notches were detected in either one or both ears. Only 28.6% of workers showed the absence of audiometric notches. The TT genotype of IL-1β polymorphisms was associated with smoking (54.5%) and audiometric notches (100%). The IL-1β gene polymorphism was dominant in 49% of the workers, whereas TC was present in 39.8% and CC in 11.2%. The CC genotype was shown to be a significant risk factor for audiometric notches in workers occupationally exposed to noise. Audiometric notches and IL-1β polymorphisms may be useful in the early diagnosis of which can prevent hearing deterioration, especially of speech frequencies.

Conclusion: This study showed an association between the CC genotype and smoking as well as audiometric notches in workers occupationally exposed to noise. Workers showing audiometric notches should be followed up regularly. Further studies are required to confirm the results.

* Corresponding author: Environmental & Occupational Medicine Department, Environmental Research Division, National Research Centre, El-Buhouth Street, Dokki, Giza, 12622, Egypt. E-mail: hebamahdy91@gmail.com (H.M. Abdallah)

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confirm the interrelationship amongst tinnitus, audiometric notches, and IL-1β genotypes.

**Keywords:** Audiometric notches; CC genotype; IL-1β genotypes; Smoking; Tinnitus

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**Introduction**

Noise, the high exposure to which is hazardous to human health, is present in the different branches of economic activity.1 Occupational noise exposure is one of the major contributing factors to adult-onset hearing loss and tinnitus,2 where workers exposed occupationally to noise commonly complain of tinnitus and hearing impairment.1 Noise-induced hearing loss (NIHL) may be accompanied by psycho-social consequences, such as social isolation, frustration, and depression.3

It is known that NIHL may be mediated by the interaction of genetic and environmental factors.4,5 but the mechanisms involved are still not clearly understood. It has been suggested that the etiopathogenesis may include a direct mechanical injury to the cochlea, inner ear cell apoptosis and necrosis resulting from oxidative stress, or metabolites released during signal transduction.5,6

Tinnitus, a common and annoying accompaniment of hearing loss, is an auditory phantom sensation defined as ‘ringing in the ear’. It is a frequent disorder that affects all strata of the population and is a major health concern. It is often linked to different forms of hearing loss of varying degrees of severity.7 Hearing loss and acoustic environmental exposures are causing increasingly known health disorders.8 Noise-induced and age-related hearing loss (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5484347/-lary26256-bib-0012 can be differentiated by the presence of audiometric notches. In the clinic, a notch is often regarded as a strong indication of NIHL. Philips et al.10 postulated that the audiometric notch may be regarded as a phenotype for identifying the genetic contributions to hearing loss. However, experts disagree about the definition of a true notch11 and various definitions of notch have been put forward.12 Recently, studies have shown that audiometric notches also occur frequently in workers who are not exposed to loud work-related noise.13,14

A relationship between prolonged working hours and hearing impairment in both low and high frequencies, with a dose—response relationship, has been detected.15 Common causes of hearing loss in the general population include aging, a history of ear diseases, diabetes, and cigarette smoking. However, the major cause in the working population is occupational noise exposure.

Smoking is an intrinsic risk factor for hearing loss. Moreover, it may synergistically influence hearing when associated with occupational noise exposure, particularly in males and older subjects.16 Noise over-stimulation induces inflammatory responses and the up-regulation of pro-inflammatory cytokines in the inner ear.7

The structure and expression of cytokines can be affected by genetic alteration, causing pathological conditions. Various studies have examined single nucleotide polymorphisms (SNPs) as risk factors for inflammatory disorders.17-19 Those SNPs may affect the expression, secretion, and cellular transport of the interleukin-1beta (IL-1β) protein.20

IL-1, the gene of which is located on the long arm of chromosome 2,21 is a polypeptide consisting of subtypes IL-1α and IL-1β, with the latter subtype being the predominant form. IL-1β is secreted mainly by macrophages, dendritic cells, monocytes, natural killer cells, and B cells. The IL-1β gene polymorphism at +3954C/T may cause a 4-fold increase in interleukin expression, increasing catabolism and resulting in structural deficit.7 Noise exposure caused the overexpression of IL-1β, interleukin-6, tumour necrosis factor-alpha, heat shock protein 70, heat shock factor 1, and cyclooxygenase-2 in the cochlea of rats.22

Polymorphisms of IL-1β, IL-1 receptor 1, or IL-1 receptor antagonist are associated with an increased risk of different solid malignant tumours. Human carriers of IL-1β polymorphisms (IL-1β-511T and IL-1β-31C) show enhanced IL-1β production and increased levels of circulating cytokines, which can result in a high risk of cancers.23

The biological activity of IL-1 cytokines is controlled at the level of their production and the maturation of receptor binding, and through post-receptor signalling by naturally present inhibitors. The association of severe inflammatory syndromes with genetic deficiencies in some of these regulatory molecules has considerably increased our understanding of the biology of IL-1 cytokines.24 Noise exposure induces the production of such cytokines by the cochlear structure. Consequently, these cytokines may trigger an inflammatory response and play a role in the mechanism of noise-induced cochlear injury.

This study aimed to assess hearing impairment in workers occupationally exposed to noise. Audiometric notches and IL-1β gene polymorphisms were evaluated.

**Materials and Methods**

The study was conducted in a spinning and weaving factory located at Kafr Hakeem in the Giza governorate. It was carried out in the open-end spinning section. All subjects filled a specific questionnaire that was designed to obtain data on their personal information, smoking habit, and detailed history of current and previous jobs. Histories of chronic drug intake, previous ear operation, pus discharge, or hearing problems were emphasised. Apart from a local otoscopic examination to exclude local ear problems, general clinical examinations were also performed. After exclusion of workers with previous ear diseases and those who reported former intake of ototoxic drugs (e.g. aspirin, quinolones, and aminoglycosides), 98 subjects were finally chosen for audiometric testing. This was done using a manual pure-tone diagnostic audiometer (Model GSI 67, Grason-Stadler, Inc., Eden Prairie, MN, USA). The subjects were tested in a sound isolation room that adhered to the American National Standards Institute requirements for an audiometric testing environment. All participants underwent pure-tone
audiometry at frequencies of 0.5, 1, 2, 3, 4, 6, and 8 KHz for both ears. Written consent was obtained from all examined subjects. Approval from the ethics committee at the National Research Centre was obtained prior to the study.

Environmental measures

Environmental noise assessment was carried out using a portable Sound Level Meter Standard (Model CR 306) at different sites in the open-end spinning sector.

Gene assessment

DNA extraction

Peripheral blood specimens from all study participants were collected into an EDTA vacuum tube, and DNA was extracted using the QIAmp extraction kit.

Genotyping of IL-1β polymorphism

IL-1β gene polymorphisms were assessed according to the methods described by Carvalho et al. The C-to-T polymorphism located at the +3954 positions in the IL-1β gene was amplified, resulting in a 182 bp fragment. The polymerase chain reaction (PCR) mixture contained 1X PCR buffer, 1.5 mM MgCl₂, 8 μM of each deoxyribonucleotide triphosphate, 1 μM of each sense primer and anti-sense primer, and 2 units of Taq DNA polymerase (Invitrogen, Carlsbad, CA, USA).

The primers used were as follows: Forward 5’-CTC AGG TGT CCT CGA AAG AAA TCA A-3’ and Reverse 5’-GCT TTT TTG CTG TGA GTC CCG-3’. PCR amplification was performed in a thermal cycle under the following conditions: Initial denaturation at 95 °C for 5 min, followed by 30 cycles at 95 °C for 1 min, 67 °C for 1 min, and 72 °C for 1 min, and a final extension step at 72 °C for 5 min.

The PCR product was digested overnight with 5 units of Taq I polymerase (Invitrogen, Carlsbad, CA, USA). The digested fragments were separated on a 2% agarose gel that was then stained with ethidium bromide and visualised under UV illumination. The gel revealed 85 and 97 bp fragments (allele C), a single 182 bp fragment (allele T), and three fragments (85, 97, and 182 bp), indicating the presence of both C and T alleles.

Statistical analysis

Statistical analysis of the data was performed using the SPSS package, version 17.0. The Chi-square test was used to compare the distributions of genotypes as well as the frequencies of alleles for the +3954 IL-1β polymorphism. The analysis of variance test was used to compare multiple variants.

Results

The environmental noise measurements revealed an elevation of the sound level at four tested sites in the open-end spinning section. The noise level ranged between 100 and 110 dB, with a mean of 105.5 dB, which exceeded the 90 dB level recommended by the Egyptian Environmental Law No.4 (1994).

Table 1 shows the general characteristics of the studied workers. The percentage of smokers was 21.4%, whereas those with diabetes constituted only 5% of the examined individuals. Workers who complained of tinnitus represented 27.6% of the studied population.

| Table 1: General characteristics of the examined workers. |
| ---------------- | --------- | --------- |
| --- | ------- | ------- |
| Age (years) | 39.47 ± 5.94 |
| Duration of exposure (years) | 17.20 ± 4.76 |
| Systolic blood pressure (mmHg) | 127.68 ± 17.66 |
| Diastolic blood pressure (mmHg) | 81.78 ± 10.79 |
| No (%) | 77 | 78.6% |
| Smoking Yes | 21 | 21.4% |
| Tinnitus Yes | 27 | 27.6% |
| Diabetes Yes | 5 | 5.1% |
| Diabetes No | 93 | 94.9% |

Table 2: Hearing impairment in the workers.

| Hearing impairment | Right Ear | Left Ear |
|--------------------|-----------|----------|
| No (%) | No (%) |
| Normal (0–25 dB) | 36 (36.7%) | 44 (44.9%) |
| Mild (26–40 dB) | 44 (44.9%) | 37 (37.7%) |
| Moderate (41–60 dB) | 18 (18.4%) | 17 (17.3%) |

Table 3: Analysis of audiometric notches in the workers’ audiograms.

| Audiometric notch at 4000 Hz | Right ear | Left ear |
| -----------------------------|-----------|----------|
| No (%) | No (%) |
| <25 dB | 36 (36.7%) | 47 (48.0%) |
| ≥25 dB | 62 (63.3%) | 51 (52.0%) |
| Total | 98 | 98 |

Percentage of Interleukin 1 Beta genotype distribution in workers.

![Figure 1: IL-1β genotype distribution amongst the examined workers.](image)
The mean age of the workers and duration of noise exposure had no significant difference amongst the three genotypes with regard to smoking habits and audiometric notches amongst the workers. There was no significant difference in the percentages of hearing impairment in the right and left ears of the examined workers, as shown in Table 2. None of the workers suffered from profound hearing loss (i.e., of >60 dB).

Table 3 demonstrates the presence of audiometric notches in one or both ears in the examined workers. Only 28 (28.6%) of the audiograms of the workers were free of notches in both ears. Majority of the workers suffered notches of ≥25 dB in both ears (45.9%). However, 25 workers (25.5%) showed notches of <25 dB. The TT genotype showed the highest percentage of distribution amongst the workers (49%). There was no significant difference amongst the three genotypes with regard to the mean age of the workers and duration of noise exposure (Figure 1).

Significant differences were detected amongst the three IL-1β genotypes with regard to smoking and audiometric notches, as shown in Table 4. The CC genotype was associated with smoking (54.5%) and with audiometric notches (100%).

No significant difference was observed in the IL-1β genotype distribution according to workers with and without tinnitus complaints, as shown in Table 5.

**Discussion**

Occupational noise is one of the most common occupational hazards worldwide. Moreover, the noise is linked to numerous non-auditory health effects, such as sleeping disorders, electrocardiogram abnormalities, and hypertension. Previous studies have shown that excessive noise exposure is the most pervasive preventable aetiology of hearing loss. It has been estimated that over 12% of the world population are susceptible to hearing loss from noise, where approximately one-third of all cases could be related to acoustic exposure.

The prevalence and significance of hearing injuries induced by noise levels not previously known to cause permanent hearing impairment have recently become obvious. Whereas the damage to, and loss of, afferent terminals of auditory nerve fibres at the cochlear inner hair cells are well documented, the effects of noise exposure and terminal loss of the inner hair cells are less recognized.

The majority of workers in the present study were non-diabetic (93%) and normotensive. Smokers represented 21.4% of the workers studied. It has been shown that cigarette smoking can hinder cochlear circulation by increasing the blood viscosity and decreasing oxygenation. Recently, cigarette smoking has been implicated in the increased susceptibility to noise damage. The combination of smoking and occupational noise exposure can synergistically affect hearing, especially in elderly male participants. In addition, individuals with the longest acoustic exposure duration and maximum pack-years are more at risk.

A considerable portion of the workers complained of tinnitus (27.6%), denoting early symptom of hearing affection. Audiograms of the workers revealed the deterioration of hearing acuity in either the right or left ears, but none of the individuals showed a profound hearing loss. Those results are attributed to the reluctance of the workers to use the available hearing protective equipment.

A high percentage of the workers presented with audiometric notches of ≥25 dB either for the right ear (62.3%) or left ear (52.0%) or for both ears (45.9%). The appearance of such notches at a frequency of 4000 Hz has been well documented as evidence of occupational noise exposure and is considered as a classical sign of NIHL.

In the present study, 49% of the workers had the IL-1β TT genotype and 11.2% were homozygous for the CC genotype. The heterozygous TC genotype was detected in 39.8% of the workers. Fujioka et al. reported the production of pro-inflammatory cytokines, including IL-1β, in the early phase of the noise over-stimulated cochlea. These cytokines, which are generated by the cochlear structure itself in response to noise exposure, may initiate an inflammatory response and have some role in the mechanism of acoustic-induced cochlear damage. IL-1β is a mediator of both bacterial and viral otitis media. It has an influence on numerous target cells, stimulating the pro-inflammatory network in the middle ear. Hence, it could result in the activation and proliferation of fibroblasts and the growth of osteoclasts in the middle ear, possibly leading to fibrosis and bone erosion.

We found that 54.5% of the workers with the CC genotype were smokers and 45.5% were non-smokers (Table 4); additionally, 36.4% complained of tinnitus whereas 63.6%
did not (Table 5). The study suggests that there is an association between polymorphisms in the IL-1β gene and smoking.

Smoking is common in all social classes, with some differences between them. Current smoking was shown to be associated with hearing impairment in both speech-relevant frequency and high frequency across all ages.31 Recently, inflammatory responses were shown to occur in the inner ear under various injurious conditions, including noise over-stimulation. Therefore, it can be concluded that smoking represents a confounding factor, in addition to noise, in workers with the CC genotype of the IL-1β gene, causing hearing impairment.

This work is considered the first study in Egypt to have correlated audiometric notches in audiograms and gene polymorphisms in workers occupationally exposed to noise. Furthermore, a significant association was shown between workers with the C allele and audiometric notches at ≥25 dB (Table 4). These results raise the hypothesis that a particular genotype could predispose individuals to hearing impairment by enhancing the inflammatory processes.

Um et al.32 suggested that the IL-1β-511 and +3953 loci may play an outstanding role in the etiopathogenesis of NIHL. IL-1β is produced and released by many distinct immune and non-immune cell types as an immediate response to inflammatory signals. Bent et al.33 demonstrated that IL-1β acted as an amplifier of immune reactions. For a fairly long time, IL-1 was widely acknowledged as being required for the effective initiation of innate immune responses and shaping of adaptive immune responses to resolve acute inflammations. However, the view of IL-1β as a beneficial immune regulator has been affronted by the finding that gain-of-function mutations in components of inflammation result in increased IL-1β production that lead to auto-inflammatory diseases.

Recommendations

Hearing conservation programs are strongly warranted in the textile industry. Hearing protective equipment should be provided to all workers. Regular follow-up is needed for workers whose audiograms present notches at their periodic hearing tests. Smoking cessation programs are important to encourage smokers to quit smoking, especially in noisy workplaces.

Conclusion

An association exists between the CC genotype and smoking as well as audiometric notches in workers occupationally exposed to noise. We suggest that an interrelationship exists amongst tinnitus, audiometric notches, and IL-1β genotypes, which requires further studies.

Source of funding

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Conflict of interest

The authors have no conflicts of interest to declare.

Ethical approval

Ethical approval certificate (Registration Number 16401) was obtained from the Medical Research Ethics Committee.

Authors’ contributions

HMA and MMT designed the study. HMA conducted the audiometry. NMA interpreted the audiogram results. MMT and KSE conducted the molecular study. All authors collected and organized the data. EME analysed and interpreted the data. MMT wrote the manuscript. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

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