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Heat Shock Protein 70-1 Gene mRNA Expression in Noise Exposed Workers

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Abstract:
High levels of occupational noise exposure remain a global concern. Over 30 million workers are exposed to hazardous noise, causing cellular stress and potentially leading to noise-induced hearing loss (NIHL). Noise exposure increases the expression of the cellular stress marker HSP70-1, which can be detected in blood samples. The present study is carried out to investigate the differences of HSP70-1 gene mRNA expression between exposed and non-exposed workers to noise. An analytical observational study with cross-sectional design is conducted. The subjects underwent examination of HSP70-1 gene mRNA expression by using quantitative real-time polymerase chain reaction (rt-qPCR). In addition, it was used to determine whether there were differences between noise-exposed and non-exposed groups with statistical significance set at $p < 0.05$. The noise levels were measured in the production area ranged from 96 to 111 dB. The investigation has been carried out on 60 male individuals, of which 30 individuals were in the exposed group and 30 samples were in the non-exposed group. Furthermore, the average age of the noise-exposed participants was 37.47±7.23 years, and the average duration of employment was 10.53±4.50 years. This research recorded that there was a statistically significant difference in the expression level of HSP70-1 gene mRNA between the two groups ($p<0.001$), in which exposure group (10.04±1.37) was higher than that of those non-exposed individuals (8.05±1.15). Significant variation was shown in HSP70-1 gene between exposed and non-exposed workers ($p<0.001$).

Keywords: *noise, hearing loss, mRNA expression, HSP70-1 gene*

1. Introduction

Noise-induced hearing loss (NIHL) is a hearing impairment resulting from prolonged exposure to loud noise, typically caused by occupational noise. The nature of the impairment is cochlear sensorineural hearing loss, which generally affects both ears[1]. Noise exposure is able to originate from industrial, military, and recreational sources. High levels of occupational noise remain a significant issue worldwide. In addition, over 30 million workers exposed to hazardous noise levels2.

Approximately 1 of 8 workers in the US experiences hearing difficulties. Among those with hearing difficulties, 1 of 4 individuals are exposed to noise at work. About 1 of 13 workers in the US suffers from tinnitus, and 1 of 25 experiences both hearing difficulties and tinnitus. Moreover, based on the 2011-2012 National Health and Nutrition Examination Survey, the prevalence of NIHL is 24.4% among US adults aged 20-69 years. A hearing screening study using pure-tone audiometry in textile factory workers in Bandung showed a prevalence of hearing impairment of 68.1% in men compared to 37.2% in women[3]. Heat shock protein (HSP) genes can be overexpressed in the inner ear through stimuli such as physiological stress, ototoxic drugs, high temperatures, and noise. Severe noise exposure can induce antibodies against members of the heat shock protein 70 (HSP70) family in industrial workers. While severe noise exposure can induce HSPs. Moderate noise exposure has been reported to provide protection against noise-induced hearing loss, whether there is a relationship between genetic variations in constitutive and inducible HSP70 genes and noise-induced hearing loss is currently unknown. The HSP70 gene family includes HSP70-1,

HSP70-2, and HSP70-hom.

Several studies have shown that the production of HSP70 in the inner ear protects hair cells against the ototoxic effects of cisplatin and aminoglycosides. The influence of HSP70 genetic polymorphisms on susceptibility to NIHL has been a focus of research for over a decade. However, these studies have not reached consistent conclusions[4]. Cellular stress caused by noise-induced hearing loss can be observed through damage to cochlear hair cells, both mechanically and metabolically. HSP70, as a cellular stress marker, can be identified by its levels in the blood and its expression in the cochlea[5]. This study aims to investigate whether there are differences in HSP70-1 gene mRNA expression between exposed ones and those not exposed to noise.

2. Research Methodology

This study is an analytical observational study with cross-sectional design to investigate the differences in HSP70-1 gene mRNA expression between exposed and non-exposed ones. The study sample consisted of exposed individuals and non-exposed ones to noise, meeting the inclusion and exclusion criteria. The inclusion criteria for noise-exposed workers were: aged 23-55 years, noise exposure >85 dB, working at least 8 hours a day, employed for 5 years or more, with no history of using ototoxic drugs, no otitis media, and no congenital ear abnormalities. While the non-exposed group, the inclusion criteria were: aged 23-55 years, not exposed to noise, working at least 8 hours a day, with no history of using ototoxic drugs, no otitis media, and no congenital ear abnormalities. All eligible workers were recorded using a questionnaire that included information on age, gender, length of employment, and daily noise exposure duration. The comparison group consisted of workers employed in areas with no high-intensity noise exposure. ENT examinations were conducted using diagnostic tools such as headlamps and otoscopes, as well as noise levels were measured with a sound level meter. Subjects provided informed consent before venous blood samples were taken for HSP70-1 gene mRNA expression analysis using rt-qPCR.

Analysis of HSP70-1 Gene mRNA Expression with PCR

Nucleotide extraction results were stored at -80°C before PCR analysis. The mRNA primers for real-time PCR and PCR conditions were based on previous research protocols. The HSP70-1 (HSPA1A) Human qPCR Primer Pair included a forward sequence of 5’-ACC TTC GAC GTG TCC ATC CTG A-3’ and a reverse sequence of 5’-TCC TCC ACG AAG TGG TTC ACC A-3’. The qPCR cycling conditions started with an initial reverse transcriptase temperature at 51°C for 30 minutes, followed by PCR activation at 95°C for 6 minutes, then 40 cycles in the following steps: 95°C for 20 seconds and 58°C for 60 seconds. Amplification was conducted using specific oligonucleotide primers for the HSP70-1 gene, with GAPDH serving as the housekeeping gene (internal control), using the forward primer 5’-CTTCATTGACCTCAAGACA-3’ and the reverse primer 5’-ACTCCACGACATACTC AGC-3’[6-8].

Data Analysis

The data in this study were analyzed using SPSS version 26 consisted of univariate and bivariate analyses. Univariate analysis was conducted to describe the characteristics of the participants expressed as counts (n) and percentages (%), or means ($\bar{x}$) and standard deviations (SD). Bivariate analysis was performed using tests for differences in proportions and mean values. The test was conducted by using the Chi-Square test or alternative tests when Chi-Square test assumptions were not fulfilled. On the other hand, the test for differences in mean values was conducted by using the independent t-test if parametric test assumptions were met, as determined by Shapiro-Wilk test for normality. If the assumptions were not met, the non-parametric Mann-Whitney U test was conducted.

3. Results and Discussion

Results

Noise-exposed workers were those employed in the Production Department of PT. X in the industrial area of Makassar City, South Sulawesi, Indonesia. The noise levels were ranged from 96 to 111 dB. There were 60 workers in that environment continuously exposed to machine noise, divided into 3 shifts, and each worked 8 hours per shift. Of all the workers in the production unit, 30 individuals participated in this study. As a control, participants employed in locations without high-intensity noise exposure.

Demographic Characteristics

All participants in this study were male. The average ages of the noise-exposed and non-exposed ones were respectively 37.47 years and 35.77 years. On the other hand, the average durations of employment for the noise-exposed and non-exposed groups were 10.53 years and 9.70 years respectively. Furthermore, there was not significant variation shown between the two groups (p=0.195 and 0.213), as shown in Table 1.

Table 1. General Characteristics of Subjects

Characteristics	Noise-exposed (n=30)		Non-noise-exposed (n=30)		p-value
	Mean	SD	mean	SD	
Age (Years)	37.47	7.23	35.77	8.50	0.195
Duration of Employment (Years)	10.53	4.50	9.7	8.01	0.213

HSP70-1 Gene mRNA Expression

Noise-exposed individuals showed higher level than non-exposed ones (10.04, 8.05). In addition, the difference in HSP70-1 gene mRNA expression between these two groups was statistically significant ($p<0.001$, Table 2).

Table 2. Differences in HSP70-1 Gene mRNA Expression Levels Between Non-noise-exposed and Noise-exposed Groups

	Group		p-value
	Non-noise-exposed	**Noise-exposed**	
HSP70-1 Gene mRNA Expression	8.05 ±1.15	10.04 ±1.37	<0.001

Mann-Whitney U test

Discussion

The noise exposure limit in the workplace is appointed at 85 dB for an 8-hour workday and 5 days a week. Measurements using a sound level meter in the production area shows noise levels ranging from 96 to 111 dB, with continuous noise at 96 dB. According to the Indonesian Ministry of Manpower Regulation Number: PER.13/MEN/X/2011 on Occupational Physical Factor Threshold Values, exposure to noise at 97 dB is allowed for only 30 minutes per day. The findings from this study indicate that the noise exposure for workers exceeds the permitted noise threshold[9].

The interaction between noise-induced permanent threshold shift (NIPTS) and age-related hearing loss has been a subject of debate. Epidemiological studies often assume additivity; that is, the net hearing loss is the sum of the decibel shifts due to aging and noise exposure[10]. Research by Anwar, Savitri, and Diah found that the majority of fishermen working in the village of Barombong experienced hearing loss. The decrease in hearing thresholds was significantly related to age, length of employment, and noise exposure intensity[11].

In studies investigating the relationship between HSP70 expression and age, measurements of HSP70 mRNA stability and HSP70 transcription have revealed that age-related decreases in HSP70 expression result from reduced HSP70 transcription[12].

In this study, the mRNA expression of HSP70-1 in noise-exposed workers was significantly higher than those of non-exposed individuals, with mean values of 10.04 and 8.05 respectively, as well as a statistically significant difference ($p<0.001$). Research by Bahalo et al. revealed that noise exposure increases the expression of HSP-70, TGF-β1, Prestin, and decreases the expression of NOX3[13]. Gratton et al. (2011) reported significant upregulation of the HSPA70 (HSP1A1) and HSP40 genes in 129 rats that were resistant to noise exposure. The upregulation of these factors contributes synergistically to the inhibition of apoptosis[14].

Heat shock proteins are the key components of the cellular protective response to stress. Rats which are lacking HSP70 exhibit more extensive infarctions due to uncontrolled apoptotic mechanisms[15]. HSP27, HSP70, and HSP72 are known to be expressed in the cochlea and have been shown to be specifically regulated by excessive exposure to noise[16-19].

The heat shock response in tissues is necessary to maintain balanced inflammatory status due to the cytoprotective and anti-inflammatory roles of iHSP70 expression. This is crucial for proteostasis against harmful challenges such as oxidative stress. Various stressors

mayns11S1 induce iHSP70 expression in the cochlea of rodents, including exposure to noise. Both groups highlighted the importance of Heat Shock Factor 1 (HSF1), the main transcription factor of the HSP70 family, in preventing and repairing cochlear damage following intense exposure to noise in hsf−/− knockout rats. The heat shock response begins immediately after exposure to noise (106 dB NPS for 2 hours), peaking HSP70 mRNA expression after 4 hours[20].

Study by Henderson et al. demonstrated a persistent stress response in the auditory system following noise-induced hearing loss (NIHL). Rats in the NIHL group showed sustained increased of iHSP70 levels for 14 days post-noise exposure. Additionally, there were elevated levels of iHSP72 and eHSP72 in the noise-induced hearing loss group. The HSP72 index (plasma/cochlear eHSP72/iHSP72 ratio) was also increased in the noise-induced hearing loss group[21]. In this study, the investigation of HSP70 mRNA expression was conducted on the blood plasma of the study subjects. Moreover, the findings from the above-mentioned animal studies support our research, indicating that exposure to noise also leads to increased plasma HSP70 levels.

4. Conclusion

The noise levels were measured in the production area ranged from 96 to 111 dB. The investigation has been carried out on 60 male individuals, of which 30 individuals were in the exposed group and 30 samples were in the non-exposed group. Furthermore, the average age of the noise-exposed participants was 37.47±7.23 years, and the average duration of employment was 10.53±4.50 years. This research recorded that there was a statistically significant difference in the expression level of HSP70-1 gene mRNA between the two groups ($p<0.001$), in which exposure group (10.04±1.37) was higher than that of those non-exposed individuals (8.05±1.15). The findings of this study showed that the mRNA expression of the HSP70-1 gene was significantly higher in exposed workers than that of those non-exposed ones ($p<0.001$).

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