MEDIAL OLIVOCOCHLEAR SYSTEM AND GENOTOXICITY IN STUDENTS OF THE TOBACCO-PRODUCING REGION

Sistema olivococlear medial e genotoxicidade em escolares de região fumicultora

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ABSTRACT

Purpose: to evaluate the association between the function of the medial olivocochlear system and the biomarkers of genotoxicity in resident students from the tobacco-producing region. **Methods:** the study group was composed by 21 normal-hearing students from the tobacco-producing region and the control group by 25 normal-hearing students who did not live in the countryside. The medial olivocochlear system was assessed by the of distortion product otoacoustic emissions and genotoxic biomarkers, such as: comet assay, micronucleus test and fluorimetric assay for the quantification of DNA. The data were subjected to statistical analysis. **Results:** by comparing the occurrence of suppression of emissions between the groups, no significant association was detected. Considering the comet assay and the fluorimetric assay for quantitation of DNA, the mean of the study group was considerate significantly higher than the mean of the control group. In the micronucleus test, it was found a significant difference in the sum of abnormal cells and in the frequency of binucleated cells, with the mean of the study group higher than the one found in the control group. And, in relation to the frequency of cells with micronucleus, it showed no significant difference between both groups. No association was found between the occurrence of suppression and the results of genotoxicity biomarkers. **Conclusion:** the study group had no change in medial olivocochlear system, which was evidenced by the presence of emissions suppression, but it presented injury rates considerate significantly higher in relation to the genotoxic biomarkers. However, there was no association between suppression of emissions and genotoxicity.

KEYWORDS: Pesticides; Tobacco; Hearing; Efferent Pathways; Genotoxicity; Child

INTRODUCTION

Brazil is one of the largest producers and exporters of tobacco in the world, being tobacco farming an activity of great social and economic importance in the country. It is usually carried out by small farmers who get aid from family members, residing near the plantation. That is the reason why they end up exposed to several toxic substances. In tobacco culture, different types of pesticides, classes and toxicities are widely used, especially organophosphates. The indiscriminate use of these chemicals ensures greater productivity and reduces losses of the season, but the health harms in short, medium and long term are not considered[1]. Besides the pesticides, the population is exposed to other highly toxic organic compounds present in the leaves of tobacco, among them, there is nicotine.

Children constitute a group with particular characteristics of exposure and special vulnerability to environmental toxicants. In addition to the fact they are in development, they differ from adults because they have relative immaturity of physiological and biochemical functions of the systems in

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the proportion of body components (water, protein, fat and minerals), the anatomical structure of the organs, in the competence of metabolizing and excreting toxic substances[2]. They also present more specific habits, such as "putting hand to mouth", which increase the chances of ingesting toxic compounds present in water, soil and household dust[3].

The range of adverse health effects, resulting from pesticides, includes acute and chronic damages[3]. Currently, several studies have demonstrated a close relationship between hearing loss and exposure to pesticides[4,5]. Organophosphate pesticides induce alterations in the auditory and vestibular system, being also verified its neurotoxic potential[4], affecting the central auditory system. In relation to the chronic health effects resulting from dermal exposure to the nicotine of the tobacco leaf, there is no much information about it. The main discussion of the toxic effects of nicotine in the smoke is related to the smoking habit. Some studies report that nicotine may have direct ototoxic effect and cause cochlear ischemia[6], as well as interfering in the neural transmission of auditory information[7].

The central auditory system consists of afferent and efferent auditory pathways. The efferent auditory pathway is divided into two parts: lateral olivocochlear system and medial olivocochlear system (MOCS). MOCS consists of myelinated fibers and predominantly crossed, which will innervate the outer hair cells (OHC) [8]. The normal functioning of the MOCS can be evidenced by the abolition or reduction of OAE with the application of an ipsilateral competing noise or contralaterally[9]. The registration of OAE and analysis of the suppression effect can be used for early detection of hearing impairment of cochlear and retrocochlear origin and also for the establishment of preventive actions in audiology[10].

Human biomonitoring is the most efficient way to prevent and diagnose early damage from human exposure to chemicals with genotoxic potential[11]. The human body is subjected to oxidative stress, which is defined as the imbalance between oxidant systems (reactive oxygen species) and antioxidants in favor of the first ones[12], causing damage in many cellular constituents, such as unsaturated lipids, proteins and DNA[13]. Several pesticides have been tested and they showed to have genotoxic potential[14]. Smoking has also been reported as the cause of increased rates of genotoxicity[15]. Genotoxicity is the capability that some substances have to induce alterations in the genetic material (DNA) of organisms exposed to them[16]. This alteration is considered a primary risk factor for long-term effects.

Although there are a variety of biomarkers available for evaluating the genotoxic damages, transient or permanent, the majority of the biomonitoring studies have used the micronucleus (MN) test and comet assay. These two techniques have been widely used to investigate DNA damage in people who were occupationally exposed and they have shown to be rapid and sensitive tests[3]. Currently, the use of fluorescent probes, such as the fluorimetric assay for quantification of DNA with the use of the Picogreen reagent, has been highlighted. This is an objective and highly sensitive technique, which detects small amounts of DNA in solution. Moreover, it plays an increasingly important role in different studies and biological applications, being quite used in molecular biology[17]. It may also be used to analyze the genotoxic or genoprotector effect of a given compound.

Based on what was described above, the aim of this study was to evaluate the association between the function of the medial olivocochlear system (MOCS) and genotoxic biomarkers in students from the tobacco-producing region.

METHODS

This is an observational, prospective, cross-sectional study. It was approved by the Ethics and Human Research Committee of the institution, registered under the protocol number of 0237.0.243.000-11. It had the support of the Reference Center for Occupational Health (CEREST) of Santa Maria. All subjects agreed to participate of the research and they presented the Written Consent Form (WCF) signed by their guardians.

The study subjects were selected from public schools in two cities of the central region of Rio Grande do Sul. All schools allowed the dissemination of research and they signed an Institutional Consent Term.

The students, in order to participate in this study, presented the following inclusion criteria: being aged between seven and 14 years, being normal-hearing people, presence of DPOAE, not being continuously exposed to intense noise and to cigarette smoke. As exclusion criteria, the following factors were used: presenting a history of hearing loss presenting auditory disorders, according to the criterion adopted for this study, and presenting chronic diseases and/or making use of continuous medication.

The study group (SG) was constituted by students from the tobacco-producing region, and the control group (CG) by the students from the urban area of another municipality, outside the tobacco-producing region. The students of the CG were selected in a different municipality of the SG with the aim of

ensuring that these subjects were free of exposure to pesticide and to the nicotine derived from tobacco leaf.

In the selection of the SG, 103 students attended the inclusion criteria of the study, but only 25 showed interest in participating of the research. From these ones, 22 participated of the research. On the other hand, for the selection of the CG, three public schools were visited, being invited 250 students on mean, but only 57 subjects showed interest, and only 26 students participated of the survey.

The convenience sample of this study initially had 48 volunteers. From these ones, two presented alterations in the basic audiometric assessment, being, so, excluded from the research and they were sent to proper treatment. The final sample consisted of 46 students - 21 belonging to the SG and 25 belonging to the CG.

The audiometric assessments and the collections of biological materials, from both groups, were carried out at CEREST, preferably all of them in the same day.

Initially, parents and/or guardians and the subject herself/himself underwent a questionnaire, to identify the criteria for inclusion and exclusion. All children were subjected to visual inspection of the external auditory canal by using the Clinical Otoscope Welch-Allyn Klinic, to verify any alterations that could make difficult the testing and the audiometric assessment, consisting of: pure tone audiometry (PTA) and immittanciometry.

PTA was carried out in a sound-treated booth with the audiometer Interacoustics AC40 model and earphone TDH-39. In the PTA air conduction, thresholds were investigated in the frequencies of 500, 1000, 2000 and 4000 Hz. The chosen technique was the descending-ascending one. Normal-hearing subjects were considered those ones who presented tritone mean (500, 1000 and 2000 Hz) less than or equal to 25 dBHL (decibel hearing level).

The acoustic impedance measurements were carried out with the AT 235 equipment, Interacoustics and 226 Hz probe tone, for research of the tympanometric curve and acoustic reflexes. These ones were investigated in the frequencies from 500 to 4000Hz bilaterally, in a contralateral mode. Only children with type A tympanogram and acoustic reflexes were present in the sample.

Subsequently the subjects were assessed by DPOAE in both ears, first in the absence and, after, in the presence of contralateral acoustic stimulation. DPOAE was carried out in a silent place, using portable equipment Otoread Screening of Interacoustics. To obtain DPOAEs (2F1-F2), two pure tones in the ratio F2/F1 = 1.22, where F1 is presented in the intensity of 65dBSPL and F2 in L2 = 55dBSPL. For DPOAE measures, the frequencies 1500, 2000, 3000, 4000 , 5000 and 6000Hz were tested. DPOAE was considered present when the signal/noise relationship was greater than or equal to 6 dBSPL at least three frequencies.

The contralateral acoustic stimulation was a white noise, in the intensity of 60 dBHL (25), generated by the audiometer which was mentioned above (Interacoustics model AC 40 via earphone TDH -39). In order to avoid manipulation of the DPOAEs probe, the phone was attached to the ear in a contralateral way to the recording of DPOAE before the beginning of the test. In this research, it was respected the following order of tests: DPOAE in the right ear (RE) without noise, DPOAE in the RE with noise, DPOAE in the left ear (LE) without noise and DPOAE in LE with noise.

The calculation of DPOAE suppression was performed by subtracting the level of DPOAE without contralateral acoustic stimulation of the answer level of DPOAE with contralateral acoustic stimulation. The analysis of suppression effect was a response (overall response). The response is calculated from the geometric mean of the frequencies under test. This study considered the effect of this deletion with decreasing of DPOAE amplitudes of at least 0.5 dB and suppression effect absent when the difference was less than 0.5 or negative. According to Collet et al. [18], an effect of suppression from 0.5 to 1.0 dB shows the integrity of MOCS.

Finally, the collection of biological material to perform genotoxic tests was performed, however; only 18 subjects from the SG and 18 subjects from the CG performed this procedure, reducing the sample to these evaluations.

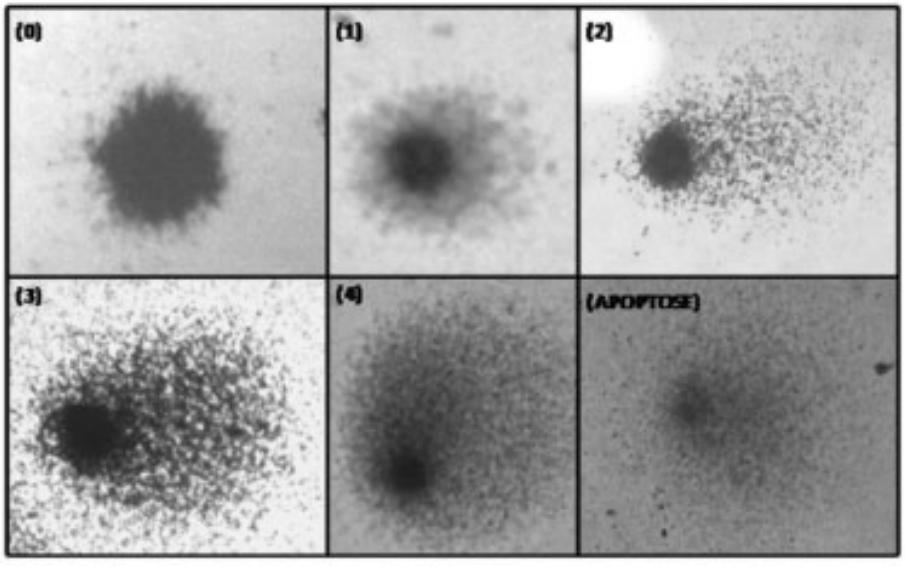
Genotoxicity tests performed in this study were: comet assay, micronucleus test (MN) and fluorimetric assay for quantification of DNA. The collection of biological material (blood and epithelial cells of the oral mucosa), for the testing, was performed by an accomplished nursing technician. After the collection, the blood was immediately stored in a tube with anticoagulant heparin and used in the comet assay and fluorimetric assay for quantifying DNA. The epithelial cells, for the MN test, were deposited in a Falcon conical tube containing 2 mL of saline solution or PBS pH 7.4 solution.

The Comet assay was carried out according to the method proposed by Singh et al.[19] and modified by Collins Ma and Duthie[20]. For each subject, slides were prepared in duplicate . All the steps were conducted without direct light to prevent additional DNA damage. In order to apply the technique, 5µL of sample (leukocytes) with 90µL of 0.75 % agarose were mixed in an eppendorf. The solution was added

to a pre-covered slide with 1 % normal agarose and covered by a coverslip, being in the fridge for five minutes. After the coverslip was removed and the slide placed in a vat with lysis solution for one day at 4 °C. The slides were removed from the lysis solution, washed with distilled water. Then, they were placed in a horizontal vat containing electrophoresis solution. The slides were left in this solution for 20 minutes at rest to permit the DNA unwinding, subsenquently, the electrophoresis was performed for 20 minutes at 25 Voltz (V) and 300 microamperes (mA). Then, the slides were placed in a vat with neutralizing solution for five 45 minutes, being washed three times with distilled water and dried until the next day at room temperature. The slides were rehydrated for five minutes and placed in a vat containing fixative solution for ten minutes. After that, they were washed three times and left to dry at room temperature. They were rehydrated again for five minutes and placed in a bowl containing staining solution for 25 minutes at 37 °C. After staining, the slides were washed three times in distilled water and again left to dry in room temperature.

The slides were analyzed using the binocular optical microscope Olympus®, model CX40, with an increase of 400 times. A score was made for each sample, of 100 cells (50 per slide). The slides were analyzed by two independent observers, and, for the damage index (DI), it was considered the mean damage of the two analyzed slides. The calculation of the damage index (DI) was made from the formula proposed by Cavalcanti et al.[21]: ID = (x 0 n0) + (1 x n1) + (2 x n2) + (3 x n3) + (4 x n4), where n = number of nuclei for each analyzed class.

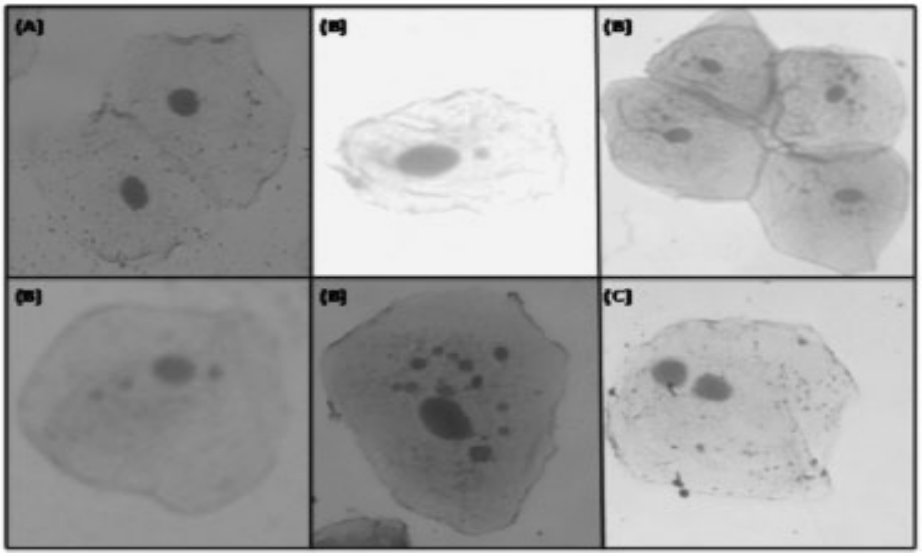
The five categories that were used for the classification of the Comet are those ones proposed by García et al. [22] and shown in Figure 1.



Fígure 1 – Classification of Damage to DNA. (Fronza et al., 2011)

In the MN test, the samples of the epithelial cells of the oral mucosa were centrifuged at 1000-1500 RPM during ten minutes at room temperature. Then, the supernatant was discarded using individual Pauster pipettes, being careful to not remove the cell pellet. So, it was added 1.5 ml of fixative solution and centrifuged again at 1000-1500 rpm for 1-2 minutes. Again the supernatant was discarded, keeping some of the fixative solution in the tube, and then the content was homogenized with Pauster pipettes to resuspend the cells. The contents were deposited in clean slides and directly identified, which ones were left to dry at room temperature for 10-15 min. After, it was carried out the staining by using the panoptic stain. Finally, the slides were washed with distilled water in a way to remove the excess stain and they were left to dry at room temperature during 20-25 min.

Once dry, the material was observed in the binocular optical microscope Olympus ®, model CX40, with magnification of 400x for counting of the micronuclei present and subsequent data analysis. 1000 cells were counted and classified into: normal cell (no alteration), cell with MN, binucleated cells (BN) cell with nuclear bridges (NBs) and cells with nuclear Buds (BUD) or “Broken Eggs”, according to Figure 2.



Fígure 2 - Classification of cells of MN test: A (cell without alteration); B (cell with MN); C (BN cell). (FRONZA et al., 2011)

For comparison purposes, only the index results of DNA damage in comet assay were used. And, for the MN test, we considered the sum of abnormal cells, the total cells with MN and the total binucleated cells.

In the fluorometric assay for quantification of DNA, initially the total blood was centrifuged at 2000 RPM for ten min. For this technique, the blood plasma was used.

Before starting the experiment it was done the reading of the Elisa plate (empty). Then, 10μL of plasma was pipetted into the Elisa plate (black) in, at least, quadruplicates. After that, 10μL of the reagent PicoGreen ® was added. After the completion of pipetting, the plate was incubated for 5 min at room temperature and protected from light (once the Picogreen reagent is photosensitive). After the incubation period, it was carried out the verification of the fluorescence plate, with 480nm of excitation and 520 nm of emission. The interpretation of the obtained values is given so that the higher the fluorescence value rises; the more DNA is verified in the environment, which indicates cell death.

Statistical analyzes were performed with the help of the SPSS software (Statistical Package for the Social Sciences) version 17.0. To determine the normality of the variables, it was used the Kolmogorov-Smirnov test. In all analyzes, we adopted a significance level of 5%.

To analyze the occurrence of suppression of DPOAE, Fisher exact test was used. Comparing the comet assay, fluorimetric assay quantification of DNA and micronucleus test (MN) results, we used the Student-t test for independent samples. And, to compare the occurrence of suppression effect and genotoxic tests, the Mann Whitney test was used.

RESULTS

All subjects, in both groups, showed DPOAE in both ears.

When comparing the occurrence of suppression between RE and LE, both SG and CG, it was verified no significant difference ($p < 0.05$). As a result, we started to consider the presence or absence of suppression of DPOAE in RE and LE simultaneously, taking into account only the group to which the students were part of. It was considered absence of suppression of DPOAE when it was absent in both ears.

In relation to the occurrence of suppression of DPOAE between groups, it was not detected statistically significant association ($p > .05$), indicating a relative independence between groups and occurrence of DPOAE regarding suppression (Table 1).

Table 1- Comparative analysis of the occurrence of suppression effect of DPOAE between the study and control groups (n=46)

Suppression	Groups		Value-pƒ
	Study (n=21)	Control (n=25)	
Present	19 (90,5%)	25 (100,0%)	0,203
Not present	2 (9,5%)	0 (0,0%)	

ƒ: Exact Fisher Test; * p<0,05

In comparison to the mean percentage of damage in comet assay, the results showed that the mean of the SG (48.4 ± 3.2) was significantly higher than the one found in the control group (37.1 ± 17.2). A significant difference is also configured in the comparison of the fluorimetric assay test score for quantification of DNA, where the mean was higher in SG than in the CG (46.1 ± 18.9 vs.24, 5 ± 4.9, p <0.001) (Table 2).

Table 2 - Comparative analysis of the average (mean and standard deviation) values of the damage index of the comet assay and fluorimetric assay for quantification of DNA between the study and control groups (n=36)

	Groups						Value-p
	Study (n=18)			Control (n=18)			
	Mean	Standard Deviation	Amplitude (Min-Max)	Mean	Standard Deviation	Amplitude (Min-Max)	
Comet Essay	48,4	3,2	28,5 – 70,5	37,1	17,2	11,0 – 74,0	0,033¶
Fluorimetric assay for quantification of DNA	46,1	18,9	25,7 – 84,8	24,5	4,9	19,1 – 38,4	<0,001§

Captions: Min-Max = minimum and maximum;
¶: Student-t Test for independent groups, assuming homogeneity of variances; §: Student-t test for independent groups, assuming heterogeneity of variances;
*p< 0,05;

In the MN test, when the sum of abnormal cells was compared between the groups, it was verified significant statistical difference (p <0.01), so that the mean of the SG (24.9 ± 9.8) showed to be higher than the mean of the CG (14.9 ± 7.8) (Table 2). Regarding the results of the comparison between groups in the frequency of binucleated cells, it was detected a statistically significant difference (p <0.01), in a way that study group (12.2 ± 6.5) presented higher mean than the control group (6.3 ± 2.2). Comparing the frequency of cells with MN, the SG also showed the rate of cells with MN higher than the one found for the CG MN, but the significant difference was not configured (p> 0.05), indicating that variations of means between the SG and CG occurred at random (Table 3).

Table 3 - Comparative analysis of the average (mean and standard deviation) values of the variables of the MN test between the study and control groups (n = 36)

Variables	Groups						Value-p
	Study (n=18)			Control (n=18)			
	Mean	Standard Deviation	Amplitude	Mean	Standard Deviation	Amplitude	
Abnormal cells	24,9	9,8	3,0 – 42,0	14,9	7,8	7,0 – 40,0	0,002§*
Binucleated	12,2	6,5	2,0 – 27,0	6,3	2,2	3,0 – 11,0	0,002§
Micronuclei	11,6	7,2	0,0 – 24,0	8,2	7,2	1,0 – 32,0	0,168¶

Captions: MN = micronuclei

¶: Student-t Test for independent groups, assuming homogeneity of variances; §: Student-t test for independent groups, assuming heterogeneity of variances;

*p< 0,05;

Based on the comparison of the presence and the absence of the effect of DPOAE suppression, it was compared in each group, the damage index of the Comet assay and fluorimetric assay for quantifying DNA, and the frequency of abnormal cells in the MN test. As the results in Table 4, no statistically significant differences (p> 0.05) were detected, indicating that the means of the compared variables are not related to suppression in the SG. In the information regarding to the CG, the absences of DPOAE suppression were not registered, which prevented the performance of a comparative analysis (Table 4).

Table 4 - Association between DPOAE suppression effect and index of DNA damage in genotoxic tests between the study and control groups

Variables	Groups				
	Study			Control	
	Suppression			Suppression	
	Present (n=16)	Not present (n=2)	pε	Present (n=18)	Not present (n=0)
Comet essay	48,2±13,7	50,2±10,2	0,844	37,1±17,2	±
Fluorimetric assay for quantification of DNA	45,1±18,6	53,2±27,2	0,569	24,5±4,9	±
Abnormal cells – MN test	24,9±10,3	25,0±7,1	0,994	14,9±7,8	±

ε: Mann Whitney Test; *p < 0,05;

DISCUSSION

The potential health effects associated to the exposure of children to pesticides are matter of constant concern. However, the chronic effects of continuous exposure to pesticides, as well to nicotine (tobacco leaf), on the human body in development are not well understood.

As previously mentioned, the pesticides can affect the peripheral and central auditory system. In this study, it was studied the effect of pesticides and nicotine on MOCS by DPOAE suppression. When the occurrence of suppression of DPOAE between groups are compared, no statistically significant association was detected (p> .05), which may indicate a relative independence between groups and occurrence of DPOAE suppression. These results suggest that exposure to toxic substances (pesticides and nicotine) did not affect the functions of the MOCS in the population who was studied.

It is noteworthy that, due to the shortage of studies that evaluate the MOCS with exposure to pesticides and nicotine, the results of this survey were compared to similar studies wherever possible.

In the absence of those ones, this research was related to studies with subjects exposed to noise and/or to other kinds of ototoxic substances and also to studies that assessed the central auditory system by means of auditory evoked potentials.

The most used organophosphate pesticides, in the tobacco plantation, present a mechanism of action based on the inhibition of acetylcholinesterase, which consequently increases the level of the neurotransmitter acetylcholine in synapses[24]. According to Harkrider, Champlin, McFadden[7], nicotine also has effect on the neurotransmitter acetylcholine. The same researchers evaluated the results of OAE and BAEP of ten normal-hearing subjects and nonsmokers, after administration of nicotine, realized that it interferes in the neural transmission of auditory information. The effect of nicotine on high neural centers may have an inhibitory effect on the OHC. That is justified by the acceleration of acetylcholine, which is the neurotransmitter of the efferent auditory system, implying an increase in the effect of OAE suppression.

Another study evaluated the effect of smoking on the auditory system, comparing the results of conventional and high-frequency audiometry of TOAE and the suppression effect between smokers and nonsmokers, concluding that cigarette smoking has an adverse effect on hearing. Specifically in relation to the evaluation of MOCS, the researchers have noted a reduction in the response level of otoacoustic emissions (suppression) in 100% of cases in both groups, and the group of smokers showed higher suppression values when compared to the non-smoking group[25]. These results disagree with the two other already mentioned studies, once it was found no increase in DPOAE suppression effect of the students exposed to pesticides and nicotine.

A study that evaluate the condition of the MOCS of subjects exposed to organic solvents through the suppression effect of TEOA observed that the presence of TEOA suppression effect was higher in the control group (72%) if compared to the study group (58%), but this difference was not statistically significant[26]. In this study, the presence of the suppressive effect was also higher in the CG (100%) when compared to the SG (90%), but this difference was even less significant.

Other authors[1] investigated the effect of organophosphates on the hearing of guinea pigs. DPOAE, BAEP and histological analysis of the cochlea of three groups of guinea pigs (control, exposed to low and high dosage of pesticides) were carried out. The authors perceived no functional alterations of the cochlea and auditory nerve; they just found that the two groups that received pesticides showed alterations in OHC cytoarchitecture, with greater loss of animals which received high dosage. In this research, it was also observed no functional alteration in the MOCS.

Hearing loss may be an early manifestation of the pesticide poisoning, injuring the peripheral component and also the central hearing[27]. The OAE and the analysis of suppression can be used for early detection of hearing impairment with cochlear and retrocochlear origin[10]. Our study showed no alteration in the auditory system, which was evidenced by the presence of DPOAE and suppression effect.

Another way to prevent risk regarding development of various diseases from the exposure to toxic substances is through biomonitoring. This is a useful tool to estimate the genetic risk from an integrated exposure to a complex mixture of chemic products[14]. In this study, genotoxic biomarkers were: comet assay, fluorimetric assay for quantification of DNA and MN test.

In the comet assay, the group of exposed students showed a significantly higher damage index than the unexposed one. These findings corroborate the results of other authors[28] who, when studying genotoxic effects on tobacco growers through the comet test also showed that the treatment group presented a significant increase of DNA damage if compared to the CG. Da Silva et al.[29] also observed that the DNA damage by comet assay in tobacco growers had increased three times when it was compared to the unexposed group.

The possible genotoxic damages caused by nicotine are still unknown[30]. Other authors[31] observed genotoxic effect of nicotine through the comet assay.

In fluorimetric assay for the quantification of DNA, the group of exposed children also presented higher mean regarding the CG, this difference was highly significant ($p < 0.001$). The fluorometric assay for the quantitation of DNA is a test that uses the ultrasensitive fluorescent reagent Quant-iT™ PicoGreen® dsDNA (Invitrogen), which detects small amounts of double-stranded DNA in solution[32]. The PicoGreen (PG) binds to DNA and, when that happens, its fluorescence increases > 1000 times, and this is proportional to the amount of present DNA (circulating)[33,34].

In the literature, studies that used the fluorimetric assay for the quantification of DNA as a biomarker of pesticide exposure are limited. However, it is a technique that plays an increasingly important role in many studies and biological applications, such as molecular biology and diagnostic techniques as biomarker for severe diseases such as Dengue Hemorrhagic Fever Dengue Hemorrágica[35].

In this study, the fluorimetric assay for quantification of DNA, using the reagent Picogreen, was shown to be an effective and highly sensitive technique for analyzing the genotoxic effect of pesticides on the exposed population. These results corroborate the findings of Parra, Sanchez-Fortún and Castanõ[36], although these tests had been made in different kinds of cells (lymphocytes and tissue cells). They studied the applicability of the reagent Picogreen in the quantification of DNA to determine the genotoxic effects induced by organophosphorus pesticides in tissue cells of fish. They concluded that this method was able to put in evidence the degree of DNA damage induced by these pesticides. Therefore, it can be used to prevent the genotoxic effects of pesticides in these kinds of cells. They also note that the necessary amount of chemicals, the cost and time required for the test is drastically reduced, so it could replace other genotoxicity tests.

On the other hand, the MN test is a widely used technique in studies of biomonitoring of populations exposed to different kinds of toxic substances. In our study, in the MN test, it was detected a statistically significant difference when the sum of abnormal cells and the frequency of binucleated cells between the groups are compared, with the mean of the SG higher than the one for the CG. However, in comparison of the frequency of cells with MN, the difference was not set, although the mean of the SG is higher than the one for the CG. These findings agree in parts with the results of other researchers who found significant differences in all types of abnormal cells when comparing the exposed group with the CG[3,29,37].

The majority of the studies have only analyzed the frequency of cells with MN, unlike this study. Several studies have reported a significant increase in the frequency of MN in subjects exposed to pesticides in comparison with the control group[37,38]. Other authors had specifically studied children exposed to pesticides and they also observed a significant increase in the frequency of cells with MN in the exposed group[3,39]. The results of our study disagree with the findings of these authors. However, these results are consistent with the findings of Pastor et al [40] who also found no difference between subjects exposed and not exposed to pesticides in relation to the frequency of MN .

In this study, we tried to examine the association between the MOCS functions (presence and absence of DPOAE suppression) and genotoxic damages. The results of this study indicate that the damage index which was found in genotoxic tests do not depend on the DPOAE suppression in the SG, ie, there are no positive association between the variables. Fronza et al.[18] verified that smokers have elevated levels of genotoxicity, evidenced by the Comet assay, however, no significant associations between the lack of effect of DPOAE suppression and genotoxicity were observed.

CONCLUSION

In this study, no alterations in MOCS were observed, which was evidenced by DPOAE suppression in students who live in the tobacco-producing region. However, significantly elevated damage indexes of genotoxic biomarkers in exposed students have been observed. However, no association between DPOAE suppression and Genotoxicity was verified.

These findings show that there is no alteration in relation to the auditory function (MOCS), but the results of genotoxic biomarkers indicate the presence of a susceptible genetic profile for the development of future pathologies resulting from the exposure to these pesticides, being the hearing loss one of them.

RESUMO

Objetivo: avaliar a associação entre a função do sistema olivococlear medial e biomarcadores genotóxicos em escolares residentes de região fumicultora. **Métodos:** trata-se de um estudo observacional, prospectivo e transversal. O grupo estudo foi composto por 21 escolares normo-ouvintes residentes de região fumicultora e o grupo controle por 25 escolares normo-ouvintes que não residiam na zona rural. O sistema olivococlear medial foi avaliado por meio da supressão das Emissões otoacústicas produto de distorção, e os biomarcadores genotóxicos foram: ensaio cometa, teste de micronúcleos e ensaio fluorimétrico de quantificação de DNA. Os dados obtidos foram submetidos à análise estatística. **Resultados:** ao comparar a ocorrência do efeito de supressão das emissões entre os grupos, não foi detectada associação significante. Tanto no ensaio cometa como no ensaio fluorimétrico de quantificação de DNA a média do grupo estudo mostrou-se significantemente mais elevada que a do grupo controle. No teste de micronúcleos, verificou-se diferença significante quanto ao somatório de células alteradas e à frequência de células binucleadas, sendo a média do grupo estudo mais elevada que a do grupo controle. Já referente à frequência de células com micronúcleo, não se observou diferença significante entre os grupos. Não foi detectada associação entre ocorrência do efeito de supressão e os resultados dos biomarcadores genotóxicos. **Conclusão:** o grupo estudo não apresentou alterações no sistema olivococlear medial, evidenciado pela presença de supressão das emissões, porém apresentou índices de dano significantemente mais elevados dos biomarcadores genotóxicos. Entretanto, não se verificou associação entre supressão das emissões e genotoxicidade.

DESCRITORES: Praguicidas; Tabaco; Audição; Vias Eferentes; Genotoxicidade; Criança

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