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Neurological Evaluation in Quilombolas Individuals Exposed to Organophosphorus Pesticides in the Brazilian Amazon Population

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***Abstract*—*The aim of the present study was to investigate the relationship between the biochemical changes of this enzyme and the occurrence of neurological symptoms. In addition, an attempt is made to link the neurocognitive damage to environmental and human pollution near oil palm monocultures in the Amazon. This is a descriptive cross-sectional study in a quilombola community in the state of Pará exposed to pesticides from 2017 to 2019 through epidemiological, clinical, biochemical, and toxicological investigations. Clinical, epidemiological, and laboratory data of the subjects were used for the study, as well as a neurological examination using a pesticide poisoning examination form from the Instituto Evandro Chagas. The sample of the study includes 195 people. Of these, more than half (54.9%) were male and the rest were female. As for the age group of the participants, 28.7% were children and adolescents, 58% of the subjects were between 20 and 59 years old, and another 13.3% were elderly. It was found that the reduced AChE levels were significantly lower in individuals with neurological complaints. In addition, the most frequently expressed signs and symptoms were weakness in 28.7%, memory impairment in another 24.6%, insomnia in 21%, and motor disorders in 15%. And it was found that the female gender manifested the neurological clinical picture the most and showed the greatest reduction in AChE enzyme. Therefore, it is necessary to biologically monitor populations exposed to the environment OP.*

I. INTRODUCTION

The main effect of organophosphates (OP) in the human body is the inhibition of the enzyme cholinesterase in nerve endings. Therefore, measurement of acetylcholinesterase activity (AchE) in blood is often used as a biomarker of effect to characterize exposure to pesticides. Neurodegeneration can occur from exposure to OP at small subclinical doses and may be referred to as organophosphate-induced chronic neurotoxicity. [1]

Incidence rates of pesticide poisoning have increased in recent years. At the same time, the use of pesticides in agriculture is expanding. At the global level, the use of these agents has gradually increased in recent years, with about 4 million tons of pesticides used or sold in agriculture.[2] In the world ranking, Brazil has been the largest consumer of pesticides on the planet since 2009, followed by the United States, China and Japan. [3]

According to SINAN (Sistema Nacional de Agravos de Notificação Compulsória), from 2007 to 2021, 157,382 cases of agricultural and household pesticides were registered in the country, resulting in 2639 cases of poisoning. And in the state of Pará, there were 1801 reports of exogenous poisoning by these agentes. [4]

It is worth noting, however, that the World Health Organization - WHO - estimates that for every reported case, there are 50 unreported cases, which ends up being about 500,000 new cases.[5] Thus, it is believed that the epidemiological profile does not match the reality of pesticide use and human exposure. This makes it difficult to scale the problem and make the public sector more visible in dealing with these problems and diseases in exposed communities.[6, 7]

Oil palm is the main oilseed production chain in the world, occupying 20% of permanent cropland. [8] Brazil ranks ninth in the world and is responsible for the production of 395 thousand tons per year. Pará is the largest national producer with 98.47%, followed by Bahia. In the northeast of the state, the municipality of Concórdia do Pará ranks third in the microregion with an annual production of 276 thousand tons.[9]

The Santo Antônio Community, in the municipality of Concórdia do Pará, there are agricultural activities related both to family farms, where pesticides are not used, and to large-scale projects such as the monoculture of “dendê”, where the use of pesticides is quite common. Therefore, a strong epidemiological link between occupational and environmental exposure is suspected. The study aims to gain knowledge about the coexistence of individuals of a traditional population with organophosphate pesticides used in the monoculture of “dendê” in the northeastern region of Pará State in the Brazilian Amazon.

II. METHOD

This is a descriptive cross-sectional study with a quantitative approach conducted between 2018 and 2019 in the Quilombola municipality of Santo Antônio, in the municipality of Concórdia do Pará/Brazil. This study was approved by the local research ethics committee. The municipality belongs to the northeastern mesoregion of the state of Pará and is located at latitude 02° 00' 06" South and longitude 47° 56'59" West, at an altitude of 440 meters above sea level.

This municipality is located about 150 km from the capital, Pará State, and has the privilege of being located in an easily accessible region that is economically linked to the capital. The main economic activities are black pepper and subsistence agriculture with cassava flour as the main product. Currently, the introduction of large-scale projects with oil palm monocultures is intensifying, aiming at the production of biodiesel.[10]

The Quilombola community of Santo Antônio lives in the vicinity of this oil palm monoculture, where the use of pesticides is widespread, so there is a strong epidemiological link between occupational and environmental exposure. The selection of study participants was based on the criteria of duration of residence (at least one year) and complaints transmitted by indicating the local management. Thus, the demographic epidemiology of the sample was characterized by sex (male and female) and age group (2-19 years; 20-39 years; 40-59 years and over 60 years), giving a total number of n - 195 (100%).

Factors influencing the occurrence of neurologic signs and symptoms, such as previous neurologic disease and clinical and/or drug conditions (diabetes associated with peripheral neuropathy), hypothyroidism or hyperthyroidism, hypovitaminosis B12, alcoholism, HIV/AIDS, syphilis, and leprosy) were used as exclusion criteria.

Study Design

Neurological Examination and Medical Examination

The study of neurotoxic effects was based on the neurological examination using the Evandro Chagas Institute (IEC) Pesticide Poisoning Examination Form, which includes epidemiological and clinical questions and a medical examination. The standardized neurological questionnaire used during the medical interview assessed the clinical tremor scale, sleep disturbances (insomnia), fatigue, memory difficulties, tingling in the hands, upper and lower limbs, hand and eyelid tremor, where single investigator observed and assessed tremor. The parameter of presence or absence of involvement was used for statistical analysis of all clinical data.

Enzymatic Activity of Erythrocyte Acetylcholinesterase

As for the toxicological analysis, the AChE densitometry values were processed in the chromatography laboratory of the Environmental Department (SEAMB) of the IEC using the modified Elmann method. The reference values used in the data analysis correspond to the assessment method for AChE (2.6-4.1 IU/mL).

Statistical analysis

All tests were performed using Bioestat 5.5 software. Quantitative variables and AChE enzyme activity were described by mean and standard deviation, and qualitative variables were described by frequency and percentage.

The Mann-Whitney test was used, as in the correlations between sex and average cholinesterase activity and the presence or absence of signs and symptoms with the community enzymatic average. The Kruskal-Wallis test was used, as in the division of age groups with respect to enzyme means. To determine the number of individuals with enzymatic inhibition (below 2.6 IU/ml) correlated with the variables sex and age and neurological signs and symptoms by sex, frequency and percentage by test. of chi-square was used to test independence or association between two categorical variables.

The significant result was detailed by multiple comparisons between two groups with adjustment of p value. Results with $p \leq 0.05$ (two-sided) were considered statistically significant.

III. RESULTS

The study includes a representative sample with the inclusion of 195 individuals. Of these, more than half (54.9%) were male and (45.1%) were female. As for the age characterization of the participants, 28.7% were in the age group of 2-19 years, 58% of the subjects were between 20 and 59 years old, and another 13.3% belonged to the elderly population.The mean AChE value was 5.8 ± 3.3 IU/mL.

In the female subjects, the mean AChE level was 5.1 ± 3.1 IU/mL, and in the male group, it was 6.1 ± 3.5 IU/mL. There was a significant difference between these groups (p=0.0266). It was found that, taking the modified Ellmann method as a reference value for erythrocyte AChE (2.6-4.1 IU/mL), the inhibition of acetylcholinesterase occurred in 75 quilombola subjects. In addition, it seems that 55 individuals with reduced AChE values were under 39 years of age, and the age group with the greatest change was between 20and 39 years. On the other hand, there was a greater balance of occurrence of the data among the sexes, as shown in Table 1.

Despite the correlation between the age group and the AChE classification (lower or higher than the reference level), the indication of a p-value (p=0.054) is considered relevant because the significance value is in a large proximity. This is different with respect to gender (p=0.847) (Table 2).:

Table 1. Acetylcholinesterase levels in the Quilombola population of the municipality of Santo Antônio, Concórdia do Pará.

Variable	Mean AchE ±SD	p-value	FIND >2.6	THINK <2.6	p-value [2]
community population	5.4± 3.3	-	120	75	-
GENRE					
Feminine	5.1± 3.1	0.0266 [1]	53 (44.2)	35 (46.7)	0.0847 [3]
Male	6.1± 3.5		67 (55.8)	40 (53.3)	
AGE GROUP					
2-19 years old	5.5 ±3.0	0.0502 [2]	34 (28.3)	22 (29.3)	0.054 [3]
20-39 years old	5.2± 3.4		34 (28.3)	33 (44)	
40-59 years old	7.0 ±3.6		34 (28.3)	12 (16)	
>60 years	5.6± 2.9		18 (55.8)	8 (10.7)	

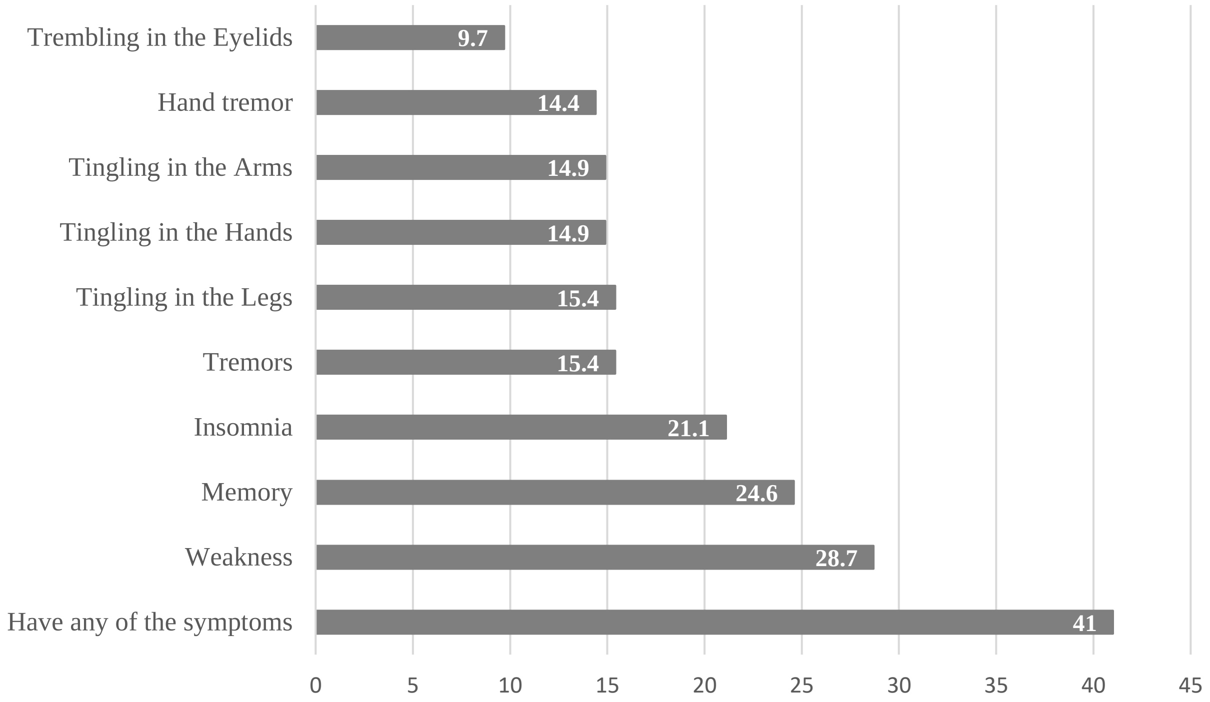
[1] Numerical variables of AchE are represented as mean ± standard deviation. The Mann-Whitney test was used. [2] Numerical variables of AchE by age group are represented as mean ± standard deviation. The Kruska -Wallis test was used.[3] Categorical variables are displayed as n (%). The percentages are relative to the total of each column. The chi-square test was used.

Source: Author's research. Instituto Evandro Chagas, Environment Section.

Neurological Clinical Manifestations

The signs and symptoms were shown in Figure 1. It is noted that 56 subjects (28.7%) complained of weakness, another 48 subjects (24.6%) had a memory problem, 21% suffered from insomnia, which were the most common symptoms. In addition, 80 (41%) of the respondents had one of the symptoms studied (at least 1 or more).

Fig.1 - Presence of neurological signs and symptoms in the quilombola community, in the municipality of Concórdia do Pará.

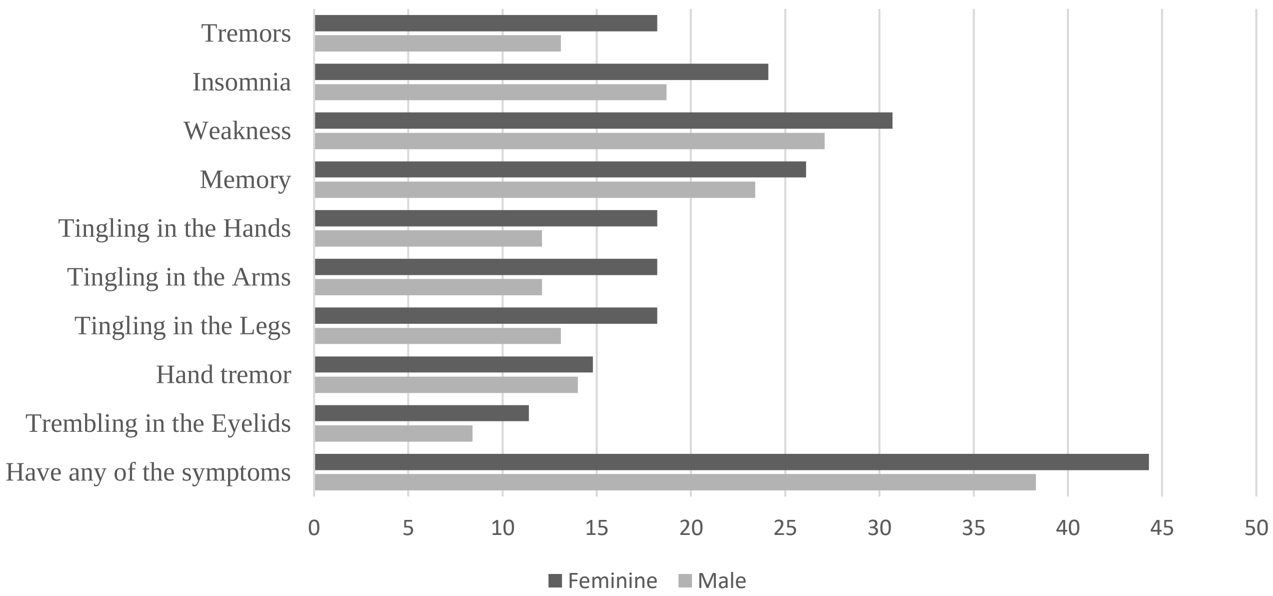


The percentages are relative to the total number of participants (n=195).

Source: Author's research. Instituto Evandro Chagas, Environment Section.

Relating these signs and symptoms to gender, we find that tremor, insomnia, weakness, memory impairment, tingling in the hands, tingling in the arms and legs, and tremor of the hands and eyelids were more common in women (Figure 2). However, there was no significant association between gender and symptoms (non-significant p-values) based on the Chi-Square Test.

Fig.2 - Association between the presence of signs and symptoms and gender.



Source: Author's research. Instituto Evandro Chagas, Environment Section.

The results of the comparison between the mean values of AChE activity and the presence or absence of signs and symptoms are shown in Table 4. In the subjects who did not have weakness, the mean AChE value was 6.6 ± 3.3 and in the group with weakness, 5.4 ± 3.3, differing significantly (p=0.017), i.e., AChE values were significantly lower in subjects with weakness.Thus, it was found that in subjects without memory problems the average AChE was 6.8 ± 3.1 and in the group with problems it was 5.1 ± 3.3, with significant significance (p=0.0007).Regarding the symptom insomnia, the mean score of AChE was 5.4 in those who had this symptom, differing from those without this symptom, in whom the mean score was 6.1, also showing significance (p= 0.032) between groups.In the participants who did not have symptoms, the mean AChE was 6.6 ± 3.3 and in the group with symptoms it was 5.2 ± 3.2, with a significant difference between the groups (p= 0.003) (Table 2). The other symptoms previously reported in this study had no significant correlation.

Table 2 - AchE levels in relation to the presence of signs and symptoms in quilombola patients in the municipality of Concórdia do Pará.

Variable	Absence	Presence	p-value
tremors	5.8 ± 3.3	5.8 ± 3.3	0.890
Insomnia	6.1 ± 3.2	5.4 ± 3.4	0.032
Weakness	6.6 ± 3.3	5.4 ± 3.3	0.017
Memory	6.8 ± 3.3	5.1 ± 3.3	0.0007
Tingling in the Hands	5.8 ± 3.3	5.6 ± 3.3	0.733
Tingling in the Arms	5.8 ± 3.3	5.6 ± 3.3	0.790
Tingling in the Legs	5.8 ± 3.3	5.6 ± 3.2	0.753
hand tremor	5.7 ± 3.3	6.0 ± 3.5	0.728
Trembling in the Eyelids	5.8 ± 3.3	5.7 ± 3.3	0.981
Have any of the symptoms	6.6 ± 3.3	5.2 ± 3.2	0.003

Numerical variables are represented as mean ± standard deviation. In all cases, the Mann-Whitney test was used.Source: Author's research. Instituto Evandro Chagas, Environment Section.

IV. DISCUSSION

There are few data in the literature that shed light on this problem, especially in traditional Brazilian populations. However, this study allows us to establish a possible association between neurological signs and symptoms and changes in the AChE biomarker with the general use of pesticides in the quilombola population living with the practice of palm oil cultivation in the Amazon. The chronic manifestations presented can be attributed to a strong and long association with environmental exposure and low levels of organophosphates applied seasonally, at least once a month, by spraying.

Much is known about the acute clinical consequences associated with occupational use of pesticides or direct handling of them. However, data are still lacking on the chronic manifestations. The main mechanism of action of OPs in the human body is the inhibition of the enzyme cholinesterase in the nerve endings, resulting in the accumulation of the neurotransmitter acetylcholine in the neuromuscular junctions, which triggers the occurrence of the cholinergic syndrome, nicotinic and muscarinic symptoms with CNS involvement.[1] Measurement of erythrocyte AChE activity in blood is commonly used as a biomarker for pesticide effects.[11]

In this study, the mean values of enzymatic activity are in the normal range, but it was observed that 38% of the participants showed enzymatic inhibition of AChE (< 2.6 IU/mL). Regarding gender, there was a significant difference between these data (p -value=0.0266), with the highest enzymatic inhibition occurring in females. In addition, the age group with the lowest mean enzymatic value was 20-39 years old, economically active, and moved around the area the most and generally engaged in extractive activities.

This result is like that of Farahat et al. (2003),[12] who studied a cohort of young people exposed to OP in Egypt. They showed that there was a reasonable proportion of individuals with enzymatic inhibition, but they were still within the normal range. This contrasts with the study conducted in Nova Friburgo (RJ) in an agricultural community, where an extreme decrease in acetylcholinesterase levels was found in 102 farmers. However, there was no significant difference between the sexes in terms of the mean value of enzyme activity. The age group with the greatest change was under 40 years old, a similar result to this work

When Ramírez-Santana et al. (2020) [13] compared two groups from the same agricultural region exposed both environmentally and occupationally to OP, they found that the frequency of inhibition of AChE was 25-30% and that there was little difference in the mean enzyme between these groups. They therefore concluded that environmental exposure OP was as high as occupational exposure.

To make matters worse, workers know the health risk and use biosafety equipment. Surrounding populations may not have this knowledge and are therefore more exposed to the effects of pesticide exposure. This observation was also made in the municipality of this study, where houses were built near the monocultures during territorialization, which can lead to direct and indirect risk to the population without any protection on a seasonal basis.

As in studies conducted in other populations, the most common general and cognitive signs and symptoms noted in this study were: weakness in 28.7% of subjects, followed by memory impairment in another 24.6%, and insomnia in 21%. In addition, symptoms indicative of motor changes, such as hand and eyelid tremors and tingling in the extremities, were noted in a percentage of about 15%; and 41% of those affected experienced at least one of these symptoms.

In addition, it was found that these complaints occurred mainly in women, a gender that also has a greater decrease in the enzyme AchE, which is due to the fact that they stay longer near the oil palm plantation, as few work outside the community.

Exposure to OP causes biochemical changes and clinical syndromes in humans. OP-induced chronic neuropsychiatric disorders may be caused by repeated exposure to low doses, which may be associated with alterations in the axonal transport system. Clinical effects with the most common signs include memory impairment, concentration and learning difficulties, anxiety, depression, and extrapyramidal symptoms such as tremor.[14, 15, 16]

When these neurological complaints were related to cholinesterase levels, it was found that AChE levels were significantly lower in individuals with weakness, as well as problems with insomnia, which was a significant value in those affected ($p=0.032$), and memory problems, where not only were lower AChE levels found in individuals with this symptom, but also showed important significance in this relationship ($p=0.0007$). In relation to the other manifestations, the mean value of esterase is lower in individuals with neurological changes, although there are no significant correlations.

These findings are consistent with recent publications linking clinical neurological findings to AChE enzyme dosing in developing countries. The increased prevalence of neurological symptoms has been associated with inhibition of cholinesterase enzymes in erythrocytes in 03 studies.[13, 17, 18]

The above data indicate that it is necessary to monitor populations exposed to pesticides because clinical signs and symptoms of enzymatic alteration of the biomarker may precede the effect. In addition, the use of validated tests that assess cognitive, motor, and behavioral functions could provide the study with greater consistency in the data observed in recent work. For example, the use of psychometric tests used in previous studies is an effective method to track chronic neurotoxic effects with moderate exposure over time. [13, 17, 12]

For the development of this study, instruments such as questionnaires within a survey were used, which made it possible to obtain epidemiological data and general health conditions; tests of neurosensory functions; laboratory tests to measure AChE enzyme levels. However, there was some limitation to better characterize the community, which could be improved by using other tools discussed in other articles. For example, a survey to complete profile of pesticide exposure at work, home, and family members; use of tests such as the Mini Mental State Examination (MMSE), Wechsler adult intelligence Scale-Revised (WAIS-R) to assess neurological functioning.[17]

In addition, there are other limitations as the only measurement of AChE activity is without consideration of spray times. Therefore, the need for biomonitoring of this quilombola population, which is susceptible to chronic intoxication by low doses of OP, is emphasized to understand the progression of neurological sequelae and to verify the relationships between enzyme dose and clinical changes over a long period of time.

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V. CONCLUSION

The present study is a contribution to knowledge as it attempts to link neurological clinical manifestations to environmental exposure of OP in monoculture areas in the Amazon, in a Quilombola population living near these agricultural areas. The average activity of the effect biomarker is within the normal range. However, in relation to the presence of neurological clinical symptoms, these dosages show reduced values. It is worth noting that these results are the result of a cross-sectional study, so it is important to conduct longitudinal studies to establish the causal relationship.

Therefore, the implementation of biomonitoring in the region is useful for early detection of possible greater participation in this Quilombola community. Therefore, publicizing and recognizing this possible association through the environmental health surveillance program is essential, which must pay attention to the risks from environmental and occupational contamination, especially at low doses and over a long period of time.

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