Putri *et al. BMC Pharmacology and Toxicology* (2025) 26:165
https://doi.org/10.1186/s40360-025-01013-y

BMC Pharmacology and Toxicology

RESEARCH

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Influence of chronic pesticide exposure on malondialdehyde and 8-OHdG levels among Wuluhan farmers, Jember, Indonesia: a cross-sectional study

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Abstract

Background Chronic pesticide exposure causes oxidative stress and DNA damage in humans, with long-term health implications. This study aims to assess chronic pesticide exposure with oxidative stress and DNA damage biomarkers among farmers in Wuluhan District, Jember, Indonesia.

Methods Seventy-four farmers from two villages in Wuluhan, Jember, were interviewed regarding pesticide exposure using a questionnaire, and serum MDA and urine 8-OHdG were examined. Thirty-six subjects from the first village had low-moderate intensity, and 38 subjects from the second village had high spraying intensity before the test. Data were analyzed by Spearman correlation tests between the chronic pesticide exposure index, MDA serum, and urinary 8-OHdG in each group.

Results All subjects in this study were classified as experiencing high chronic pesticide exposure. In farmers with a low-moderate intensity of exposure, the chronic pesticide exposure index was negatively correlated with MDA but not with the 8-OHdG level. In farmers with high spraying intensity before the test, 8-OHdG increased significantly compared to another group. In this group, most of the farmers with a working duration of less than 15 years had higher urinary 8-OHdG levels, suggesting impaired DNA repair and cellular adaptation after long-term pesticide exposure.

Conclusion Farmers experience high oxidative stress and DNA damage due to chronic pesticide exposure. Nevertheless, as the working duration with low-moderate intensity increases, the level of oxidative stress will decrease as cellular adaptation. At high spraying intensities of pesticides, urinary 8-OHdG significantly increases. 8-OHdG may be helpful as a biomarker of acute exposure to pesticides, and MDA can be used for evaluating chronic pesticide exposure.

Keywords 8-OHdG, DNA damage, MDA, Oxidative stress, Pesticide exposure

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Background

Agricultural workers are a vulnerable population exposed to pesticides. In agricultural practices in Indonesia, many farmers still need to apply good agricultural practices (GAP), especially in applying pesticides, so it is often done not according to procedures and results in health problems [1]. Several studies examining pesticide exposure's effects on Indonesian farmers' health have also revealed that farmers are vulnerable to chronic obstructive pulmonary disease, dermatitis, neurological complaints, and degenerative diseases such as hypertension, diabetes mellitus, and cancer [2–5].

Pesticides, one of the exogenous compounds often used in agriculture, are known to increase oxidative stress and lead to DNA damage [6, 7]. Oxidative stress is a condition of imbalance between the production and accumulation of oxidative compounds and the biological detoxification process in cells or tissues [8]. When there are many reactive oxygen species (ROS) in the body, mainly hydroxyl compounds, they will oxidize the bases that make up DNA, especially in guanosine-rich regions or promoter regions. This oxidation will cause several conditions, including base mutations and truncation of the oxidized bases, ultimately disrupting the transcription process that can affect gene expression. DNA damage accumulation leads to hyperglycemia, hypertension, neurodegenerative diseases, aging, immunodeficiency, and cancer [6, 9].

Malondialdehyde (MDA), due to lipid peroxidation due to ROS, is commonly used as a biomarker of oxidative stress. As a reactive oxygen metabolite (ROM), MDA can also bind to deoxyguanosine and deoxyadenosine in DNA to produce mutagenic DNA adducts [10]. The presence of a DNA adduct on deoxyguanosine initiating base excision that produces 8-OHdG. Various studies have reported the involvement and increased levels of 8-OHdG in people exposed to radiation and patients with cancer, neurodegenerative diseases, and metabolic diseases [11–13]. Therefore, this study assessed the relationship between chronic pesticide exposure through the pesticide exposure and MDA and 8-OHdG levels, which are biomarkers of oxidative stress and DNA damage, in farmers in Wuluhan District, Jember.

Methods

Study design and subjects

This study used a cross-sectional approach with purposive sampling method. Based on the standard deviation in the previous study [14], we calculated the sample size of our study with the Lameshow hypothesis test for population means. By the power of test 80%, the minimum sample size should be 67 subjects. We added 10% from the minimum value to anticipate exclusion after the data taken.

Seventy-four farmers involved in the study were taken from two villages at different times. The first group of 36 people was from Tamansari Village in October-November 2022, when the intensity of pesticide spraying was low-moderate. The second group of 38 people was from Lojejer Village in March 2023, when the spraying intensity was high near harvesting.The inclusion criteria were healthy adult farmers (>18 years old) who were exposed to pesticides at least one year before sampling and agreed to be a participant in the study by signing an informed consent. Exclusion criteria were no history of degenerative disease and alcohol consumption.

Assessment of chronic pesticide exposures

We collected data on age, length of time working as a farmer, frequency of spraying each month and its accumulation in a year by questioner in Bahasa. All the data mentioned were calculated by the following algorithm:

$$chronic\,pesticide\,exposure\ (CPE) = log10\left[\left(\frac{Y\ x\ D}{age-18}\right)+1\right]$$

where Y is the length of exposure to pesticides in years and D is the estimated number of days exposed to pesticides in one year. Index values of 0.698 to 2.757 or more are categorized as high exposure, and 0.697 to 0.00 are categorized as low exposure [15, 16]. We also collected sociodemographic profile and type of pesticide they used.

Biospecimen collection and storage

Blood sample

Experts carried out blood collection in the median cubital vein using a 22 G needle 5 mL syringe. Blood taken will be divided into two 2.5 mL red vacutainer tubes. The collected specimens were then stored in a cooler box with a temperature of 4–8 °C during transportation to the laboratory. In the laboratory, serum and sample aliquots are separated. The samples were then stored at -20 °C for analysis within 24 h.

Urine sample

Urine samples taken on the study subjects were spot urine. Urine was collected in 60 mL urine tubes. Each pot was labeled with the patient's identity, put into a cooler box equipped with a dark lining, and kept at a temperature of 4–8 °C from the time of collection and during transportation. After entering the laboratory, samples were stored in dark microtubes at -80 °C until the time of analysis.

Examination of serum MDA levels

One milliliter of serum was mixed with 2 ml of TCA (Sigma-Aldrich, USA)-TBA (Merck, USA)-HCl stock

solution and then vortexed. Heat the test tube using a bath for 15 min. Under hot and acidic conditions, malondialdehyde (MDA) reacts with TBA. Then, take the test tube and wait for it to cool down. Centrifuge 3000 rpm for 10 min, and take the supernatant using a micropipette. The absorbance of the supernatant was read on spectrometer (Genesys 100) at a wavelength of 535 nm. The absorbance reading results were then calculated using the formula obtained from the linear equation of the standard curve. Our standard curve for MDA was shown in Fig. 1.

Examination of urinary 8-OHdG level

The urine was removed from the refrigerator, then it was centrifuged for 20 min at 1000xg in 4 °C. The supernatant was used for ELISA Kit 8-OHdG (Catalog No : E-EL-0028, Elabscience, USA) and Jaffe method urine creatinine levels (Dialab, Austria). Procedure of examination based on the kit. The ELISA results are optical density (OD) data read by ELISA reader (Biopharm, Germany). The OD value of the standard was made into a 4-parameter logistic (4PL) curve through MyAssay.com to produce an equation with an R-value above 0.90. The standard curve for 8-OHdG was shown in Fig. 2. The OD values of the samples were input on the equation to determine the concentration of each sample. The results of the concentration calculation in ng/mL were then recalculated with the ratio of 8-OHdG concentration to creatinine. The final result of 8-OHdG concentration is expressed in ng/mg creatinine.

Statistical analysis

Characteristic of subject between two groups was analyzed with mann-whitney test for numeric variable and chi-square test 2 × 2 for categorical variable like smoking status (Yes or No) and PPE status (Using PPE and Not Using PPE).

Each data point will be tested with Spearman correlation test,. Analyses will also be conducted on each sample group using Mann Whitney test and Spearman correlation test. Statistical tests will be conducted with SPSS v26, and data will be presented in graphs using GraphPad Prism v9.5.

Results

Subject characteristics

This study was conducted in two villages in the Wuluhan district, Tamansari and Lojejer. These two villages are geographically close and have similar regional characteristics, so the agricultural commodities grown have almost the same cycle. It can be seen from the characteristics of the subjects who mostly use pesticides with almost the same commodity objectives, such as rice, corn, chili, and onions. The subject characteristics are listed in Table 1 and the type of pesticide they used are listed in Table 2.

All subjects in this study were male. Based on these characteristics, group B, on average, had a longer working period than group A, and more did not use PPE when applying pesticides. In addition, the median serum MDA and urinary 8-OHdG levels in the second group were higher than those in the first group, as shown in Fig. 3.

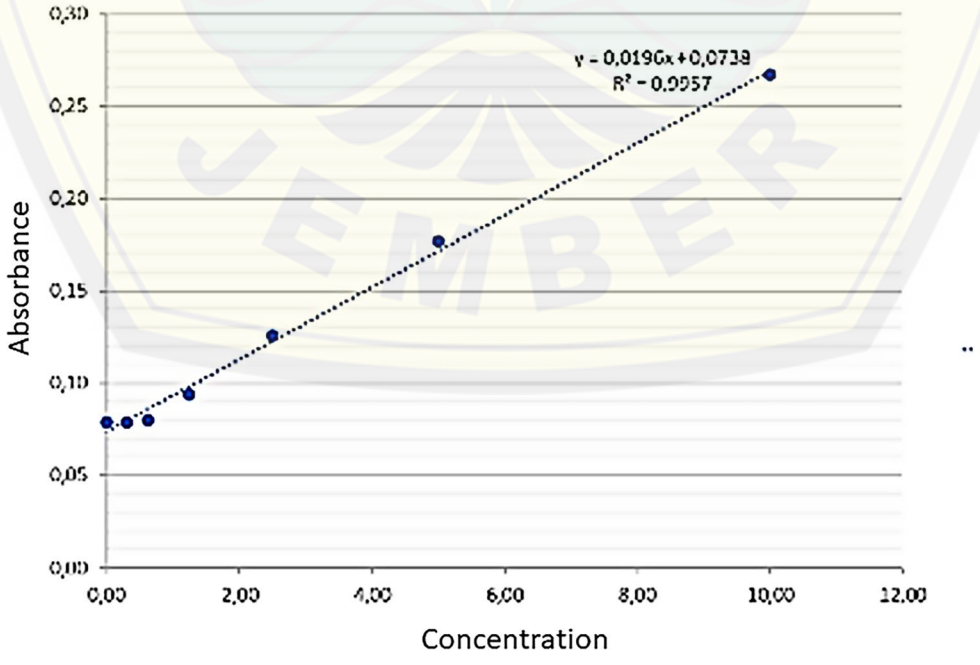

Fig. 1 The standard curve for serum MDA

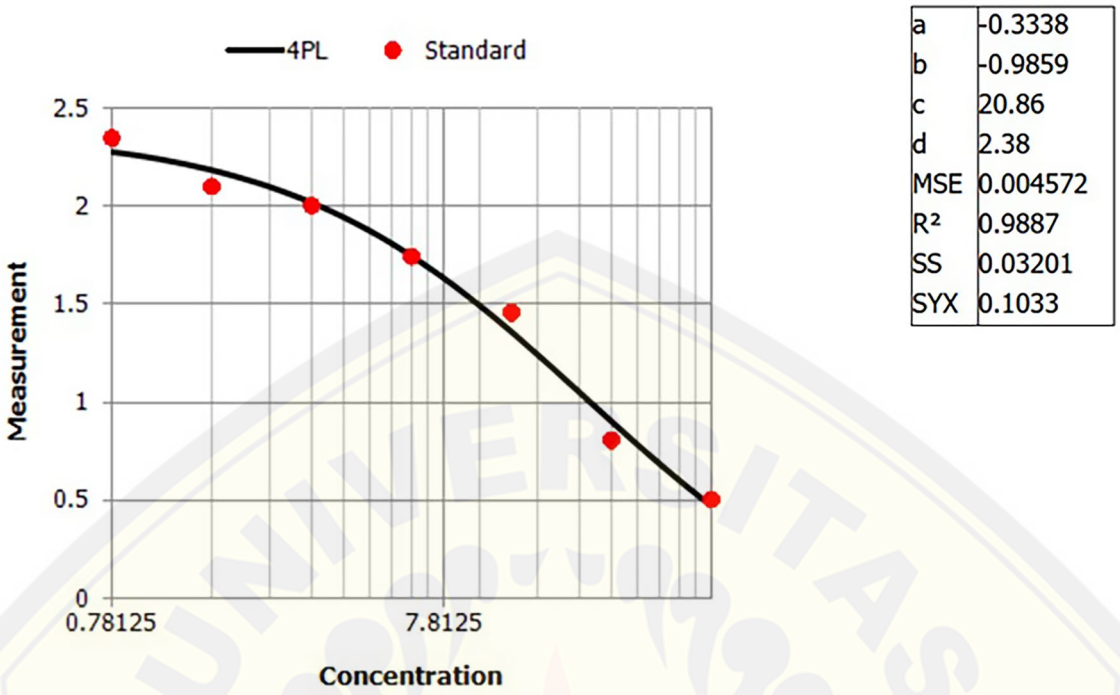

Fig. 2 The standard curve for urinary 8-OHDG

Table 1 Subject characteristic

Indicator	Grup A (*n* = 36)	Grup B (*n* = 38)	*p*
Age (y.o)	49 (23–70)	51 (30–74)	0,267
Length of works (year)	20 (4–40)	29 (5–50)	0,028**
Spraying frequencies in a year	48 (24–106)	36 (20–102)	0,151
Chronic pesticide exposure index	1,48 (1,02 – 1,99)	1,47 (0,88 – 2,01)	0,770
Serum MDA level (µmol/L)	9,48 (2,77 – 24,86)	10.43 (7,57 – 18,29)	0,040**
Urinary 8-OHdG level (ng/mg creatinine)	13,54 (4,7–87,10)	54,5 (6,11–163,27)	0,000**
PPE-status			0,030*
Using PPE	35 (97,2%)	31 (81,6%)	
Not Using PPE	1 (2,8%)	7 (18,4%)	
Smoking status			0,057
Yes	28 (77,8%)	22 (57,9%)	
No	8 (22,2%)	16 (42,1%)	

*) $p < 0.05$ significant by Chi-square test

**) $p < 0.05$ significant by Mann Whitney test

Correlation between chronic pesticide exposure index, MDA serum, and urinary 8-OHdG in the low-moderate spraying intensity group (Group A)

Based on the characteristics of the subjects, the chronic pesticide exposure index in Group A is higher than that in Group B because Group A has a median value of more spraying frequency in a year even though the median length of work is shorter. If the pesticide exposure index, MDA, and 8-OHdG levels are analyzed in group A, it will result in a correlation matrix, as shown in Table 3.

MDA levels in this group were negatively correlated with the chronic pesticide exposure index (R=-0.338; $p = 0.022$) and length of work as a farmer (R=-0.452; $p = 0.003$), indicating that chronicity without an increase in spraying intensity was negatively correlated with MDA levels in farmers. Urinary 8-OHdG levels did not correlate with the chronicity variable. The pattern of serum MDA and urine 8-OHdG levels against chronicity of pesticide exposure in Group A is shown in Fig. 4.

Correlation between chronic pesticide exposure index, MDA serum, and urinary 8-OHdG in the high spraying intensity group (Group B)

The relationship between chronicity parameters such as chronic pesticide exposure index, length of work, and frequency of spraying, none of which were significantly

Table 2 Type of pesticides used by subject

Type of pesticide	Chemical ingredient
Organophosphate	Chlorpyriphos
	Profenofos
Carbamate	Methomyl
	Carbofuran
	Fenubucarb
Neonicotinoid	Imidacloprid
	Dinotefuran
	Nitenpyram
Fungicide	Abamectin
	Emamectin benzoate
	Difenoconazole
	Mancozeb
	Propineb
	Tebuconazole
	Propiconazole
Pyrethroid	Cypermethrin
Oxadiazine	Indoxacarb
Ryanoid	Flubendiamide
Herbicide	Glyphosate
	Paraquat dichloride
	Atrazin

correlated to oxidative stress and DNA damage in this group. The graph in Fig. 5 shows a spike in urinary 8-OHdG levels, which then tends to decrease in farmers with longer working years, while serum MDA is persistently high. This may be due to high exposure to pesticides before sampling. In addition, in the second group that experienced high-intensity pesticide exposure, MDA levels were negatively correlated with 8-OHdG levels (R=-0.293; p = 0.037). The correlation matrix of Group B is shown in Table 4.

Table 3 Correlation matrix in Group A

Indicator	MDA	8-OHdG
MDA	1	
8-OHdG	0.097	1
Chronic pesticide exposure index	-0.338*	0.159

*) $p < 0.05$, significant by spearman correlation test

Discussion

In our study, there were significant differences in MDA and 8-OHdG levels between the first and second subject groups, suggesting the possibility that exposure time or the last exposure dose may have a role in increasing the levels of both biomarkers, especially 8-OHdG levels in urine. Our findings concord with research published by Kaur et al. in India in 2011, where in the case of acute exposure, there was a significant increase in DNA damage compared to the time of low-dose exposure 5–6 months later using comet tail examination [17]. In addition, a study on the effects of nanoparticle exposure on the increase in oxidative biomarkers in the urine of photocopy workers showed that 8-OHdG levels increased the fastest in the first 6 h after exposure compared to other biomarkers and began to decrease until the third day of examination. In chronic exposure, 8-OHdG increased significantly compared to the control, but there was no significant increase every week, and it even tended to settle down [18].

Out of the two groups, the effect of chronicity of pesticide exposure was only apparent in the MDA levels of Group A, who were exposed to low-moderate intensity before sampling. In this group, MDA tended to decrease as pesticide exposure increased. Our finding is in line with in vivo organophosphate exposure studies in rats that also showed that chronic exposure to pesticides for 90 days decreased MDA levels compared to those on days 15 and 30, and the effect was independent of the

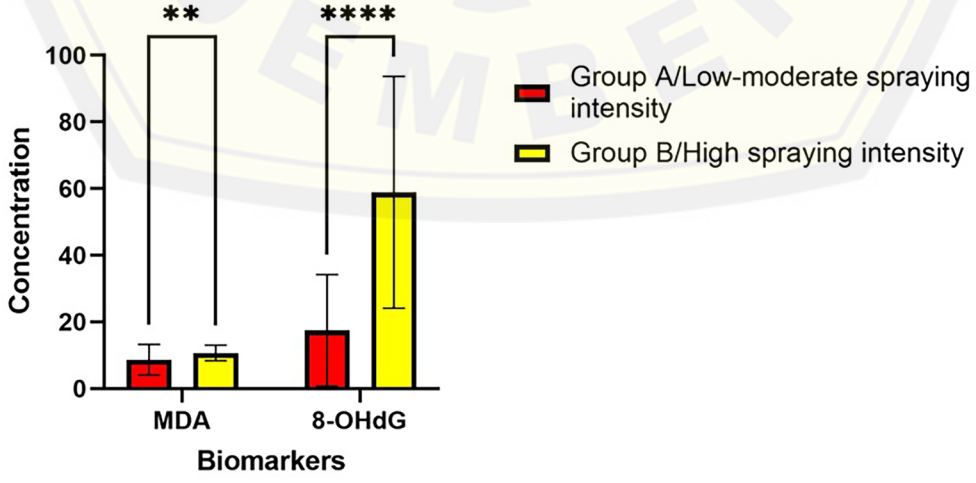

Fig. 3 Differentiation MDA level and 8-OHdG between low-moderate spraying intensity (Group A) and high spraying intensity (Group B)

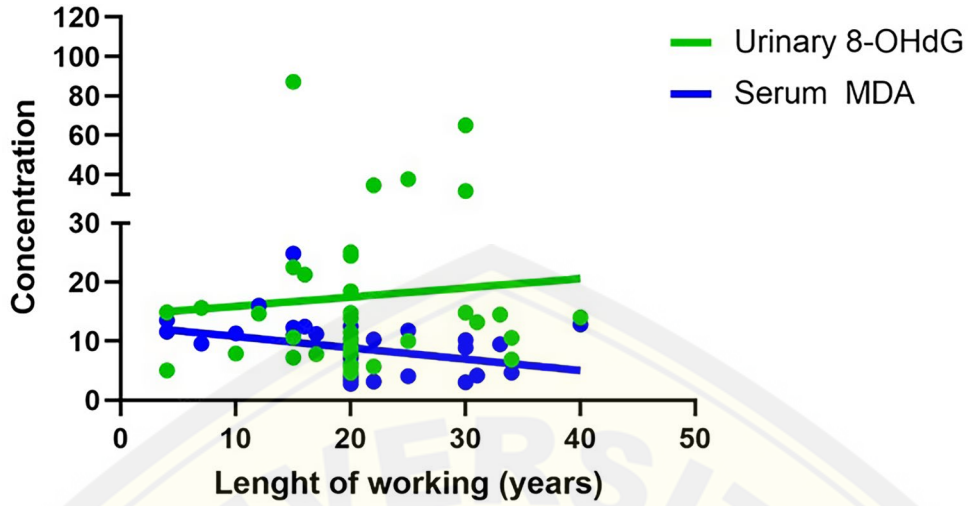

Fig. 4 Linear trend MDA serum and urinary 8-OHdG to the length of working in Group A

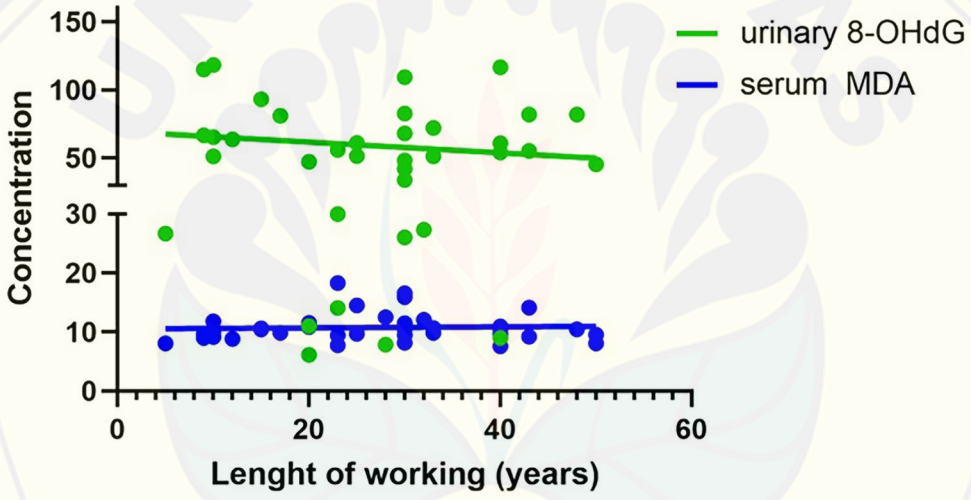

Fig. 5 Linear trend MDA serum and urinary 8-OHdG to the length of working in Group B

Table 4 Correlation matrix in Group B

Indicator	MDA	8-OHdG
MDA	1	
8-OHdG	-0,293*	1
Chronic pesticide exposure index	0,064	-0,085

*) $p < 0.05$, significant by spearman correlation test

dose of organophosphate given. This indicates the body's compensatory mechanism against oxidative stress [19]. While the levels of 8-OHdG in each group did not show a significant relationship with chronicity, only Group B, which received high-intensity exposure before sampling, showed that urinary 8-OHdG levels tended to be high in workers less than 20 years and then tended to decrease. The trend pattern indicates the possibility of a decreased DNA repair ability in farmers with longer pesticide exposure. The relationship of chronicity with biomarkers of oxidative stress and DNA damage under acute and chronic exposure conditions is summarized in Fig. 6.

In the second group with high spraying intensity near harvest, there was a negative correlation between serum MDA biomarkers and urine 8-OHdG levels. This may occur because the two biomarkers have different speeds in responding to oxidative stress. The Griseofulvin toxicity test using hepatic cells showed that MDA would increase faster in acute conditions and reach its highest point in the first week, then tend to settle down and begin to decline in week five or subchronic conditions. In the same study, 8-OHdG levels derived from cells only increased significantly in subchronic exposure and tended to remain at that level. In contrast, 8-OHdG levels from hepatic mitochondrial preparations alone increased in second week [20]. In addition, 8-OHdG is also more rapidly excreted, so in acute exposure, it tends to increase sharply [18]. However, no other study has compared the

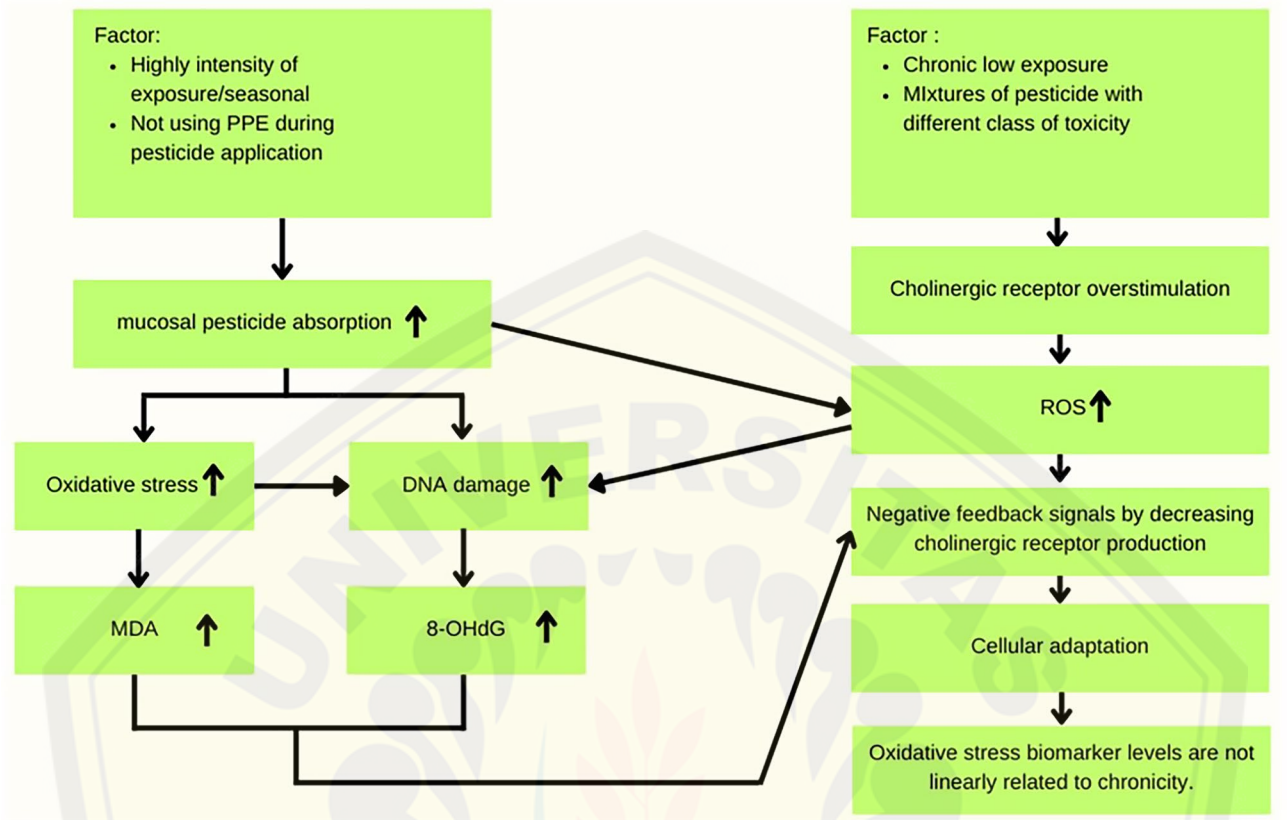

Fig. 6 Schematic of the relationship between pesticide exposure and oxidative stress in farmers. The left diagram shows the relationship between acute or sub-chronic exposure with increased levels of MDA and 8-OHdG as biomarkers of oxidative stress. In contrast, the right-side diagram shows the relationship between chronic exposure to low doses of pesticides with oxidative stress conditions. These two diagrams will be interconnected if there is acute exposure to farmers chronically exposed to pesticides

release rate of MDA in serum with 8-OHdG in urine, so these two biomarkers may run independently.

Our study did not have a control group, so the correlation pattern does not mean causality. But, we revealed how pesticides impact stress oxidative regulation in farmers. It could have contributed to managing the health impact of pesticides. We recommend routinely monitoring pesticide usage and making interventions to protect them from illness in the future.

The limitation of our study is that we cannot measure pesticide residue in the subject as concrete evidence of pesticide exposure. Therefore, we cannot distinguish whether pesticides are the only factors that affect MDA and 8-OHdG or if there are other factors that influence chronic stress oxidative levels, like smoking and nutritional status. Other factors that may influence oxidative stress conditions in farmers, such as behavior and hygiene, behavior patterns before, during, and after spraying, the last day of spraying before the sample was taken, polymorphism analysis of the subject mainly related to genes involved in xenobiotic metabolic processes, oxidative stress regulation, and DNA repair, need to be further elaborated to answer questions that still cannot be answered in this study.

Conclusion

The chronic pesticide exposure index negatively correlated with MDA but not 8-OHdG in low-moderate spraying intensity farmers. However, although they had different response rates, MDA and 8-OHdG significantly increased when there was acute exposure. 8-OHdG could be helpful as a biomarker of acute pesticide exposure, and MDA could be used for monitoring the effect of long-term pesticide exposure. Routinely monitoring the health of farmers can prevent illness in the future. We recommend that the government make a policy to protect our farmers from excessive pesticide exposure. Given the limited scope of molecular epidemiology research on pesticide exposure, further studies are required to validate the novel findings presented in this study.

Abbreviations

8-OHdG	8-hydroxy-2′-deoxyguanosine
MDA	Malondialdehyde

Acknowledgements
We would like to thank the Agricultural Department and Health Department in Jember, who gave us permission and support to perform our study.

Author contributions
ERMP made substantial contributions to the study design, research process, and data analysis, and AHS made substantial contributions to the research idea and process. ERMP and AHS were involved in preparing the manuscript and revising the intellectual content. S made substantial contributions during the research process, interpreting the data, and preparing the manuscript. All authors read and approved the final manuscript.

Funding
This research was supported by the Educational Fund Management Institution (LPDP), Ministry of Finance, Republic of Indonesia as postgraduate research.

Data availability
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical approval
The sampling and research were conducted under ethical approval No. 1641/H25.1.11/KE/2022 and No. 1746/H25.1.11/KE/2023 issued by the Ethics Committee of the Faculty of Medicine, University of Jember. Each subject received an explanation of the study and agreed to participate by signing an informed consent. All methods were conducted in concordance with relevant guideline and regulation.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Received: 13 July 2023 / Accepted: 17 May 2024
Published online: 15 October 2025

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