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Neurodegenerative and Neurobehavioral Symptoms in Jember Agricultural Workers Caused by Oxidative Stress and Neurotransmitter Disturbance

Muhammad Ihwan Narwanto,[1] Azham Purwandhono,[2] Kristianningrum Dian Sofiana,[3] Zahrah Febianti,[4] Muhammad Afiful Jauhani,[5] Inke Kusumastuti,[6] Elvia Rahmi Marga Putri[7]

[1]Anatomy Department, Faculty of Medicine Jember University, East Java, Indonesia
[2]Pathology Department, Faculty of Medicine Jember University, East Java, Indonesia
[3]Physiology Department, Faculty of Medicine Jember University, East Java, Indonesia
[4]Biochemistry Department, Faculty of Medicine Jember University, East Java, Indonesia
[5]Forensic Department, Faculty of Medicine Jember University, East Java, Indonesia
[6]Psychiatry Department, Faculty of Medicine Jember University, East Java, Indonesia
[7]Clinical Pathology Department, Faculty of Medicine Jember University, East Java, Indonesia

Abstract

Increased use of pesticides can have detrimental health consequences, one of which is chronic neurotoxicity. The symptoms include degenerative and neurobehavioral issues. Chronic neurotoxicity occurs through oxidative stress, inflammation, and neurotransmitter disturbances. This study aimed to determine chronic neurotoxicity and test malondialdehyde and cholinesterase levels as neurotoxicity biomarkers among agricultural workers in Wuluhan, Jember, Indonesia. The 60-person research sample was divided into two groups: agricultural and non-agricultural workers. The interview utilized a mini-mental score examination, Chan's questionnaire, and the Patient Health Questionnaire to analyze the cognitive impairment, Parkinsonism, and depressive symptoms. The examination of serum malondialdehyde levels was performed using the TBARS method and cholinesterase levels by photometric kinetic method at a biochemistry laboratory from October to November 2022. Results showed cognitive impairment and depressive symptoms in agricultural workers, as well as high levels of malondialdehyde and low cholinesterase levels. This study concludes the presence of chronic pesticide neurotoxicity among agricultural workers in Jember, Indonesia, and that malondialdehyde and cholinesterase levels might serve as biomarkers of pesticide-induced neurotoxicity.

Keywords: Cholinesterase, cognitive, depression, malondialdehyde, pesticides

Introduction

The agricultural sector is the prime source of income for the inhabitants of Jember as the regency has the second largest agricultural land in East Java Province. Wuluhan is one of the central agricultural sub-districts in Jember.[1] In this area, pesticides are commonly used to control, maintain, and optimize agricultural raw products.[2]

The increased use of pesticides might harm one's health. Neurotoxicity, both acute and chronic, is one effect that could occur. Chronic neurotoxicity occurs upon repeated exposure to small amounts of pesticides over time. It potentially leads to complex health problems manifesting as degenerative symptoms like cognitive impairment, Parkinsonism and neurobehavioral symptoms such as depressive symptoms.[3]

Chronic neurotoxicity occurs through various pathways, including oxidative stress, inflammation, and neurotransmitter disturbances.[3,4] Research in Italy showed higher malondialdehyde (MDA) levels among farmers using synthetic pesticides than farmers using organic pesticides.[5] Moreover, in Tanzania, there was a decrease in acetylcholine esterase activity in agricultural workers.[6] A study in Bandung, Indonesia, discovered a cognitive impairment among farmers exposed to organophosphate insecticides.. There was also a correlation between the use of herbicides and depression

Corresponding Author:
Muhammad Ihwan Narwanto
Anatomy Department, Faculty of Medicine Jember University, Indonesia
Email: muhammadnarwanto@unej.ac.id

among agricultural workers in France.[8]

Early detection of chronic neurotoxicity is critical for maintaining agricultural workers' health and productivity. This research is the first study conducted on a population of agricultural workers in Jember that takes the complexity of pesticide neurotoxicity symptoms, including neurodegenerative and neurobehavioral symptoms, and combination biomarkers in pesticide neurotoxicity, namely MDA and acetylcholine esterase, as a topic. Therefore, this study aims to determine chronic neurotoxicity and to test malondialdehyde and cholinesterase levels as neurotoxicity biomarkers of agricultural workers in Wuluhan, Jember.

Methods

This study recruited a sample of two groups of 30 participants each: the agricultural workers as the exposed group and the non-agricultural workers as the control group. This number is the minimum sample in analytical observational research. The samples were obtained using a purposive sampling method, they were selected based on inclusion and exclusion criteria. The exposed group of agricultural workers should have been directly exposed to pesticides, had worked as agricultural workers for at least a year, and were willing to become research subjects. The control group consisted of subjects with similar demographic aspects who lived in the same area but were not agricultural workers and were willing to participate. Subjects with a history of diabetes mellitus, high blood pressure, alcoholism, malignancy, liver disease, pregnancy, and treatment with acetylcholinesterase inhibitors were excluded.

The participants interviewed to obtain data on neurodegenerative and neurobehavior symptoms, followed by examinations of serum malondialdehyde and cholinesterase levels as neurotoxicity biomarkers. We utilized mini-mental score examination (MMSE), Chan's questionnaire, and Patient Health Questionnaire (PHQ) to assess cognitive impairment, Parkinsonism, and depressive symptoms, respectively. Serum MDA level was measured using the TBARS method with the thiobarbituric acid purchased from Tokyo Chemical Industry, Portland, USA. This study also measured the plasma cholinesterase level. Plasma cholinesterase is an enzyme sensitive to the presence of inhibitors (pesticides) and is applicable as an indicator of pesticide exposure. Cholinesterase levels were measured by a photometric kinetic method using butirylthiocoline as a cholinesterase substrate, with the reagent kit purchased from Diasys Diagnostic Systems GmbH, Germany. The MDA and cholinesterase levels were measured in October 2022 at the Biochemistry Laboratory, Faculty of Medicine, University of Jember. Reagents and other materials were obtained from the laboratory.

For serum preparation, the blood sample was transferred into a red tube and left for 1 hour at room temperature (25°C) to induce coagulation. Then, the sample was centrifuged at 3000 rpm for 15 minutes to separate the serum and the clot. Serum was stored in a microtube and ready to be processed or stored in a -80°C freezer for later examination.

The MDA levels (nmol/ml) were measured by mixing 1 ml of serum with 2 mL of TCA-TBA-HCl stock solution (15% w/v trichloroacetic acid; 0.375% w/v thiobarbituric acid; 0.25 N hydrochloric acid). The solutions were put into the boiling bath for 15 minutes. After the cooling process, the test tubes were centrifuged for 10 minutes at 3000 rpm. Subsequently, the supernatant was measured at the 535 nm absorbance. The cholinesterase levels were determined by mixing 20 μL serum with 1000 μl reagent containing pyrophosphate (pH 7.6 95 mmol/L) and potassium hexacyanoferrate (III) 2.5 mmol/L then incubating them for 3 minutes. The solution was then added with 250 μl of butyrylcholine, and the absorbance was measured with a spectrophotometer at λ 405 nm.[9]

The Mann-Whitney test was utilized to evaluate the outcomes of demographic data, cognitive impairment, Parkinson's symptoms, and depressive symptoms between the control and agricultural workers groups. The t-independent test was performed to compare MDA and cholinesterase levels between the control and agricultural workers groups. Finally, the Spearman correlation test was employed to examine the correlation between the MDA and cholinesterase levels and cognitive impairment, Parkinson's symptoms, and depression in the agricultural workers' group. The significance level for all tests is $p<0.05$. This research has obtained ethical approval from the Faculty of Medicine, University of Jember (certificate number: 1641/H25.1.11/KE/2022).

Table 1 Subject Characteristics

	Control groups (n=30)	Exposed group (n=30)	p-value
Sex			
Male	30	30	1.000
Female	0	0	
Age (years old)			
20–40	11	4	
40–60	16	24	0.130
>60	3	2	
Education			
Uneducated	2	1	
Elementary-middle school	16	25	0.060
Senior high school	12	4	

Results

Table 1 details the demographics of the respondents in both groups. The two groups showed no significant differences in gender, age, and educational level. The results of the measurement of cognitive impairment, Parkinson's symptoms, and depression status are shown in Table 2.

More than half of the respondents in the exposed group experienced mild cognitive impairment, and some experienced severe cognitive impairment. In the control group, most respondents showed no cognitive impairment, a small number experienced mild cognitive impairment, and none experienced severe cognitive impairment. There are significant differences in cognitive impairment between the two groups. Most respondents showed no Parkinsonism hence the insignificant inter-group differences. Twenty-six percent of respondents from the exposed group experienced mild depressive symptoms and 6% had mild depression, while 90% of the control

Table 2 Cognitive Impairment, Parkinsonism, and Depressive Symptoms Among Respondents

	Control group (n=30)	Exposed group (n=30)	p-value
Cognitive impairment			0.003*
No cognitive impairment	19	9	
Mild cognitive impairment	11	16	
Severe cognitive impairment	0	5	
Parkinsonism			0.305
Normal	29	27	
Parkinson	1	3	
Depression status			0.026*
No depressive symptoms	27	20	
Mild depressive symptoms	3	8	
Mild depression	0	2	
Moderate depression	0	0	
Severe depression	0	0	

Noted: No cognitive impairment: 30-24 point of MMSE; Mild cognitive impairment: 23-18 point of MMSE; Severe cognitive impairment: 17-0 point of MMSE; Normal: 0-5 point of Chan's questionnaire; Parkinson: >5 point of Chan's questionnaire; No depressive symptoms: 0-4 point of PHQ; Mild depressive symptoms: 5-9 point of PHQ; Mild depression: 10-14 point of PHQ; Moderate depression: 15-19 point of PHQ; Severe depression: >20 point of PHQ

Table 3 Correlation between Neurotoxicity Biomarkers and Neurotoxicity Symptoms

Biomarker	Neurotoxicity symptoms	r	p-value
Cholinesterase	Cognitive impairment	0.422	0.020*
	Depression status	-0.075	0.695
MDA	Cognitive impairment	-0.487	0.006*
	Depressive symptoms	-0.015	0.937

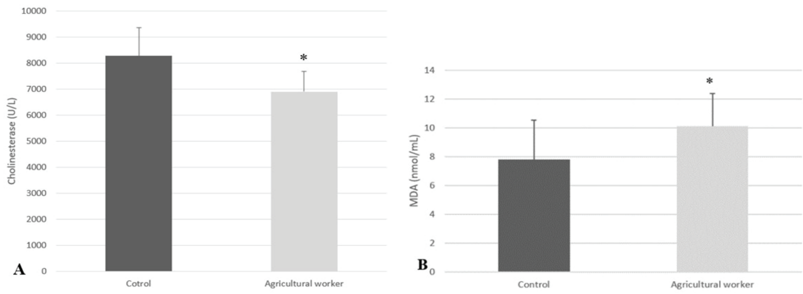

Figure 1 Differences in Cholinesterase Level (A) and MDA (B) between the Agricultural Workers and The Control Groups

group respondents had normal results, with the rest having mild depressive symptoms. The two groups showed significant differences in depressive symptoms. Figure 1 depicts the cholinesterase and MDA levels in both groups. The exposed group had lower cholinesterase levels than the control group (p=0.000). MDA levels were significantly higher in the exposed than in the control group (p=0.001).

The exposed group demonstrated a significant positive correlation between cholinesterase levels and cognitive measurements (r=0.422, p=0.02). A significant correlation was also evident between the MDA levels and cognitive impairment in the exposed group (r=-0.487, p=0.006). However, no correlations with depressive symptoms were found for both biological markers (p=0.695 and p=0.937 for cholinesterase and MDA, respectively) (Table 3).

Discussion

This study showed that agricultural workers had lower cholinesterase levels than the control group. However, this decrease was still within the normal range. This finding followed Malueka et al.[10] study that demonstrated lower cholinesterase levels among children exposed to pesticides. Likewise, research by Nepane *et al.*, on farmers in Nepal showed decreased plasma cholinesterase levels following the habitual spraying with organophosphate pesticides.[11] As pesticides inhibit acetylcholinesterase activity, this decreased cholinesterase level is expected. Pesticide compounds bind acetylcholinesterase which subsequently inactivates the enzyme.[12] As a significant amount of acetylcholinesterase binds to pesticides, free acetylcholinesterase level would decrease, hence the finding of its lower levels among agricultural workers than the control group.

This study also found significant differences in MDA levels between the exposed and the control group, with the exposed group having higher MDA levels. A previous study among farmers in Wonosari Village, Bondowoso Regency, demonstrated similar results.[13] Pesticide exposure at the cellular level might cause an imbalance between the pro-oxidant and antioxidant systems and increase the production of reactive oxygen species (ROS).[14] High MDA levels indicate lipid peroxidation due to increased production of ROS in pesticide exposure.[15]

The exposed group also showed a higher incidence of cognitive impairments than the control group. This finding was concordant with a study in South Korea which stated that farmers in rural areas experienced cognitive decline upon examination with the Korean-Montreal Cognitive Assessment (KMoCa).[16] Additionally, significant correlations by Spearman test were found between cognitive impairment and MDA and cholinesterase levels among agricultural workers. Pesticides that enter the body might induce oxidative stress, as evidenced by increased serum MDA levels among farmers who use pesticides.[15] An in-vivo study showed similar findings.[17] The presence of reactive oxygen species (ROS) caused by oxidative stress might lead to long-term potentiation (LTP) disorders as the basis for memory disturbance.[18] Research on experimental animals showed that pesticide exposure caused a decrease in cholinesterase levels that led to damages in the hippocampus complex, an area of importance in memory and cognition.[19]

Another finding of this study is that depressive symptoms were more prevalent among agricultural workers than the control group. This result is concordant with research in Madhya Pradesh, India.[20] However, no significant correlations were evident between depression and MDA and cholinesterase levels within the exposed group. This study's findings differ from research conducted in Iowa and North Carolina which showed a correlation between depression and acute and chronic pesticide use in farmers.[21] Conversely, this study agreed with a study in California that found no correlation between exposure to pyrethroid pesticides among residents living close to agricultural areas with mild and moderate depressive symptoms, as measured with the Geriatric Depression Scale short form-15 (GDS).[22] Depression is a very complex entity involving biological, social, and psychological factors. Biological factors thought to play a role include the involvement of the amygdala, hippocampus, neurotransmitter disorders, inflammation, oxidative stress, and genetic factors.[23] The insignificant correlations of this study might be due to highly-varied risk factors for depression that were not completely controlled in this study, such as psychological and socio-economic conditions. Another limitation of this study is the need for identification of the types of pesticides used.

This study showed no significant difference in Parkinsonism between agricultural workers and controls. This result differs from other studies, implying pesticides are a factor in the occurrence of Parkinson's among agricultural workers.[24] In both studies, the diagnosis was established clinically. Despite the validity and reliability of Chan's questionnaire for screening for Parkinsonism, direct clinical examination by clinicians might provide better results. The relatively small sample size of this study might also affect the results. Additionally, Parkinson's disease is rare and has various clinical manifestations. This was exemplified by a study by Del Brutto that recruited a sample of 642 residents working as carpenters with exposure to toxic materials while working, yet only found two subjects with Parkinson's disease.[25] Due to the negative health effects of pesticides, workers agricultural are advised to be careful and comply with the rules for pesticide use.

This study concludes that chronic pesticide neurotoxicity among agricultural workers in Jember might manifest as cognitive impairment and depression. The high MDA and low cholinesterase levels might serve as biomarkers of pesticide-induced neurotoxicity. Further research with a larger sample size, identification of the type of pesticide used and neurotoxicity symptoms, and more varied biomarkers of chronic neurotoxicity should be considered.

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