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Hearing screening and cholinesterase activity among rural workers exposed to pesticides

Triagem auditiva e dosagem das colinesterases em trabalhadores rurais expostos a agrotóxicos

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ABSTRACT | Background: Agricultural activities and exposure to pesticides increase the risk of hearing loss and might reduce cholinesterase activity. **Objective:** To describe and analyze the correlation between hearing screening and plasma and erythrocyte cholinesterase activity relative to rural workers exposed to pesticides. **Methods:** Cross-sectional, quantitative, descriptive and explanatory study conducted with 71 rural workers exposed to pesticides. Data were collected by means of an instrument to characterize exposure to pesticides, hearing screening and blood samples to measure plasma and erythrocyte cholinesterase activity. **Results:** Each participant used three different pesticides, on average, glyphosate being the most frequently mentioned. About 60.6% of the sample exhibited hearing abnormalities and 32.4% complained of tinnitus. Erythrocyte cholinesterase activity was within the normal range in all the cases; one single participant exhibited reduced plasma cholinesterase activity. There was not significant correlation between the hearing screening and cholinesterase levels. **Conclusion**: Audiological evaluation should be included in the assessment of rural workers. Monitoring workers exposed to pesticides should not be restricted to cholinesterase activity alone, because this method is insufficient to detect actual exposure to pesticides.

Keywords | agrochemicals; occupational risks; occupational health.

RESUMO | Introdução: A atividade agrícola e a exposição a agrotóxicos aumentam o risco de perda auditiva e a possibilidade de redução das colinesterases. **Objetivos:** Descrever e correlacionar os resultados da triagem auditiva e da dosagem das colinesterases plasmática e eritrocitária de trabalhadores rurais expostos a agrotóxicos. **Método:** Pesquisa de natureza quantitativa, com alcance descritivo e explicativo, com delineamento transversal, realizada com 71 trabalhadores rurais expostos a agrotóxicos. A coleta de dados foi realizada de três formas: instrumento de coleta para caracterização do contato aos agrotóxicos; triagem auditiva; e coleta de sangue para dosagem das colinesterases plasmática e eritrocitária. **Resultados:** Cada trabalhador utilizava "em média" três agrotóxicos diferentes e o mais citado foi o glifosato. Sobre a triagem auditiva, 60,6% possuíam alterações auditivas e 32,4%, zumbido. Na dosagem da colinesterase eritrocitária, todos os trabalhadores rurais apresentaram valores de dosagens de colinesterase dentro da normalidade. Na dosagem da colinesterase plasmática, um participante apresentou valor de dosagem inferior ao normal. Não houve correlação significativa entre a triagem auditiva e o valor das colinesterases. **Conclusões:** A avaliação audiológica deve ser incluída no cuidado à saúde dos trabalhadores rurais e o acompanhamento ocupacional de trabalhadores expostos a agrotóxicos não deve ser restrito à dosagem das colinesterases, pois tal medida é insuficiente para diagnosticar exposição real a agrotóxicos.

Palavras-chave | agrotóxico; riscos ocupacionais; saúde do trabalhador.

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INTRODUCTION

Frequent exposure to pesticides might damage the health of rural workers. These substances have been detected in human blood and milk and food residues[1]. Reducing the use of pesticides is recommended as preventive measure against cancer[2-4].

Several procedures are required to detect pesticide poisoning, including clinical evaluation, reported information and laboratory tests. Plasma and erythrocyte cholinesterase activity measurement is one of the laboratory tests included in protocols for investigation of pesticide poisoning[5,6].

Cholinesterases are enzymes involved in the hydrolysis of acetylcholine, namely, a neurotransmitter that acts as chemical mediator in the transmission of the nervous impulse in synapses[6,7]. Carbamate and organophosphate pesticides are cholinesterase inhibitors and thus might make acetylcholine accumulate in the body, which is associated with several clinical signs, including bradycardia, diarrhea, tremor, muscle stiffness and paresis. Accumulation of acetylcholine in the central nervous system might trigger seizures, depression, restlessness and even coma[8].

For these reasons, the Brazilian Ministry of Labor and Employment Regulatory Standard no. 7 (RS 7) indicates monitoring workers exposed to organophosphates and carbamates through measurement of the blood cholinesterase activity. According to RS 7, the maximum permitted levels are 30% reduction of the erythrocyte cholinesterase, 50% reduction of the plasma cholinesterase or 25% of both by comparison to baseline.

In addition to their implications for health in general, according to some studies pesticides might also be harmful to hearing, i.e. they are potentially ototoxic[9,10]. Hoshino et al.[11] observe that hearing loss might be an early sign of pesticide poisoning. Therefore, hearing evaluation is also recommended in protocols for assessment of chronic pesticide poisoning[5].

The Ministry of Health National Policy of Hearing Health Care stresses the responsibility of the public sector in the identification of determinants and conditioning factors for diseases and hazards associated with hearing loss. It is worth reminding that hearing is a subject of multidisciplinary and intersectoral interest, and that not only society should be sensitized in this regard[12], but also the agencies responsible for the conditions under which work is performed, such as the Ministry of Labor and the Public Prosecutor Office.

As Lobato[13] and Körbes et al.[14] point out, the Brazilian legislation does not indicate hearing monitoring for workers exposed to chemicals, such as pesticides. As per NR 7, only workers exposed to noise above permitted levels should be subjected to this procedure.

Given the aforementioned considerations, the aim of the present study was to describe the results of hearing screening among rural workers exposed to pesticides and analyze their correlation with the plasma and erythrocyte cholinesterase levels.

METHODS

The present cross-sectional, quantitative, descriptive and explanatory study was conducted with rural workers exposed to pesticides residing in Santa Rosa municipality, in the northwest of Rio Grande do Sul, Brazil. According to the Brazilian Institute of Geography and Statistics (Instituto Brasileiro de Geografia e Estatística–IBGE)[15] agriculture is one of the main local production activities, the most prevalent crops being soybean, wheat and maize.

All the participants resided in the area of coverage of one rural Health Basic Unit (HBU) included in the Unified Health System (Sistema Único de Saúde–SUS). We surveyed the HBU medical records to establish the total number of local male agricultural workers (n=293). The inclusion criteria were: age 18 or older, male sex, using pesticides in their properties and working 15 hours/week at least (this is the IBGE criterion to define rural workers). Individuals with abnormalities of the external auditory canal on visual inspection or with previous non-work–related hearing loss were excluded. Women were not considered due to the possible influence of hormones on cholinesterase levels.

Probabilistic sampling was performed to recruit a stratified sample proportional to the number of individuals in each considered age range. The calculated sample size was 96 participants, who were selected by means of the lottery method. Originally, we intended to contact this group by telephone to inform them about the study

aims and request them to visit the HBU on preset dates and times. However, not all the initially selected subjects could be reached or agreed to participate, so we attempted to contact additional individuals. On the whole, only 82 of the recruited subjects effectively visited the HBU on the appointed dates, and 11 were excluded, nine for failing to meet the inclusion criteria and two who refused to take the hearing screening test. Therefore, the final sample comprised 71 participants.

Data collection was performed on three different occasions: first, on the participants' initial visit to the HBU, when we administered an instrument to investigate exposure to pesticides and past and present medical history; the second was for the hearing screening test, which was also performed at the HBU; and finally, blood sample collection at the HBU reference laboratory for later plasma and erythrocyte cholinesterase measurement.

The instrument for data collection was designed based on the protocol for assessment of chronic pesticide poisoning developed by the State Secretariat of Health of Parana, Brazil (2013)[5]. Hearing screening was performed in a silent room at the HBU with Interacoustics AD229 audiometer. We investigated the air conduction auditory thresholds for frequencies 250, 500, 1,000, 2,000, 3,000, 4,000, 6,000 and 8,000 Hz. The degree of hearing loss was established according to the World Health Organization (WHO) classification, which considers the average thresholds for frequencies 500, 1,000, 2,000 and 4,000 Hz. We could not measure the cholinesterase levels before exposure, because the technique demands a 30-day period free from exposure to pesticides. Therefore, the reference values considered were those recommended by the laboratory where the tests were performed: erythrocyte cholinesterase 10,229–23,761 U/L and plasma cholinesterase 4,620–11,500 U/L. The former was measured by means of the photometric method and the latter with the colorimetric method. Only 50 participants (70.4%) visited the laboratory on the appointed date for sample collection; the probable reason is that this facility is located downtown and thus difficult to access by rural residents.

The present study complied with the ethical standards described in the National Health Council Resolution no. 466/2012, which regulates research involving human beings, and was approved by the institutional research ethics committee (CAAE: 61963416.3.0000.5564). All the participants signed an informed consent form.

Statistical analysis was performed with software R and included measures of position and variability for quantitative variables and absolute and relative frequencies for qualitative variables. The significance level was set to 5%. Pearson's correlation coefficient was used to test associations between quantitative variables, or alternatively Spearman's coefficient when the data did not exhibit normal distribution.

RESULTS

The average age of the sample was 55±10.5 years old and their average length of exposure to pesticides 27.6±13 years, varying from 4 to 66 years.

Fifty different pesticides were mentioned; each participant used three different pesticides, on average. The pesticide most frequently used was glyphosate, a glycine analogue, which was mentioned by 88.7% of the participants.

On hearing screening 31 (43.7%) participants exhibited mild-to-moderate bilateral hearing loss, and 12 unilateral hearing loss (Figure 1). The former were referred for audiological evaluation at an auditory rehabilitation center and the remainder of the participants were oriented to undergo audiological evaluation annually. Twenty-three participants (32.4%) reported tinnitus. Four participants complained of itch and vertigo and were referred to an otolaryngologist.

Table 1 describes the erythrocyte and plasma cholinesterase levels of the 50 participants who attended the laboratory appointments. The plasma cholinesterase level was below the normal range (3,638 U/L) for one single participant, who was referred for medical evaluation at the HBU. Table 2 describes the participants' erythrocyte cholinesterase levels according to their length of exposure to pesticides.

Although measuring the erythrocyte cholinesterase level is indicated for monitoring individuals long exposed to pesticides, we did not find significant correlation (p=0.944) between this variable and length of exposure to pesticides (Table 2).

Table 3 describes the hearing screening results and erythrocyte and plasma cholinesterase levels. We did not find significant correlation between enzyme levels and four- (500, 1,000, 2,000 and 4,000 Hz) or three-tone (500,

1,000 and 2,000 Hz) average or acute frequencies (3,000, 4,000 and 6,000 Hz).

DISCUSSION

Some studies evidenced toxic effects of pesticides on the vestibulocochlear system of albino guinea pigs. Administration of organophosphates was associated with morphological abnormalities in the cochlea, including lesions in all three canals and ciliary abnormalities in the saccule and utricle[14].

In regard to humans, several studies reported abnormal results on hearing screening among rural workers exposed to pesticides[9,10,16-19], which agrees with our findings. It is believed that pesticides might induce abnormalities in the auditory and the vestibular systems through a slow and silent action[20].

As shown in Figure 1, when one considers the 31 participants (43.7%) with mild-to-moderate bilateral hearing loss and those with unilateral hearing loss jointly, 60% of the sample in total exhibited hearing problems. This prevalence is higher than that reported in other studies[1,13].

Agricultural work and exposure to pesticides increase the risk of hearing loss. In other words, hearing is poorer among agricultural workers by comparison to the overall population[9,19]. However, the occurrence of auditory problems among rural workers cannot be exclusively attributed to pesticides, because this population of workers is also exposed to other factors liable to damage the auditory system, such as noise and vibration, which together give rise to a multifactorial process[10,21].

Tinnitus was also a frequent complaint, reported by 23 (32.4%) participants. This condition was described also in

Table 1. Erythrocyte and plasma cholinesterase levels of rural workers exposed to pesticides, Health Basic Unit, Bela Uniao, Santa Rosa, Rio Grande do Sul, Brazil, 2017 (n=50).

Cholinesterase*	Mean±SD (U/L)	Median (U/L)	Minimum (U/L)	Maximum (U/L)
Erythrocyte	14,430±2,202	14,100	10,850	19,930
Plasma	7,756±1,461	7,604	3,638	10,600

*Reference values according to the laboratory where the tests were performed — erythrocyte cholinesterase: 10,229 to 23,761 U/L; plasma cholinesterase: 4,620 to 11,500 U/L; SD: standard deviation.

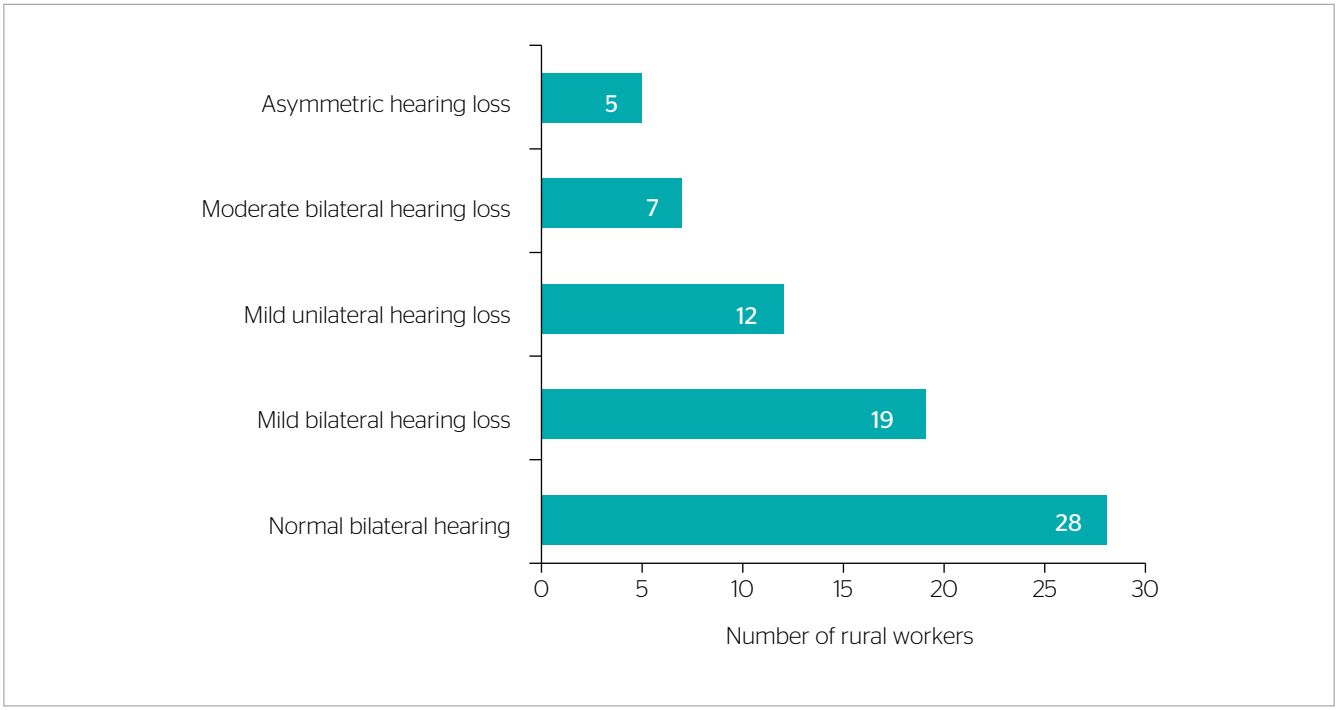

Figure 1. Hearing screening results of rural workers exposed to pesticides, Health Basic Unit, Bela Uniao, Santa Rosa, Rio Grande do Sul, Brazil, 2017 (n=71).

other studies, such as that by Delecrode et al.[16], in which it affected 28.05% of the 82 participants. The prevalence of tinnitus in the study by Foltz et al.[22] was 29.1%.

Although tinnitus is often associated with hearing loss, it is an expression of cochlear damage[16]. Therefore, in addition to noise-related hearing loss (NRHL) it might also be due to the neurotoxicity of chemicals harmful to the vestibulocochlear system as a whole[13]. Tinnitus might create problems at work and impair the quality of life of workers[16].

According to Guida et al.[17] hearing health programs should consider not only exposure to noise, but also other factors, such as pesticides, to effectively prevent the occurrence of hearing problems.

Table 2. Erythrocyte cholinesterase levels according to length of exposure to pesticides among rural workers, Health Basic Unit, Bela Uniao, Santa Rosa, Rio Grande do Sul, Brazil, 2017 (n=50).

Years of exposure	Erythrocyte cholinesterase* (U/L)
	Mean (SD)
Less than 10	15,093 (2,357.494)
10 to 19	14,123 (2,042.668)
20 to 29	14,422 (1,943.442)
30 to 39	15,114 (2,579.921)
40 to 49	14,134 (2,543.236)
50 or more	12,770#
r (p)	-0.01 (0.944)

*Reference values according to the laboratory where the tests were performed: 10,229 to 23,761 U/; SD: standard deviation; #standard deviation was not calculated because this group included one single participant; r: Pearson's correlation coefficient and corresponding p value.

As Table 1 shows, the erythrocyte and plasma cholinesterase levels were within the normal range. The plasma cholinesterase level was below the reference values for one single participant. This was a 56-year-old man who had been exposed to pesticides for 10 years, having had had the last contact with glyphosate — a glycine analogue — and (aryloxy)alkanoic acid-derived herbicides 90 days earlier. Since the plasma cholinesterase decreases in acute poisoning, i.e. up to 10 days after exposure, and this participant had not been exposed to organophosphates, the reduction found in the enzyme activity was possibly due to some other reason; his medical records indicated he suffered from a heart disease.

Neither bivariate analysis, shown in Table 2, nor investigation of correlation between the hearing screening and cholinesterase levels (Table 3) yielded significant results. These findings agree with those reported in other studies[1,13,23] in which the enzyme levels were also within the normal range and did not exhibit association with other variables.

The fact the enzyme levels were within the normal range might be explained by the low frequency of use of cholinesterase inhibitors, such as organophosphates, by the participants; indeed, only eight of them reported having had contact with this class of pesticides. Another possibility, also mentioned by Murakami et al.[1], is that the blood samples might have collected during a time in the year when this class of pesticides is not used.

According to Araújo et al.[24], cholinesterases are biological markers of acute or chronic exposure to organophosphate

Table 3. Correlation between hearing screening results and erythrocyte and plasma cholinesterase activity relative to rural workers exposed to pesticides, Health Basic Unit, Bela Uniao, Santa Rosa, Rio Grande do Sul, Brazil, 2017 (n=50).

Audiological evaluation	Cholinesterase			
	Erythrocyte		Plasma	
	RE	LE	RE	LE
	r (p)	r (p)	r (p)	r (p)
500, 1,000, 2,000, 4,000 Hz	0.04 (0.770)	0.01 (0.927)	-0.10 (0.501)	-0.13 (0.368)
500, 1,000, 2,000 Hz	0.04 (0.757)	0.06 (0.700)	0.05 (0.737)	0.03 (0.856)
3,000, 4,000, 6,000 Hz	-0.03 (0.823)	-0.07 (0.644)	-0.21 (0.134)	-0.19 (0.194)

RE: right ear; LE: lef ear; r: Spearman's correlation coefficient and corresponding p value.

and carbamate pesticides, which are mainly used for cultivation of seeds, such as those of soybean, wheat and maize. Measuring these enzymes is recommended by WHO for the purpose of occupational toxicology research. In turn, the Brazilian legislation (RS 7) indicates measuring both erythrocyte and plasma cholinesterase activity for biological monitoring of occupational exposure to organophosphates and carbamates.

Cholinesterase activity is the single parameter currently considered in employer-sponsored poisoning control campaigns, even though the available studies point to substantial limitations in their ability to assess the state of health of workers[1,20,23].

One the limitations of this method derives from the fact that RS 7 indicates comparing measurements to baseline values, for which workers should be free from any exposure to pesticides for 30 days at least[25]. Since such requirement is often difficult to meet, the alternative is to perform comparisons to reference values obtained from non-exposed populations, even though specific reference values should be ideally obtained for each particular occupational group[7].

One should further bear in mind that cholinesterase activity might vary as a function of other conditions, such as cirrhosis, hepatitis, liver cancer, myocardial infarction, duodenal ulcer, acute or chronic infections, anemia, pregnancy and alcoholism[1,7].

In a study, cholinesterase activity was compared between samples collected during periods of low (baseline) and peak exposure to pesticides. The criterion used in comparisons was that established by RS 7, i.e. 50% reduction as the maximum permitted, which was found for only one of 103 analyzed rural workers. Even when other cut-off points were tested, to wit, 20% and 30% reduction, only three participants (2.9%) exhibited reduced enzyme activity[23].

Studies to investigate new markers sensitive to combined pesticide exposure and molecular toxicology research are needed to enable early and reliable detection of cases of poisoning. Higher availability of laboratories for biological monitoring and providing training to healthcare professionals to associate problems exhibited by workers to exposure to pesticides and other substances (causal link) might contribute to increase the precision of diagnosis[26].

Since establishing a diagnosis is currently difficult, several procedures are needed simultaneously, including clinical evaluation, physical examination, analysis of the occupational history of workers and exposure to toxic substances all along their working life[1,5]. Given the limitations of the available biomarkers, administering questionnaires to investigate symptoms is a valuable strategy to investigate pesticide poisoning[23].

CONCLUSION

The results of the present and other studies in the literature indicate that rural work is indeed associated with risk to the hearing health of workers, as a considerable proportion of the participants exhibited hearing problems and tinnitus.

Therefore, healthcare providers should include audiological evaluation in the assessment of this population of workers. Similarly, the criteria grounding recommendations for audiological monitoring in the labor legislation for should be revised, and this approach should be extended to cover also workers exposed to other factors harmful to the auditory system, as is the case of pesticides.

Monitoring workers exposed to pesticides should not be restricted to measuring the cholinesterase activity alone, as it might lead to serious errors and wrong conclusions. As a fact, normal results might even lead to "enhance" to use of pesticides: if workers are made to believe that this test suffices to establish their state of health, normal results might persuade them they may continue using these substances without any of the measures required to ensure their safety.

In addition, measuring cholinesterase activity in clinical practice is not practical, since pre-exposure levels are virtually impossible to be obtained, given that subjects should be free from exposure for a quite long period of time.

As limitations, in the present study we did not consider the time when sowing was performed — and the corresponding use of organophosphate pesticides — for data collection, which might have interfered with the results for cholinesterase activity.

Nevertheless, other parameters should be considered in the follow up of workers exposed to pesticides in addition to those discussed in the present study. The latter exhibit considerable limitations, while in actual practice workers

are exposed to multiple pesticides from different chemical classes and working conditions.

Finally, effective knowledge of determinants and factors conditioning chronic exposure to pesticides is characterized by high complexity and thus requires continuous investigation. The present study pioneeringly focused on the northwestern area of Rio Grande do Sul; additional studies are needed to help SUS health professionals find resources to provide integral care to rural workers. Other institutions should develop efforts through intersectoral actions to afford this population healthier farming options, including adequate orientation on pesticides.

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