Chronic pesticides exposure on the farmers in Tanjung Wangi Village: Focus on Alpha-Synuclein as a Biomarker of Parkinson-sporadic

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Abstract. The study aim was to analyze the effect of chronic pesticide exposure on farmers in Tanjung Wangi village. Observational study with male adult farmer as respondents. 100 questionnaires were given to farmers and 56 returned. The average age of respondents was 46.5 years, education was primary school, length of work/day was 4-6 hours, length as farmer was more than 10 years. The results were no respondents have Parkinson symptoms with a median score were 57, 61, and 67.5 on 1 to 5, 5 to 10, and more than 10 years pesticide exposure respectively. From 56 respondents selected 30 persons randomly for physical examination (PE) and blood examination Parameters measured were PE, concentration of glutathione-total and alpha-synuclein in erythrocyte membranes. Data analysis was performed with IBM SPSS 21. The results were no respondents have digestive and balance disorders. Alpha-synuclein concentrations were not significantly different between the length of exposure as well as total glutathione concentrations. PE significant correlation with alpha-synuclein (rho=0.366, p=0.044). Pesticides cause oxidative stress in erythrocytes. Alpha-synuclein has the potential as a biomarker for Parkinson-sporadic disease.

1. Introduction
According epidemiology data, Parkinson disease increase sharply and will double in 2030 [1]. The largest number PD was in Asian countries, including Indonesia. Parkinson disease were common in areas exposed to pesticides, such as agricultural and plantation areas [2].

The ethology of Parkinson disease (PD) is not yet clear. One of risk factor of PD was exposed to neurotoxins, could be drugs, metals, pesticides, etc. The previous study with animals models were found that Parkinson phenomenon occurs in rats induced by rotenone, a natural pesticide [3]. Thus, it could be said that exposure to pesticides in humans might cause disease too, especially Parkinson-sporadic [3].

Pesticides are use commonly, such as in: a). Houses: use of pesticides to suppress the growth of mosquitoes, cockroaches and termites; b) yard: use of pesticides to suppress the growth of weeds, insects; c) Agriculture, the use of pesticides to suppress the growth of insects, rats, weeds. In general, pesticides become needs that cannot be abandoned, both in housing and agriculture. Because they
assume that pesticides could increase agricultural productivity, so that could increase quality of life too [4]. Therefore, the use of pesticides increase without regulation, they not realize, that is poison cause disease, even death [5].

Pesticides exposure will cause cell damage while its mechanism depends on the type of pesticide. The pesticides commonly to used are organochlorine, organophosphate and carbamate. The organophosphate pesticides damage the nervous system at low doses [6]. Diazinon, chlorpyrifos, parathion, and dimethoate are pesticides from organophosphate which are suspect to cause Parkinson disease [7]. This mechanism is unclear, might be through the xenobiotic pathway that causes free radicals to increase and oxidative stress occurs [7,8]. Early detection is carried out before Parkinson-sporadic clinical symptoms appear because clinical symptoms will detect after dopaminergic neurons die more than 70% [9,10].

Tanjung Wangi is a village in west Java as one of agricultural centre which its product such as: corn, cassava, sweet potatoes, bananas, tomatoes, chili, carrots, beans, broccoli, etc. Tanjung Wangi farmers as the subject in this study who were exposure chronically to pesticide. They depend on pesticides for economic reasons without consider health impacts [4]. Was chronic exposure to pesticides affect their health? Were in this village found the farmers with Parkinson-sporadic symptoms? Could Parkinson-sporadic symptoms be known earlier? The purpose of this study was an early detection of Parkinson-sporadic disease in farmers in Tanjung Wangi Village who were exposed to chronic pesticides.

2. Methods
Observational study with respondents were farmers from Tanjung Wangi Village. This study have ethics clearance from Ethics Committee of the Faculty of Medicine, Bandung Islamic University (No 362/ Ethics Committee.FK/IV/2018) and a recommendation letter from Nation Unity Agency and Politics, West Java (070/1009 Rekomlit/ KesBak /2018).

2.1. Respondents
Male adult farmers, and at become farmer more than one year. Respondents were asked to fill out informed consent, questionnaires, and blood collection.

2.2. Questionnaire
The respondents filled out three types of questionnaires. First was self-data questionnaire, second was questionnaire on pesticide use, and third was PDQ39 questionnaire-modification.

2.3. Physical examination
Physical examination were carried out based on the results of all questionnaire that had been filled. Physical examination performed by doctors who are members of this study and were assisted by alumni doctors from Faculty of Medicine, Bandung Islamic University. Physical examination was carried out including weight and height, blood pressure, physical balance and digestive disorders.

Measurement of Glutathione-total and Alpha-Synuclein Proteins: Blood collection were carried out based on the results of all the questionnaire that had been filled. Blood collection is carried out by expert from the Clinical Pathology Laboratory, Hasan Sadikin Hospital (RSHS). Blood samples were brought to Biomolecule Laboratory, Clinical Pathology, RSHS, red blood cells were isolated and fractionated. Measurement of glutathione-total and alpha-synuclein in erythrocyte membranes. Glutathione-total measurement was in accordance with E-BC-K097 Kit and alpha-synuclein measurement accordance to E. EL Kit. H0983.

2.4. Data analysis
Data obtained were analysed using IBM SPSS 21 software.
3. Results

3.1. Farmers in Tanjung Wangi Village
The number of questionnaires distributed was 100 pieces, did not return 15 pieces, 20 pieces were returned blank, 9 pieces were incomplete and filled 56 pieces. The average age of respondents was 46.5 years (figure 1.A). Education mode of respondents with elementary school (figure 1.B). Mode of the work length/day of respondent was between 4-6 hours (figure 1.C). Mode of the length as farmers was less than 10 years (figure 1.D).

![Figure 1](image)

**Figure 1.** Demographic data of farmers in Tanjung Wangi Village who were exposed to pesticides Age of respondents were less than 25 years, between 25 to 50 and more than 50 years (A). Education of respondents were not education, elementary, junior, and high school (B). The work length of the respondent were 2-3 hours, 4-6 hours, 7-9 hours (C). The Length as farmers of respondents were between 1-5 years, 5-10 years and greater than 10 years (D).

3.2. Plants and pesticides used
In general, respondents planted such as: tomatoes, chillies, mustard green, broccoli, beans, carrots, red beans. The harvest period was between two to three months with the average pesticide spraying time once a week. The most widely used fungicide are Victory Mix, Antracol, Dakonil, and Arsenal. The most commonly used insecticides are Prevathon, DuPont, Besvidor, Demolish and Cypermethrin.

3.3. Parkinson symptoms
Median score of modified PDQ 39 questionnaires in group 1, 2, and 3 were 57, 61 and 67, respectively (figure 2.A). The Highest score was 104 in group 3, but the lowest score was 42 in group 1, 2, and 3.

3.4. Physical examination
There were 30 respondents who participated in the physical examination that were from group1 was nine people, group 2 was six person, and group 3 was 15 people. The results showed that all respondents did not experience balance or digestive disorders (figure 2.B). The highest score in group 3.
The results of questionnaires, physical examinations, and blood tests of farmers in Tanjung Wangi Village. The average score of PDQ39-modification, the lowest score on group 1, the length of farmer between one until five years (A); The average score of physical examination, the lowest score on Group 1, the length of farmer between one until five years (B); The average of glutathione-total concentration, the lowest concentration on Group 2, the length of farmer between five until ten years (C); The average of alpha-synuclein concentration, the lowest concentration on group 2, the length of farmer between five until ten years (D).

3.5. Alpha-synuclein protein
The average of alpha-synuclein protein concentration in erythrocyte membranes in group 1, 2, and 3 respectively were 11.39; 11.36; 11.41 pg/L (p=0.325) (figure 2.C). The highest concentration in group 3 and the lowest in Group 2.

3.6. Glutathione-total
The average glutathione-total concentration in erythrocyte membranes in group 1, 2, and 3 respectively were 23.39; 23.36; 23.41 µg/L (p=0.355) (figure 2.D). The highest concentration in Group 3 and the lowest in group 2.

The correlation between PDQ39 questionnaire with physical examination (PE) was positively correlated with rho = 0.227 (p=0.229), and with alpha-synuclein concentration was positive correlation with rho = 0.243 (p=0.196). While glutathione-total was negatively correlated with rho = -0.170 (p=0.369). Physical examination (PE) was positively correlated with glutathione-total concentration with rho=0.057 (p=0.727), while with alpha-synuclein concentration positively correlated with rho=0.366 (p=0.044). Glutathione-total concentration was positively correlated with alpha-synuclein concentration with rho=0.044 (p = 0.817).

4. Discussion
The modified PDQ 39 questionnaires became 42 questions [4] divided into questions about mobility, daily activities, emotions, disability, family support, communication, discomfort [11] and added questions about digestive disorders to see the onset of Parkinson's disease. The results of this study
indicate that Parkinson's symptoms were not found in Farmer in Tanjung Wangi Village, because the average score was less than 84 points, although symptoms such as muscle pain were found in some respondents but were not specific symptoms of Parkinson's disease. Pesticides will enter cells through oxidative stress pathways [8,12,13]. Increased free radicals will oxidize biomolecules randomly and a lot of factors must be considered.

Physical examination of farmers in Tanjung Wangi Village is mainly emphasized on the possibility of digestive and balance disorders which are the initial characteristics of Parkinson-sporadic symptoms. Common digestive disorders are difficulty defecating (bowl movements) or constipation. This is closely related to the process of the occurrence of the 10th cranial nerve, which regulates the innervation of the digestive system [14]. The clinical symptoms of Parkinson-sporadic are tremor, muscle stiffness, slow motion, and impaired stability when standing and walking [15,16] In this study no specific symptoms of Parkinson-sporadic disease were found. It was found that respondents experienced muscle stiffness, and joint pain, but were not followed by other signs. It is suspected that exposure to pesticides causes oxidative stress which causes inhibition of the action of the enzyme acetyl cholinesterase which is closely related to cholinergic syndrome [13,17].

Blood tests are important for diagnosing various diseases, both infectious, degenerative and genetic disorders. In this study, the examination of glutathione-total and alpha-synuclein protein could be increase in farmers who were exposed to pesticides chronically through oxidative stress pathways [8]. In this study, glutathione-total and alpha-synuclein protein showed no significant difference between exposure between 1-5 years, 5-10 years, and greater than 10 years. Exposure to pesticides causes oxidative stress and cells could homeostasis to handle oxidative stress. Cell reactions to oxidative stress conditions are very diverse and dependent on the sensitivity of the cell itself.

One component of blood that can be used as a sign that a degenerative process is occurring is erythrocytes [18]. The first thing to know is the main function of erythrocytes, which is to resemble the oxygen needed for each cell that builds an individual. The function of erythrocytes depends on the metabolism that occurs in the cell so that oxidative stress conditions will also determine the speed of metabolism [18].

In this study no research was conducted yet on the types of pesticides directly related glutathione-total and alpha-synuclein proteins. In general, the pesticides used are to eradicate fungi, weeds, and insects. Frequently used fungicides have cymoxanil active compound, as 2-Cyano-N- (ethylcarbamoyl)-2-methoxyiminoacetamide and its safe for the environment.

5. Conclusion
Pesticides commonly used to kill plant pests such as fungi, weeds and insects with the use of one spray per week for approximately 6-9 hours. The pesticides used by farmers in Tanjung Wangi Village are generally classified as safe for the environment. The duration of pesticide exposure does not affect glutathione-total concentration in the membrane. Alpha-synuclein protein is found in membrane erythrocyte. This indicates the transmission of alpha-synuclein from neurons to erythrocytes. This finding needs further research to be developed to determine the biomarkers of neurodegenerative diseases.

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