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Cognitive Disorders with High Beta Amyloid Levels in Farmers using Organophosphate Pesticides

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One of the famous tourist destinations in Bali is Jatiluwih terrace rice in Tabanan regency, with beautiful rice paddies view. Many smallholders are exposed to chronic organophosphate due to the process of mixing pesticides, spraying, and cleaning in a minimum period of 2 years. This study used a consecutive case control plan for farmers who used chronic organophosphate pesticides in the village of Penarukan Kerambitan Tabanan from January to March 2019. A total of 66 study subjects aged 40-60 years were exposed to pesticides and met the eligibility criteria. This study showed subjects with serum beta amyloid levels of more than 112.03 ng/L had a 15 times risk of experiencing neurocognitive impairment compared to subjects with serum beta amyloid levels below 112.03 ng/L and statistically significant (95% CI 3.882-60.969; p<0,001). 29 times at risk of causing neurocognitive dysfunction after adjusting for age, education and gender. The duration of exposure = 10 years is at risk of causing neurocognitive impairment of 2.6 times compared to the duration of exposure of less than 10 years (95% CI 0.95-7.63; p=0.005).

Keywords: Organophosphate Pesticides; Amyloid Levels; Cognitive Disorders.

One important sector in the Balinese economy is agriculture, but an increase in agriculture is correlated with an increase in the use of pesticides. Long-term exposure to pesticides has a negative impact on health and is thought to cause neurocognitive impairment.

Previous studies on animal models with the administration of deltamethrin and carbofuran once a day for a period of 28 days, reported a decrease in spatial memory function, decreased synaptic protein N-Methyl-D-Aspartate receptor 1 (NR 1), synaptophysin and synapsin 1, genes cAMP Related Element Binding (CREB)[1]. A meta-analysis study reported an association between exposure to chronic organophosphate pesticides and decreased neurocognitive function in workers[2].

Exposure to pesticides is thought to interfere with beta amyloid homeostasis, resulting in an increase in beta amyloid in the cortex and hippocampus resulting in memory impairment and decreased motor activity[3]. Oxidative stress causes an increase in the neuroinflamation response and progressive loss of neuronal cells and synapses. Exposure to pesticides also causes deposition of senile plaque beta amyloid protein in cerebral blood vessels associated with the incidence of dementia.

In this study it was reported that there is a risk of long-term use of organophosphate pesticides

in farmers in the Kerambitan area of Tabanan in the province of Bali with the risk of neurocognitive dysfunction especially memory and executives in chronic organophosphate pesticide users associated with serum beta amyloid levels.

MATERIAL AND METHODS

This study used a consecutive case-control design for farmers who were chronically exposed to organophosphate pesticides in Penarukan Kerambitan Tabanan village from January to March 2019.

This study was conducted on 66 subjects aged 40-60 years who were exposed to pesticides and met eligibility criteria. All research subjects were interviewed by the research team regarding demographic aspects, pesticide use activities, and neurocognitive function examinations were carried out.

Exposure to chronic pesticides in a minimum of 2 years with a history of direct exposure to pesticides through the process of mixing pesticides, spraying pesticides, when cleaning pesticide applicator[4]. Neurocognitive function is the ability to think which consists of five cognitive domains, namely attention, language, memory, visuospatial and executive functions. The neurocognitive function here is performed using a mini cog test. A mini cog test score of less than 3 indicates impaired neurocognitive function. A mini cog test score of more than or equal to 3 indicates normal neurocognitive function. The serum beta amyloid level was measured by taking a blood sample of 2 milliliters and collected in a bottle with anti-coagulant ethylenediaminetetraacetic acid (EDTA). The examination method uses the Enzyme-Linked Immunosorbent Assay (ELISA) system with a 470 reader 270 Biomerieux washer in 2002 in the Clinical Pathology laboratory of Sanglah Central General Hospital. Serum beta amyloid levels were assessed in units of ng / L. Determination of the limit of normal serum beta amyloid levels was done statistically using the Receiver Operating Characteristic (ROC) method and assessing the Area Under the Curve (AUC) method. The threshold value of serum beta amyloid levels used in this study was 112.03 ng/L with a sensitivity of 90% and specificity of 70%. Groups with high serum beta amyloid levels (=112.03 ng/L) and groups with normal serum beta amyloid levels (<112.03 ng/L). Data analysis using SPSS version 21.0 for Windows includes descriptive analysis, bivariate analysis to calculate Odds Ratio (OR), with statistical significance determined based on the p value, declared significant if $p<0.05$, and multivariate analysis.

RESULTS

This study used 66 research subjects which were then classified into two groups, namely impaired neurocognitive function (case group) and normal neurocognitive function (control group). Research subjects were dominated by women (n=40; 60.6%) and most of the study subjects were 60 years old (n=26; 39.4%). Fifty-nine percent of research subjects with educational background graduated from elementary school. Most research subjects do not smoke and do not consume alcohol. Exposure to chronic organophosphate pesticides is the length of exposure to pesticides obtained during life. Mini cog aims to evaluate neurocognitive function globally and obtained a range of scores

Table1. Participants Characteristics

Characteritics		Cases	Controls
		n (%)	
Age (years)		60 (40-60)	54 (40-60)
Gender	Male	12 (36.4)	14 (42.4)
	Female	21 (63.6)	19 (57.6)
Education (years)		6 (0-12)	6 (0-12)
Exposure (years)		15 (5-40)	5 (2-15)
Mini Cog Score		2 (1-2)	3 (3-5)
Serum Beta Amyloid Levels (ng/L)		160,25 (79.94-3035.90)	109.88 (49.24-156.79)

between 1 to 5. The range of serum beta amyloid levels is quite wide, which is between 49.24 ng/L – 3035.90 ng/L. The basic characteristics of the research subjects are presented in table 1.

The relationship between each demographic characteristic of the study subjects and neurocognitive function is presented in table 2. The age variable uses a 55-year cut point determined based on the median value of all study subjects. The analysis showed the OR value of age was 0.371 with a significance value that was not significant. The gender variable showed an OR of 0.776 with a significance value that was not significant. Educational variables, smoking, and alcohol also did not show significant difference in influencing neurocognitive function.

The ROC curve showed that the serum beta amyloid level had an AUC value obtained at 88.9% (95% CI 0.806-0.972, p<0.001). The results of the ROC coordinates showed the threshold value of the serum beta amyloid level used in this study was 112.03 ng/L with a sensitivity of 90% and specificity of 70%. Research data were classified into groups with high serum beta amyloid levels (=112.03 ng/L) and groups with normal serum beta amyloid levels (<112.03 ng/L). The relationship of serum beta amyloid levels with organophosphate exposure is shown in table 3. The analysis showed that exposure to lama 10 years risk of causing neurocognitive impairment of 2.6 times compared to the duration of exposure less than 10 years (95% CI 0.95-7.63; p=0.005) * OR= odds ratio

The relationship of serum beta amyloid levels with neurocognitive function is presented in table 4. The results of the analysis showed subjects with serum beta amyloid levels of more than 112.03 ng/L had a 15-fold risk of causing neurocognitive impairment compared to subjects with serum beta amyloid levels below 112.03 ng/L and statistically significant (95% CI 3,882-60,969; p<0.001).

Variables included in multivariate analysis were duration of exposure to pesticides, serum beta amyloid levels, age, and education. Based on the results of multivariate analysis the variables that influence neurocognitive function are serum beta amyloid levels with adjusted OR values of 29.581, p <0.001 95% CI 4.754-184.046 and duration of pesticide exposure with adjusted OR values of 21.514, p<0.001 95% CI 4.219-109.707.

Table 2. Relationship of Subjects Characteristics to Cognitive Function

Characteristics		Cases n(%)	Controls	OR (95% CI)	p-value
Age (years)	< 55	13 (39.4)	21 (63.6)	0.371	0.084
	≥ 55	20 (60.6)	12 (36.4)	(0.137-1.005)	
Gender	Male	12 (36.4)	14 (42.4)	0.776	0.614
	Female	21 (63.6)	19 (57.6)	(0.288-2.087)	
Education	≤ 6	27 (81.8)	20 (60.6)	2.925	0.057
(years)	> 6	6 (18.2)	13 (39.4)	(0-948-9.028)	
Smoking	Yes	7 (21.2)	7 (21.2)	1.00	0.805
	No	26 (78.8)	26 (78.8)	(0.307-3.255)	
Alcohol	Yes	2 (6.1)	2 (6.1)	1.000	1.000*
	No	31 (93.9)	31 (93.9)	(0.132-7.555)	

*tested with*Fisher's exact*; OR= odds ratio

Table 3. The Relationship Between Chronic Organophosphate Exposure and Serum Beta Amyloid Levels

Characteristics		Beta amyloid levels (%) High	Low	OR(95% CI)	p-value
Peticides	≥ 10 years	29 (67.4)	10 (43.5)	2.693	0.005
exposure	< 10 years	14 (32.6)	13 (56.5)	(0.95-7.63)	

Table 4. Relationship of Beta Serum Amyloid Levels with Neurocognitive Function

Characteristics		Cases n (%)	Controls	OR 95% CI	p-value
Serum Beta Amyloid Levels (ng/L)	High Normal	30 (69.8) 3 (13.0)	13 (30.2) 20 (87.0)	15.3 (3.882-60.969)	<0.001

* OR= odds ratio

DISCUSSION

This study shows that the proportion of male and female study subjects who experienced neurocognitive impairment compared to subjects without neurocognitive impairment showed no clinical significance. The relationship between age and neurocognitive function has been proven theoretically. Based on this the age of the subjects in this study was made to be homogeneous and limited to 40 to 60 years to reduce the possibility of bias in the analysis process. The difference between the gender between cases and controls was not clinically significant. Educational variables that are clinically significant affect neurocognitive function but are not statistically significant. This result is also supported by previous studies which showed that the level of education was not significantly significant in pesticide users in influencing neurocognitive function[5].

The duration of exposure received by research subjects throughout their lives varied from 2 years to 40 years with an average duration of exposure of 12.98 years. The duration of organophosphate pesticide exposure of more than or equal to 10 years significantly increases the risk of serum beta amyloid levels by 2.6 times (95% CI 0.95-7.63; p=0.005).

An in vivo study in 2011, found that organophosphate exposure can cause metabolic disorders related to amyloid-ß homeostasis, thereby causing an increase in amyloid-ß levels in the cortex and hippocampus[3]. Organophosphate exposure also causes deposition of amyloid ß senile plaque protein in cerebral blood vessels so that it is responsible for causing dementia[6].

Another study in 2019 by Jaymie *et al*. in rats given organophosphate exposure for eight months the results of high beta amyloid levels were found in the cortex and hippocampus area. This increase was significant in the two types of beta amyloid both A1-40 and A1-42 in the cortex and beta amyloid A1-42 in the hippocampus.

The pathophysiology of organophosphate exposure causing an increase in beta amyloid levels has not been described in many previous studies. Organophosphate compounds induce the formation of free radicals through increased oxidative stress. Amyloid beta protein was found to increase with increasing oxidative stress. Exposure to chronic organophosphate pesticides triggers the formation of reactive oxygen and nitrogen, then damages the neuronal cell lipid membrane and changes the composition of the neuron cell lipid membrane. In addition, chronic organophosphate exposure also causes iron metal accumulation through the Fenton reaction and subsequently is involved in the formation of free radicals[7].

Increased oxidative stress will trigger the formation of beta amyloid protein and vice versa aggregation of beta amilod protein will increase oxidative stress through increased levels of lipid peroxidation products including malondialdehyde, 4-hydroxynonenal (HNE) and acrolein[8]. These toxic products are formed as a result of changes in the cellular structure of neuron cells due to an increase in oxidative stress[7]. Increased formation of free radical species results in an imbalance of apoptotic proteins namely Bax protein and anti-apoptotic Bcl-2, Bcl-xL which alter the composition of the mitochondrial membrane and facilitate the release of cytochrome C and activation of neuronal apoptotic pathways[9].

Chronic organophosphate exposure will activate glia cells, macrophages, and oligodendrocytes thus triggering an inflammatory response. Activation of these cells then triggers the release of pro-inflammatory cytokines in the brain, such as interleukin (IL) -1ß, IL-18 and IL-33. The release of proinflammatory cytokines will

then facilitate the formation and deposition of beta amyloid[10].

The pathophysiology of beta amyloid causing neurocognitive impairment has been described. Amyloid beta is a major component of amyloid plaque, a plaque found in patients with Alzheimer's, dementia Lewy bodies as well as other dementias[11]. Beta amyloid can also form aggregates that line blood vessels in the brain called amyloid cerebral angiopathy[12,13].

Beta amyloid formed after successive division of APP (Amyloid Protein Precursor). The increase in APP results in a number of neuritic plaques, slows the learning process and shows memory impairment in line with an increase in the amount of amyloid. The anatomical pathology of beta amyloid in humans and rats is no different, namely there is an aggregation of beta peptide amyloid which is hydrophilic 38-43 amino acids. Most beta amyloid is found in the frontomedial cortex, hippocampus and enthorinal cortex as well as the occipital cortex region[14].

The results of this study showed subjects with high serum beta amyloid levels had a 15 times risk of causing neurocognitive impairment. This is consistent with previous research by Harrington *et al*. in 2017 in 335 subjects aged 60-85 years. All subjects were positron emission tomography (PET) scans and neurocognitive function examinations followed for 72 months. Subjects were categorized as Aß +, ie the value of SUVR / Before the Centiloid Kernel Transformation =1.5 based on PET scan results and Aß-, namely the value of SUVR / Before the Centiloid Kernel Transformation <1.5. The results of this study indicate the Aß + group has a longer response time in completing the attention function and processing speed checks.

A 54-month hospital-based cohort study conducted by Pietrzak *et al*. in 2014 in 333 healthy adults. This study aims to determine changes in beta amyloid evaluated by PET scans associated with changes in neurocognitive function. This study concludes that an increase in beta amyloid levels is associated with cognitive decline globally (Cohen's value d=0.78, 95% CI 0.33-1.23), language (Cohen's value d=0.51, 95% CI 0.07-0.96), and executive function (Cohen's value d=0.39, 95% CI 0.05-0.83).

Another study also evaluated the relationship between beta amyloid levels and neurocognitive function in normal individuals. The study was conducted on 907 individuals aged over 40 years. The results of this study obtained a correlation between positive beta amyloid (beta amyloid levels in cerebrospinal fluid or on abnormal PET scans) with a decrease in delayed memory function and immediate memory (Auditory Verbal Learning Test), attention and executive functions (Trail Making Test A and B)[15].

CONCLUSION

The study concluded that exposure to chronic organophosphate pesticides for more than 10 years, 2.6 times the risk of causing high serum beta amyloid levels. Subjects with serum beta amyloid levels of more than 112.03 ng/L had 15 times the risk of causing neurocognitive impairment or 29 times the risk of causing neurocognitive impairment after adjusting for age, education, and gender.

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