REVISTA NEUROCIÊNCIAS

Original

Audiological findings of gas station attendants exposed to fuels

Achados audiológicos de frentistas expostos a combustíveis

Hallazgos audiológicos de empleados de gasolineras expuestos al combustible

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Resumo

Introdução. O histórico de exposição ocupacional geralmente é mal documentado. Os dados fisiológicos apontam para uma combinação de processos de cocleotoxicidade e neurotoxicidade subjacentes aos efeitos auditivos observados nas exposições a solventes. Assim, a caracterização do risco de exposição a solventes é necessária para desenvolver e implementar medidas diagnósticas e preventivas eficazes. **Objetivo.** Avaliar o sistema auditivo periférico e central de frentistas expostos a combustíveis. **Método.** Este estudo transversal utilizou uma bateria de testes que incluiu audiometria tonal liminar (ATL), imitância acústica, potencial evocado auditivo de tronco encefálico (PEATE) e potencial evocado auditivo cognitivo (P300). Um grupo não exposto incluiu 23 participantes sem exposições conhecidas a ruído, combustíveis ou solventes. O grupo exposto a combustíveis incluiu 21 frentistas que foram expostos a combustível e solventes. **Resultados.** Apenas 2 dos 24 frentistas apresentaram audição normal em todos os testes. Individualmente, o reflexo acústico estapediano apresentou o maior número de alterações, com diferenças significativas entre os grupos, para os resultados do reflexo acústico estapediano ipsilateral da orelha direita e contralateral da orelha esquerda para o grupo não exposto. Resultados anormais do PEATE e reflexo acústico estapediano podem ser detectados antes das anormalidades de P300 e ATL. **Conclusão.** Foram observadas alterações auditivas caracterizadas por comprometimento do sistema auditivo periférico e/ou central, sugerindo ação tóxica da exposição aos combustíveis. Mais pesquisas são necessárias para melhor caracterizar os danos às vias auditivas centrais induzidos por combustíveis e para identificar uma bateria de testes audiológicos ideal para uso com este grupo de indivíduos.

Unitermos. Audição; Solventes; Combustíveis; Ototoxicidade; Potencial evocado auditivo; P300

Abstract

Introduction. Occupational exposure history is typically poorly documented. The physiological data points to a combination of cochleotoxicity and neurotoxicity processes underlying the

observed auditory effects of solvent exposures. Thus, the characterization of risk from exposure to solvents is needed to develop and implement effective diagnostic and preventive measures. **Objective.** To evaluate the peripheral and central auditory systems of gas station attendants exposed to fuels. **Method.** This cross-sectional study used a test battery that included pure-tone audiometry (PTA), acoustic immittance, auditory brainstem response (ABR), and cognitive auditory-evoked potential (P300). A non-exposed group included 23 participants with no known exposures to noise, fuels, or solvents. The fuels-exposed group included 21 gas station attendants who had been exposed to fuel and solvents. **Results.** Only 2 of 24 gas station attendants presented normal hearing results in all tests. Individually, the stapedial acoustic reflex had the greatest number of abnormalities, with significant differences between groups for the non-exposed group in the ipsilateral stapedial acoustic reflex results from the right ear and contralateral from the left ear. Abnormal ABR and stapedial acoustic reflex results could be detected sooner than P300 and PTA abnormalities. **Conclusions.** Auditory abnormalities characterized by impairment in the peripheral and/or central auditory system were observed, suggesting a toxic action of exposure to fuels. Further research is needed to better characterize the damage to central auditory pathways induced by fuels and to identify an optimal audiological test battery for use with this group of individuals.
Keywords. Hearing; Solvents; Fuels; Ototoxicity; Auditory Evoked Potential; P300

Resumen

Introducción. Los antecedentes de exposición ocupacional suelen estar mal documentados. Los datos fisiológicos apuntan a una combinación de procesos de cocleotoxicidad y neurotoxicidad subyacentes a los efectos auditivos observados de la exposición a disolventes. Por lo tanto, la caracterización del riesgo de exposición a solventes es necesaria para desarrollar e implementar medidas diagnósticas y preventivas efectivas. **Objetivo.** Evaluar el sistema auditivo periférico y central de empleados de gasolineras expuestos al combustible. **Método.** Este estudio transversal utilizó una batería de pruebas que incluyeron audiometría de tonos puros (ATL), inmitancia acústica, potencial evocado auditivo del tronco encefálico (BAEP) y potencial evocado auditivo cognitivo (P300). Un grupo no expuesto incluyó a 23 participantes sin exposición conocida al ruido, combustibles o solventes. El grupo expuesto al combustible incluía a 21 empleados de gasolineras que estuvieron expuestos al combustible y a los disolventes. **Resultados.** Sólo 2 de los 24 empleados de las gasolineras tenían una audición normal en todas las pruebas. Individualmente, el reflejo acústico estapedial mostró el mayor número de cambios, con diferencias significativas entre los grupos, para los resultados del reflejo acústico estapedial ipsilateral del oído derecho y contralateral del oído izquierdo para el grupo no expuesto. Los resultados anormales de ABR y del reflejo estapedial acústico se pueden detectar antes que las anomalías de P300 y ATL. **Conclusiones.** Se observaron cambios auditivos caracterizados por deterioro del sistema auditivo periférico y/o central, sugiriendo la acción tóxica de la exposición a los combustibles. Se necesitan más investigaciones para caracterizar mejor el daño inducido por combustible en las vías auditivas centrales e identificar una batería ideal de pruebas audiológicas para usar con este grupo de individuos.
Palabras clave. Audición; Solventes; Combustibles; Ototoxicidad; Potencial evocado auditivo; P300

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INTRODUCTION

A large body of literature documents the ototoxic effects of chemicals such as solvents, metals, asphyxiants, and pesticides in the environment or workplace[1,2]. No overarching authoritative recommendations exist to guide clinical audiologists on the selection of audiological tests to be used in the evaluation of patients who report environmental or occupational exposures to ototoxicants. This deficiency can probably be attributed to the complexity of this challenge. Environmental and even occupational exposure histories are typically poorly documented. The number of combinations of chemicals one can be exposed to is practically infinite, and the specific modes of action of these compounds are as varied. Moreover, noise is a significant co-exposure, particularly in the workplace. Solvents, the most studied class of chemicals regarding ototoxicity, have been shown to interact synergically with noise in causing auditory dysfunction[1,3]. In addition, physiological data points to a combination of cochleotoxicity and neurotoxicity processes underlying the observed auditory effects of solvent exposures[4,5]. Thus, the characterization of risk from exposure to solvents is needed to develop and implement effective diagnostic and preventive measures.

Most studies on the ototoxicity of solvents were conducted with populations from industrial environments where both ototoxic and neurotoxic effects have been reported [1]. Jet fuels have components that are known to affect some hearing functions[1,6]. The exact conditions necessary for jet fuel exposure to cause hearing effects among humans or how long one would have to be exposed to be at risk have not been identified[7,8]. Animal experiments have shown that jet fuel, itself, did not alone appear to cause hearing loss; however, when combined with noise, jet fuel enhances the damaging effects of noise[9,10]. Additionally, these studies suggest that the damage to the ear might not be detectable in an audiogram but could lead to secondary neural damage as the central auditory system adapts to a reduced input[8,9]. Few studies have been conducted with populations with low exposure to other types of fuels or exposures within occupational tolerance levels, such as gas station attendants[6,11-13]. These studies identified an increased rate of hearing disorders for this worker population.

At this time, there is no consensus as to which tests should be part of the audiological assessment of populations exposed to ototoxicants at work. Several audiological tests have been examined for this particular use[14-18]. The present study used an extensive battery of tests to evaluate the peripheral and central auditory systems of gas station attendants exposed to fuel.

METHOD

Study population

The study was approved by the Ethics Committee of Bauru Dental School, University of São Paulo–SP (CAAE #893.513,2014). All participants gave voluntary, informed written consent before participation. The study population included:

- Fuels-exposed group of 24 gas station attendants (21 male and 3 female) from six different gas stations with an average age of 43.4±11.9 years and average time of exposure to fuels of 15.4±11.1 years. Their work tasks included filling gas tanks, changing oil, washing cars, and working as cashiers.

- Non-exposed group, including 23 subjects (20 male and 3 female), with an average age of 35.5±14.3 years. These participants were volunteers recruited through ads on the campus of the Bauru Dental School of the University of São Paulo, associated with the snowball technique.

Subjects were excluded using the following criteria:

- Fuels-exposed group: a past history of occupational exposure to noise or another chemical agent and those with less than one year of exposure to fuels.

- Non-exposed group: past history or current occupational exposure to noise or ototoxic agents.

In both cases, volunteers with a tympanogram demonstrating negative pressure lower than -150 daPa or absence of maximum compliance peak, and the presence of air-bone gaps higher than 10 dB HL in pure tone audiometry in at least one frequency were excluded.

Procedures

The study protocol included a questionnaire, visual inspection of the ear canal, pure-tone audiometry (PTA), immittance audiometry, auditory brainstem response (ABR), and P300 auditory-evoked potentials. The questionnaire was administered face-to-face and included questions on medical and occupational history. PTA was conducted in a soundproof booth using an Interacoustics® AC40 audiometer, with TDH-39P headphones for air conduction testing or by a radioear B-71 bone vibrator for bone conduction testing. Air conduction pure-tone thresholds for the octave frequencies between 0.25 and 8 kHz were obtained bilaterally for all participants. Thresholds between 0 and 25dBHL were classified as normal[19]. Bone conduction pure-tone thresholds for octave frequencies between 0.5 and 4kHz were considered abnormal when greater than 25 dBHL.

Tympanometry and stapedial acoustic reflex (SAR) (contralateral and ipsilateral) thresholds were obtained using a Siemens® SD 30 immittance audiometer, from 0.5 to 4kHz. SAR results were considered abnormal in the absence of reflex at the equipment's maximum output intensity or in the presence of reflex with a sensation level higher than 90dBHL[20].

Auditory evoked potentials were recorded using Smart-EP by Intelligent Hearing Systems, with the acoustic stimuli presented through 3A insertion earphones.

The ABR recordings were made by independent monaural stimulation. Electrodes were placed at Fz (active), right and left mastoid (reference), and Fpz (ground), using the following protocol for the recordings: click stimuli at 80dBnHL, rarefaction polarity, rate of 21.1 clicks per second, 15ms recording window, two trials per ear (2048 sweeps for each trial), high-pass and low-pass filters set at 100 and 3kHz, respectively.

The criterion for determining the integrity of the auditory pathways was the presence of waves I, III, and V, as well as the analysis of absolute latencies and interpeak intervals. Thus, the average reference values used to determine normality in our research were based on two elements:

(1) biological calibration of the equipment, for normal hearing adults, considering a standard deviation of 0.2ms (wave I = 1.4 to 1.8ms; wave III = 3.5 to 3.7ms; wave V = 5.6 to 5.8ms; interpeak I-III = 2.0 to 2.2ms; interpeak III-V = 1.7 to 2.0ms and interpeak IV = 4.0 to 4.3ms) and, (2) measures matched by equipment and age range[21] (wave I = 1.69±0.13ms; wave III = 3.82±0.16ms; wave V = 5.59±0.20ms; interpeak I-III = 2.13±0.14ms; interpeak III-V = 1.78±0.18ms, and interpeak IV = 3.90±0.21ms).

Cortical evoked potential (P300) recordings were obtained using the oddball paradigm. Electrodes were placed at Fz and Cz (actives), right and left mastoid (reference), and Fpz (ground), using the following protocol: *"da"* (180 ms) as infrequent stimulus (presentation probability of 20%) and *"ba"* (180 ms) as frequent stimulus (presentation probability of 80%), intensity of 70dBHL, stimulation rate of 1.1 per second and filters of 1 to 30Hz. During the P300 recording, participants were instructed to identify rare stimuli by raising their hands each time the stimulus was heard. The P1-N1-P2 complex was identified in the record referent to the frequent stimulus and the P300 was identified in the record referent to the infrequent stimulus, as the positive wave of greatest amplitude following the P1–N1–P2 complex. Absolute latencies (ms) and amplitude (µV) of the P1, N1, P2, and P3 components were analyzed, according to previous studies: P1: 51,63±10,93ms, 0,62±0,34µV; N1: 81,38±20,11ms, -0,95±0,27µV; P2: 143,11±18,37ms, 0,81±0,41µV; P3: 341±23ms, 2,12±1,07µV[22,23].

Statistical Analises

The Student's t-test was carried out to determine whether the test battery results were significantly different between groups and between the right and left ears. Stepwise logistic regression was used to determine which interactions to include in the final model. The regression model included indicator variables for age and exposure group.

RESULTS

No means differences were found between the right and left ears. From then on, the results were analyzed for the mean results of both ears. Table 1 presents t-test results for the comparison of the results of audiological tests (mean values and standard deviation) for the exposed and non-exposed participants. Results were significantly different for the N2 ($p \leq 0.04$), P3 ($p \leq 0.03$), and the amplitude of P3 ($p \leq 0.03$).

Table 1. Comparison of the results of the audiological tests (mean values. standard deviation) for the right and left ears. obtained through the t-test for dependent samples.

GROUP	**Non-exposed**		**Fuels-exposed**		
Test	**N**	**Mean (SD)**	**N**	**Mean (SD)**	**p valor***
PTA (dB)					
0.25 kHz	46	11.1 (8,0) [a]	48	10.2 (5.4) [a]	0.540
0.5 kHz	46	11.1 (7,2) [a]	48	9.0 (5.3) [a]	0.132
1 kHz	46	8.7 (4,7) [a]	48	9.9 (5.0) [a]	0.241
2 kHz	46	9.0 (6,4) [a]	48	10.5 (7.6) [a]	0.307
3 kHz	46	13.4 (12,3) [a]	48	15.1 (13.9) [a]	0.552
4 kHz	46	16.3 (13,7) [a]	48	18.4 (17.3) [a]	0.511
6 kHz	46	15.9 (18,2) [a]	48	16.2 (13.7) [a]	0.935
8 kHz	46	16.8 (19,0) [a]	48	15.2 (12.8) [a]	0.629
STAPEDIAL ACOUSTIC REFLEX (dB)					
Ipsi 0.5 kHz	44	94.2 (7.3) [a]	41	92.8 (6.8) [a]	0.366
Ipsi 1 kHz	45	95.3 (9.2) [a]	44	93.8 (9.7) [a]	0.467
Ipsi 2 kHz	45	94.8 (8.1) [a]	44	92.6 (8.7) [a]	0.207
Ipsi 4 kHz	30	95.6 (7.1) [a]	29	97.0 (5.0) [a]	0.389
Contra 0.5 kHz	43	98.3 (8.3) [a]	39	99.6 (7.0) [a]	0.426
Contra 1 kHz	42	97.8 (7.5) [a]	37	97.1 (7.8) [a]	0.690
Contra 2 kHz	40	97.2 (8.6) [a]	38	96.3 (7.9) [a]	0.621
Contra 4 kHz	28	99.1 (8.9) [a]	30	102.5 (7.7) [a]	0.129
ABR (ms)					
I	44	1.6 (0.1) [a]	46	1.7 (0.1) [a]	0.230
III	46	3.9 (0.2) [a]	47	3.9 (0.1) [a]	0.566
V	46	5.8 (0.2) [a]	48	5.8 (0.3) [a]	0.172
I-III	44	2.2 (0.1) [a]	46	2.2 (0.1) [a]	0.815
III-V	46	1.9 (0.1) [a]	47	1.9 (0.1) [a]	0.841
I-V	44	4.1 (0.2) [a]	46	4.1 (0.1) [a]	0.925
P300 (ms)					
P1	23	69.5 (15.9) [a]	24	72.1 (25.3) [a]	0.680
N1	23	116.8 (21.3)[a]	24	117.5 (30.2)[a]	0.924
P2	23	185.3 (20.6)	24	183.3 (36.4)[a]	0.820
N2	23	221.2 (30.6)[a]	24	256.6 (76.9)[b]	**0.045 ***
P3	23	302.5 (44.3)[a]	24	335.1 (52.2)[b]	**0.029 ***
Ampl P3	23	6.2 (3.7)[a]	24	5.1 (3.5)[b]	**0.030 ***

*p valor ≤0,05. Letters **a** and **b**= Different letters indicate a significant difference between the groups. N= Number of subjects; SD= Standard deviation; kHz= kilohertz; RE= Right ear; LE= Left ear; Ipsi= Ipsilateral acoustic reflex; Contra= Contralateral acoustic reflex; ABR= Auditory Brainstem Response; I. III. V (waves - absolute latencies). I-III. III-V. I-V (waves - interpeak intervals); ms= Milliseconds; P300= Cognitive auditory-evoked potential P300; P1. N1. P2. N2 e P3= components of Cognitive auditory-evoked potential P300; Amp= amplitude; dB= decibel.

Table 2 presents the number of test results classified as either normal or abnormal for the non-exposed and fuels-exposed groups. The adjustment of the logistic model did not converge for the P300 and ABR because of the low frequency of abnormal results.

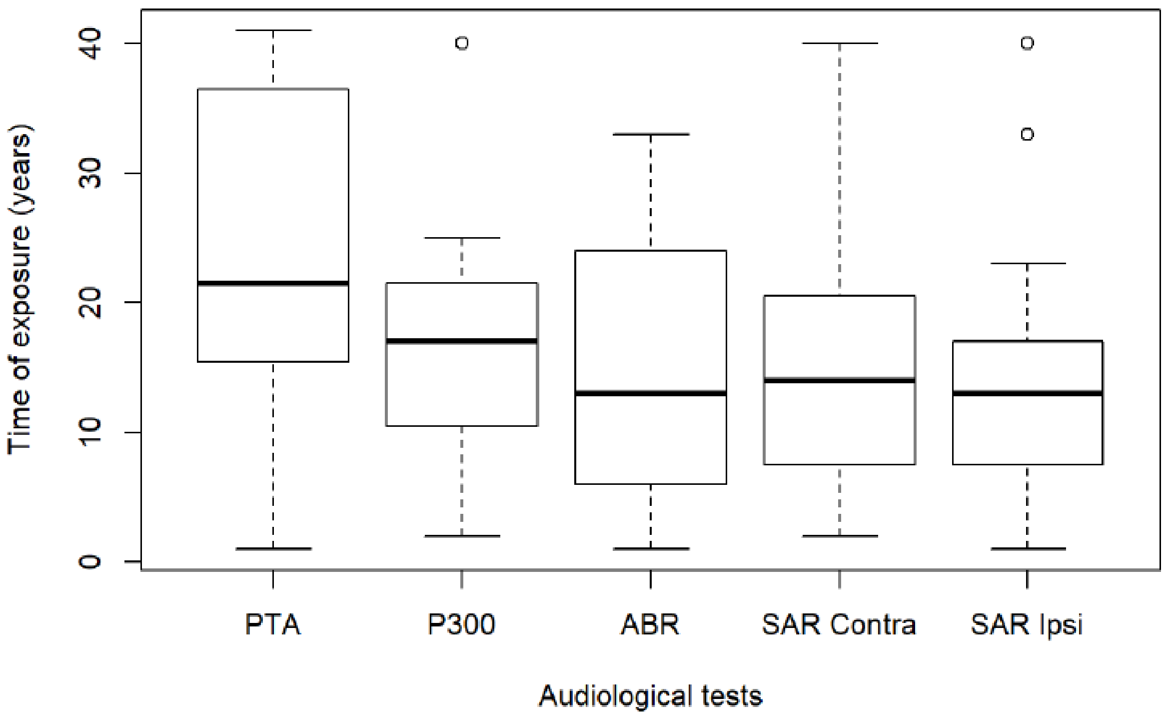
Table 2. Results of the audiological tests of the non-exposed and fuels-exposed groups as normality or abnormality, for each category of occupational exposure to fuel (logistic model).

			Exposure Categories			
			Non-exposed group	**Fuels-exposed group**		
				1 – 15 years	**>15 years**	**Total**
	Age	N Mean±SD	23 35.48±14.27	11 35±8.39	13 50.46±9.64	47 39.51±13.56
Pure-tone audiometry	**RE**	Normal	14	9	7	30
		Abnormal	9	2	6	17
		p	0.359	0.233	0.682	-
	LE	Normal	17	10	8	35
		Abnormal	6	1	5	12
		p	0.300	0.273	0.441	-
Stapedial Acoustic Reflex	**Ipsi RE**	Normal	18	4	9	31
		Abnormal	5	7	4	16
		p	0.069	0.022*	0.549	-
	Contra RE	Normal	16	5	8	29
		Abnormal	7	6	5	18
		p	0.411	0.182	0.624	-
	Ipsi LE	Normal	17	6	8	31
		Abnormal	6	5	5	16
		p	0.503	0.264	0.441	-
	Contra LE	Normal	17	3	8	28
		Abnormal	6	8	5	19
		p	0.050	0.014*	0.441	-
Auditory Brainstem Response	**RE**	Normal	19	10	13	42
		Abnormal	4	1	0	5
		p	-	-	-	-
	LE	Normal	18	7	9	34
		Abnormal	5	4	4	13
		p	-	-	-	-
Cognitive Auditory Evoked Potential (P300)	**P1**	Normal	23	11	13	47
		Abnormal	0	0	0	0
		p	-	-	-	-
	N1	Normal	23	10	12	45
		Abnormal	0	1	1	2
		p	-	-	-	-
	P2	Normal	23	10	11	44
		Abnormal	0	1	2	3
		p	-	-	-	-
	N2	Normal	23	10	11	44
		Abnormal	0	1	2	3
		p	-	-	-	-
	P3	Normal	23	11	9	43
		Abnormal	0	0	4	4
		p	-	-	-	-
	P3 Amp	Normal	23	10	9	42
		Abnormal	0	1	4	5
		p	-	-	-	-

SD= standard deviation; N= number of subjects; RE= right ear; LE= left ear; Ipsi= Ipsilateral stimulation; Contra= Contralateral stimulation; Amp= amplitude; ms= milliseconds; * p value ≤0.05 (statistically significant).

Only 2 of the 24 gas station attendants had normal results in all tests. Figure 1 shows the quartile distribution of abnormal hearing tests in the fuels-exposed group about the duration of exposure to fuels. ABR and the SAR abnormal results were detected after a shorter time of exposure to fuel compared to the other tests.

Figure 1. Boxplot chart (based on quartiles) of the distribution of abnormal results of the audiological tests obtained in the fuels-exposed group about the time of exposure to fuels.



Abnormal results in the tests - PTA (n=8); P300 (n=8); ABR (n=9); SAR Contra (n=16); SAR IPSI (n=15).

DISCUSSION

Previous studies have brought up the need for guidance for clinical audiologists regarding the selection of tests to be used in the evaluation of patients with a history of exposure to ototoxic chemicals[15,18]. Differences between mean group results were detected when test results were classified for normality. Only 2 of 24 attendants had normal results in all the performed tests (Tables 1 and 2). Abnormal results in the stapedius reflex testing (elevated or absent thresholds) were significantly more common in the fuels-exposed group, in the ipsilateral SAR for the right ear, and contralateral for the left ear. The number of abnormal results was greater for those exposed for longer periods (more than 15 years). Given the lack of detailed medical or exposure histories, we cannot speculate on whether this finding could be explained by differences in exposure levels or histories, or other differences between study groups.

Studies reported that the absence of SAR was more common than expected in fuel-exposed participants, as was the presence of SAR with elevated reflexes[6,12]. The prevalence of abnormalities in the present study was greater in the contralateral SAR responses than in ipsilateral, which had also been observed by other studies[6,14].

Besides suggesting the involvement of the auditory nerve/low brainstem[24], SAR can be indicative of retrocochlear pathology. Aromatic solvents, which are components of several types of fuels, may modify the action of the SAR in the stapedius muscle because of its anticholinergic effects on the efferent motor neurons[25,26]. They can also act on the regulation of acetylcholine release in muscles that are involved in the protective middle ear reflex and mediated by efferent motor neurons emanating from the olivary complex in the brainstem[27]. The observed SAR responses could be explained by a synaptic change, that is, in the peripheral portion of the acoustic reflex arc. Alternatively, or perhaps concomitantly, they could result from a failure in reflex activation at the level of the superior olivary complex.

The distribution of abnormal results of the audiological tests obtained in the fuels-exposed group about the time of exposure to fuels (Figure 1) shows that abnormal results could be detected through ABR, P300, and SAR, procedures that evaluate central auditory functions before they were detected by PTA. Study participants exposed to fuels showed a large concentration of abnormalities in the first years of exposure, with a statistically significant difference for ipsilateral SAR of the right ear and contralateral left ear in the category of 1 to 15 years of fuel exposure (Table 1). Previous studies have shown that fuel exposure can be associated with central auditory nervous system dysfunctions without peripheral dysfunctions and that they may be an earlier sign of fuels-induced auditory dysfunction[7,9].

Two other Brazilian studies carried out with gas station attendants observed an effect of length of exposure in years on the results of auditory exams, with a statistically significant difference in ABR latency and interpeak latencies[6,11]. Effects were detected among those exposed for more than five years. As the time of exposure to gasoline increased, the ABR absolute latencies and interpeaks also increased[6]. A significant slope was observed for the duration of employment for the group of gasoline station attendants with normal hearing for the amplitude of wave V in the left ear ($p=0.01$).

Limitations of the present investigation include a lack of detailed exposure histories or exposure measurements, a small sample size, the difference in age between the groups, and wide confidence intervals as reported in Table 2. Table 2 includes 18 p-values, three below 0.05 and one slightly above. With the assumption of no differences at the 0.05 level, one would expect about one test to be statistically significant by chance alone. If there are real effects, they also may not be detected because of the small sample size. One should interpret the significant p-values (a statistical summary of the data would be equal to or more extreme than its observed value) with caution. Non-statistically significant results can mask potentially important public and clinical health effects[28]. In summary, given our failure to recruit a larger sample of participants, our contribution towards an answer on which tests audiologists should use in a population of workers is restricted. A more impactful contribution of this study's results could be achieved if combined with the results of other studies in a meta-analysis, using summary statistics or unadjusted p-values.

Future research should investigate additional populations of workers exposed to ototoxic fuels and solvents, including miners; construction workers; heavy equipment operators, bridge and tunnel workers; railroad workers; oil and gas workers; loading dock workers; truck drivers; material handling operators; farmworkers; long-shore workers; and auto, truck, and bus maintenance garage workers[29]. Workers who are exposed to multiple agents should undergo a complete audiological evaluation. The use of pure tone audiometry is not enough to evaluate or monitor overall hearing abilities. Workers exposed to ototoxic solvents in isolation or in combination with noise should receive tests that evaluate the auditory system more comprehensively, from the cochlea to the higher auditory pathways[8,30]. PTA should be complemented with supplementary assessment tools such as acoustic reflex measures and evoked potentials.

The tests evaluated in the present study contribute to the identification of risk to allow for the proposal, implementation, and evaluation of preventive measures. In addition, the results suggest that workers exposed to fuel solvents should be included in hearing conservation interventions, regardless of the levels of their noise exposures[17].

CONCLUSION

Auditory abnormalities were observed in gas station attendants, suggesting a toxic action from exposure to fuel solvents. Considering the results of the present small-scale study and the findings from other researchers, assessing hearing in subjects with a history of fuel exposure by evaluation of pure tone hearing thresholds alone is likely insufficient for an accurate diagnosis. Central auditory assessment should be considered as part of the audiological test battery. Further research is needed to better characterize the damage to central auditory pathways induced by solvents and to determine the optimal audiological test battery for use with this patient group.

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