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Association between serum cholinesterase levels and body composition in vegetable farmers assessed by bioelectrical impedance analysis

Hubungan kadar kolinesterase serum dan komposisi tubuh pada petani sayuran menggunakan bioelectrical impedance analysis

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Abstract

Exposure to all pesticide classes can have a disruptive effect on metabolism and energy storage. The bioindicator that can be used to identify farmers exposed to pesticides is acetylcholinesterase (AChE). AChE can be a good indicator for changes in body composition due to its associations with various metabolic and physiological parameters. Abnormal AChE levels stimulate nicotinic and muscarinic receptors in various organs, causing a wide range of metabolic disorders, including changes in the body composition. This study aimed to examine the correlation between long-term pesticide exposure and potential alterations in body composition. This research was conducted in Sagarahiang village, one of the highest vegetable producers in Kuningan, West Java on July-August 2024. And it was a cross-sectional study of 46 farmers who sprayed pesticides in vegetable areas and was determined by purposive sampling. Data collected through interview using questionnaires and some tools, like indicators of pesticide exposure were measured by cholinesterase serum and history of exposure, whereas parameters of body composition were measured by bioelectrical impedance analysis (BIA) using a body fat monitor with eight electrodes. Data analysis using the Pearson correlation test. The percentage of subjects with abnormal AChE activity was four persons (8,9%). AChE levels had no correlation with all components of the exposure history, but had a significant correlation with body composition parameters (p<0,05), body weight (p=0,027), Body Mass Index (BMI) (p=0,020), total fat (p=0,038), visceral fat (p=0,030), and resting metabolism (p=0,037) with a weak correlation (r= 0,31-0,34). In conclusion, AChE is not strong enough to assess changes in body composition clinically.

Keywords: AChE, BIA, body composition, pesticide exposure, vegetable farmers

Abstrak

Pajanan pestisida pada semua golongan memiliki efek mengganggu metabolisme dan penyimpanan energi. Bioindikator yang dapat digunakan untuk mengidentifikasi petani terpajan pestisida adalah asetilkolinesterase (AChE). AChE dapat menjadi indikator yang baik dalam perubahan komposisi tubuh karena erat kaitannya dengan berbagai parameter metabolisme dan fisiologis. Kadar AChE yang tidak normal menstimulasi reseptor nikotinik dan muskarinik di berbagai organ tubuh, menyebabkan berbagai macam gangguan metabolisme termasuk perubahan komposisi tubuh. Tujuan penelitian ini adalah menguji korelasi antara pajanan pestisida jangka panjang dan potensi perubahan komposisi tubuh. Penelitian ini dilakukan di Desa Sagarahiang, salah satu penghasil sayuran tertinggi di Kuningan, Jawa Barat pada Juli – Agustus 2024dan merupakan penelitian cross sectional dengan 46 petani yang melakukan penyemprotan pestisida di lahan pertanian sayuran yang ditentukan secara purposive sampling. Pengumpulan data dengan wawwancara menggunakan kuesioner, dan beberapa peralatan seperti indikator pajanan pestisida diukur dengan serum cholinesterase dan riwayat pajanan, sedangkan parameter komposisi tubuh dengan bioelectrical impedance analysis (BIA) menggunakan body fat monitor dengan delapan elektroda. Analisis data dilakukan dengan uji parametrik korelasi pearson. Subjek dengan AChE abnormal sebanyak 4 orang (8,9%). Kadar AChE tidak berkorelasi dengan semua komponen riwayat pajanan, namun berkorelasi signifikan dengan parameter komposisi tubuh ($p<0,05$), yaitu berat badan ($p=0.027$), Index Massa Tubuh (IMT) ($p=0.020$), lemak total ($p=0.038$), lemak visceral ($p=0.030$), metabolisme istirahat ($p=0.037$) dengan kekuatan korelasi yang lemah ($r= 0,31-0,34$). Kesimpulan, AChE tidak cukup kuat untuk dapat menyimpulkan adanya dampak klinis perubahan komposisi tubuh.

Kata Kunci: AChE, BIA, komposisi tubuh, pajanan pestisida, petani sayuran

Introduction

Pesticide exposure has harmful effects on humans, especially spraying farmers, as a high-risk population in agricultural areas (Damalas & Koutroubas, 2016). All classes of pesticides (organophosphate, carbamat, pyrethroid, and organochlorin) are Metabolism Disrupting Chemicals (MDC) (Cander & Yetkin, 2023). MDC are environmental chemicals(Heindel et al., 2017)MDC are categorized as obesogenic and diabetes agonists; they can increase body weight, blood glucose, and insulin resistance despite increasing physical activity and limiting calorie intake (Cander & Yetkin, 2023).

Pesticides are persistent and bioaccumulate in various organs (Kalyabina et al., 2021). Pesticides with different active ingredients and concentrations are found in the human body. At low concentrations, pesticides can have chronic effects through bioaccumulation mechanisms, leading to outcomes in the future. Studies in Vietnam, India and China have found urinary levels of pesticide metabolites ranging from 28,9 to 13,6 ng/ml(Li & Kannan, 2018). In addition, organophosphate residues (malathion, parathion and chlorpyrifos) in the blood and urine of cotton farmers in Pakistan and Cameroon are known to cause metabolic disorders, including obesity and dyslipidaemia(Javeres et al., 2021).

Pesticide exposure also has potentially disruptive effects on metabolism and energy storage, such as changes in body composition. Pesticides, even at low doses, can bioaccumulate in the environment and within organisms, leading to significant health concerns, including metabolic disorders (He et al., 2020). This occurs through cholinergic mechanisms (Glover, Eisenberg, et al., 2022). Pesticides act as *acetylcholinesterase* (AChE) inhibitors to convert acetylcholine (ACh) to choline and acetate. Excess ACh at central and peripheral levels stimulates the activation of nicotinic and muscarinic receptors in various organs, causing complex metabolic disorders (Adeyinka et al., 2020).

Several cholinergic mechanisms, including hormonal interactions such as insulin, affect weight control (Zhu et al., 2019). The hypothalamus regulates the metabolism and storage of energy such as glucose, lipids, carbohydrates, and proteins and affects the increase in body fat or decrease in muscle mass (Glover, Eisenberg, et al., 2022). The gastrointestinal tract delay the absorption of nutrients from the intestine into the bloodstream (Ito et al., 2019). In addition, Excess ACh affect nerve and muscle function, potentially causing muscle weakness (Abdel et al., 2021; Aroniadou-Anderjaska et al., 2023).Pesticide use in agricultural fields can reduce serum cholinesterase levels and indicate pesticide poisoning among farmers (Budiawan, 2014). Therefore, one bioindicator that can be used to identify farmers exposed to pesticides is AChE (Shentema et al., 2020). Abnormal AChE levels cause a wide range of metabolic disorders via mechanisms such as adipose tissue dysfunction, inflammation (Adeyinka et al., 2020) and changes in body composition (How et al., 2020). In previous studies cholesterol, TGL and LDL levels were significantly increased in those with low AChE levels compared to those with normal AChE levels. Therefore, AChE may act as a potential biomarker in predicting the chronic effects of pesticide exposure in the occurrence of several components of metabolic syndrome (Kshatri et al., 2022). This study aimed to examine the correlation between long-term pesticide exposure and potential body composition alterations in vegetable farmers. It is expected that there is a significant positive correlation between acetylcholinesterase

(AChE) enzyme levels in the body and changes in body composition, where increased AChE levels are expected to be associated with positive changes in body composition indicators, such as increased muscle mass or decreased body fat percentage.

Methods

This study was conducted in Sagarahiang, one of the most vegetable-producing villages in Kuningan, West Java, Indonesia. Estimated study duration for 2 months in July – Agustus 2024. This was an analytical observational study with a cross-sectional design. The target population in this study was all vegetable farmers, but the target population consisted of farmers who sprayed or intensively used pesticides in vegetable areas. The sample size of about 46 farmers with subjects was determined by stratified random sampling to identify farmers who intensively using pesticides, due to sample selection based on regional strata and randomised to be involved in the study. Sample must fulfil the following inclusion criteria: 1) male, main group who sprayed and exposed to pesticides; 2) age above 30 years; 3) farming is a major occupation and has no potential exposure to pesticides from work other than as a farmer; 4) not suffering from serious illness; 5) ability to understand speak in Bahasa or Indonesian language and communicate well; 6) body condition eligible for venous blood sampling; and 7) willingness to follow the entire process of research activities. ,

Indicators of pesticide exposure measured by cholinesterase (AChE) serum and history of exposure.The history and characteristics of pesticides used by farmers were measured using a questionnaire. In this study, the instruments for measuring cholinesterase levels and body composition have used laboratory equipment that regularly undergoes periodic calibration tests, in order to maintain the validity of measurement results.

Serum AchE was analyzed in Semarang city laboratory using a semi-automatic spectrophotometer Stardust MC 15 through kinetic method. The first procedure was to collect venous blood samples of as much as 3 cc by medical laboratory staff Kuningan, who has a professional registration certificate. Blood samples were centrifuged at 2000 rpm to separate serum and plasma. A minimum of 200 μL of serum was required for analysis in the laboratory and then immediately delivered on the same day with standardized packaging techniques, thereby reducing the potential of damaging samples during transportation. Serum was examined using two reagents that previously held at room temperature for 30 minutes before used in analysis process. Blood collection was taken in a relaxed condition, no need to fast and well hydrated, blood collection taken in the morning before they did a lot of physical activity, in good health, as well as not taking any medications. This procedure was carried out to prevent things related to the test results.

Meanwhile, parameters of body composition were measured by Bioelectrical Impedance Analysis (BIA) or Biological Resistance Method using the body fat monitor brand Omron with eight electrodes type HBF-375 Karada Scan. HBF-375 was chosen due to the advantage of a larger number of electrodes. The 8 electrodes available (4 on the hands and 4 on the feet) increase the ability to measure body composition, more body composition items can be measured, as some BIA devices may only measure total fat and total muscle mass, not the distribution of visceral, subcutaneous fat and muscle mass in each limb.Standard Operating Procedures (SOPs) used to ensure more consistent BIA results including measurement time in the morning after waking up, before a lot of strenuous activity. The BIA is placed on a hard surface not on a mattress or carpet, hands and feet must in dry condition, not wet or wearing socks. The BIA calculates body composition by estimating the resistance value. Endurance was measured using a low voltage electric (50 kHz, 500 μA) through both hands and feet, which is not a stimulant and is very safe for the body. Several tissues with higherwater content in the human body tend to conduct electricity easily (such as muscles and blood vessels). Fat tissues rarely conduct electricity (Lohman et al., 2019). The body composition components measured included body weight, Body Mass Index (BMI), resting metabolism, total percent fat or whole-body fat, visceral and subcutaneous fat, skeletal muscle, and body age.

The research data were analyzed using the SPSS 25 software for Windows. Before data analysis was carried out, some data needed to be converted, and cut-off points or threshold values, such as serum cholinesterase levels categorized as normal, when AChE levels ranged from 4620 to 11500 U/L. This reference range of refers to the laboratory and analytical method used. The types and characteristics of pesticides used were based on the Insecticide Resistance Action Committee (IRAC), World Health Organization (WHO), Fungicide Resistance Action Committee (FRAC), and Integrated Risk Information System (IRIS). All data were analyzed descriptively and are presented in tabular and diagrammatic forms. The normality test value p=>0,05 using Saphiro-Wilk (n=46). Correlation analysis was performed using the Pearson correlation test with a confidence level (CI) of 95% and a significance level of p <0,05. The confounding factors of this study were controlled for by design, such as the sex of all subjects, however other factors such as diet, physical activity, or socioeconomic index were controlled in this study. This study involve six data collectors in the interview process. The data collectors had previously received training in data collection for a week. All participants received complete information about all stages of the study, knew the risks and gave written consent. Researchers applied ethical principles by maintaining data confidentiality, providing referral services to the nearest primary health care if side effects occurred. This research was approved by the Research Ethics Commission of the Institute of Health Science Kuningan, (number 64/EP/STIKKU/2024).

Result and Discussion

The mean age of the research subjects was 54 years (SD 10,78); the youngest age was 28 years, and the oldest was 74 years (Table 1). According to the Law of the Republic of Indonesia Number 13 Year 2003, the productive age of labour is 15-64 years old. where the population at that age has good physical ability to do a job. Based on these results, there were subjects who had an age exceeding the productive age. All subjects in this study were male, allowing them to work longer hours on agricultural fields despite their older age. Moreover, most of the subjects did not have any jobs other than working in the fields.

Subjects have lived for generations in the same location, so they have generally lived in the location for a long time, which is a central agricultural location, and worked as farmers from a young age. The mean of time living of the subjects was 50,4 years. According to this study, subjects were born, lived, and worked on the farm for a long time, so there was a high opportunity to be exposed to pesticides from the land. Farmers may be exposed to higher amounts of pesticides than those in other groups. Farmers who mix, transport, and spray pesticides can be exposed to chemicals due to spills and splashes, direct contact with pesticide sprays due to the use of damaged or poorly used personal protective equipment (PPE) (Liem et al., 2021) or lack of protective equipment (Setyowati et al., 2023). Farmers sometimes mix pesticides without using gloves, spray downwind, or spray while smoking cigarettes (Ibrahim & Sillehu, 2022).

Generally, farmers planted at least 1–6 vegetables, with a mean total of 2–3 types. The most common vegetables were chillis, tomatoes, spring onions, and cabbage. Spraying is applied according to the type of vegetable; however, in the rainy season, it increases three times a week for chilli and cabbage.

Table 1. Subject characteristics

Variable	Minimum	Maximum	Mean ± SD
Age (years)	28	74	54,40 (10,78)
Cholinesterase (U/ml)	2335	12635	7830,49 (1985,07)
Body weight (kg)	44,10	84,90	60,07 (11,61)
Height (cm)	142	172	160,07 (6,63)
Body mass index (kg/cm^2)	18,00	31,90	23,33 (3,76)
Total fat (%)	10,20	31,30	22,86 (6,22)
Visceral fat (%)	2,00	21,50	9,91 (5,26)
Resting metabolism (kal)	1144	1797	1425,11 (175,84)
Body age (years)	18	72	47,85 (12,31)
Subcutaneous fat whole body (%)	7,10	39,00	16,39 (5,42)
Subcutaneous fat trunk (%)	5,60	21,50	14,15 (4,32)
Subcutaneous fat arm (%)	11,40	28,40	20,81 (5,10)
Subcutaneous fat leg (%)	10,40	29,00	20,65 (5,79)
Skeletal muscle whole body (%)	23,40	37,10	29,3 (3,06)

Skeletal muscle trunk (%)	17,70	32,70	23,27 (3,88)
Skeletal muscle arm (%)	26,80	42,40	35,60 (2,81)
Skeletal muscle leg (%)	16,20	54,20	46,07 (5,42)
Time living (years)	7	74	50,40 (14,63)
Total of vegetables	1	6	2,56 (1,22)
Amount of pesticide used each farmer	1	20	7,78 (4,97)
Insecticide	Not used	13	4,76 (3,37)
Fungicide	Not used	9	2,78 (2,41)
Herbicide	Not used	5	0,18 (0,77)
Adhesive	Not used	3	0,47 (0,86)
Type of pesticide used			
Organophosphate	Not used	5	1,71 (1,63)
Yes	31 (68,9%)		
No	14 (31,1%)		
Carbamat	Not used	5	1,11 (1,35)
Yes	24 (53,3%)		
No	21 (46,7%)		
Piretroid	Not used	7	2,00 (1,93)
Yes	31 (68,9%)		
No	14 (31,1%)		
Organochlorine	Not used	1	0,02 (0,14)
Yes	1 (2,2%)		
No	44 (97,8%)		
Others	Not used	9	2,58 (2,30)

Pesticide use in vegetables is higher than in other plant species. Moreover, in the study location, many farmers have quite large agricultural land reach 202,8 ha for 46 subjects interviewed. The large number of plants and the large amount of land they owned were related to the amount of pesticides used, which reached 284 litres every growing season. Organophosphates were more likely to be used during the dry season, and carbamates during the wet season.

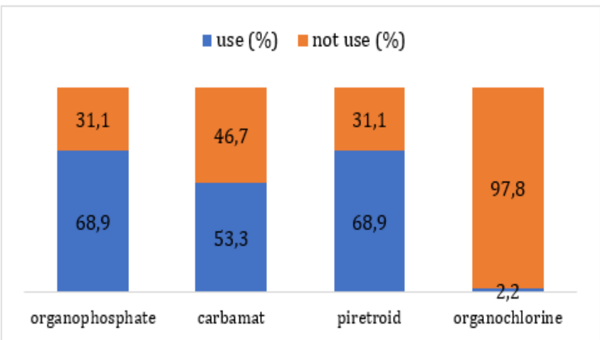

Figure 1. Type of pesticide used by vegetable farmers

The amount of pesticides used by each farmer was 1–20 types, with an average of 7–8 types. This number represents a wide variety of active ingredients, volumes, doses, and mixing activities of pesticides used by farmers. An increase in the volume of pesticide mixtures will increase the cumulative exposure level received by farmers (Liem et al., 2021) and the possible chronic effects that will be received.

Generally, the pesticide exposure that took place at the study site was chronic, which occurred as a result of repeated exposure for a period of months or years (repeated long-term exposure, which may extend for the duration of the organism's existence) (Damalas & Koutroubas, 2016). Routes of pesticide exposure to vegetable farmers are generally through respiratory and dermal routes, where for each type and active ingredient used with toxicity levels in class II - Ib, which is moderately toxic to highly toxic, can already provide adverse health effects even in low amounts or doses.

Chronic toxicity refers to a pesticide's capacity to induce harmful health consequences over a prolonged duration, typically following repeated or continuous exposure, which may persist throughout the lifespan of the affected organism (Damalas & Koutroubas, 2016). Chronic effects can develop owing to the role of pesticides as AChE inhibitors. Pesticides that are commonly used by farmers are organophosphate and carbamate, based on the chemical composition and characteristics of the active ingredients, which have irreversible behavior in disrupting AChE to break down acetylcholine (ACh) into choline and acetate. This is known as the cholinergic mechanism, which increases the amount of ACh that cannot be broken down in the body. ACh is a neurotransmitter that spreads throughout the body at both central and peripheral locations. High levels of ACh cause overstimulation of nicotinic receptors (nAChRs) and muscarinic receptors (mAChRs) in various organs, such as the hypothalamus, gastrointestinal system, heart, and blood vessels (Adeyinka et al., 2020). The stimulation of increased nAChR and mAChR with changes in body composition occurs mainly in the hypothalamus and gastrointestinal tract. The hypothalamus plays a role in regulating the metabolism and storage of energy such as glucose, lipids, and proteins. As there are many stimulation pathways that regulate metabolism in this organ, it is possible to have a wider influence on metabolic processes (Glover, Steenland, et al., 2022). In the gastrointestinal tract, it delays the absorption of nutrients through the intestine (Ito et al., 2019).

In this study, the serum cholinesterase levels were mostly normal, and only 8,9% of the subjects had abnormal AChE levels (figure 2). This value based on the group of farmers who were chronically exposed to pesticides or potentially exposed to pesticide poisoning (Wicaksono et al., 2023). However, based on the characteristics of the farmers' body composition, it can be seen that the main finding is the high total fat, with the dominance of fat stored as visceral fat, with the percentage of very high (22,2%) and high (17,8%) visceral fat, with an average of 9,91%. However, the BMI scores of the obese and overweight farmers were 4,4% and 26,7%, respectively. The BMI score was sufficient to describe the presence of visceral fat in the body, which is generally found in the abdomen and surrounding vital organs. (Lopez-Yus et al., 2024). Therefore, a person with high visceral fat is not always visible or easily observed physically, but high visceral fat leads to changes in body composition, especially body weight and BMI (Santillana et al., 2023). Additionally, the percentage of skeletal muscle was low (84,4%). This could be linked to the excess accumulation of acetylcholine in synapses, glands, smooth muscle, and motor end plates, where cholinergic receptors are found. These manifestations are due to overstimulation (characterized by myoclonic jerks, fasciculations, and muscle spasms) followed by weakness that may lead to paralysis (Miyairi et al., 2024; Zhao et al., 2024).

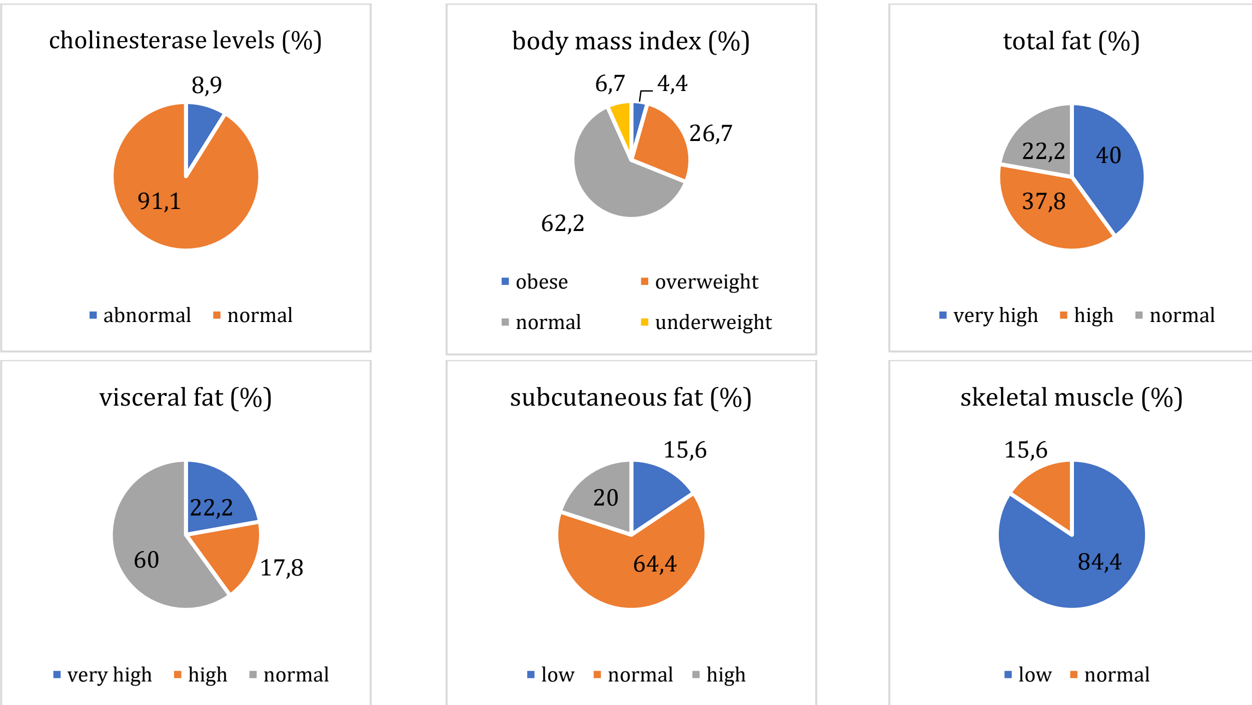

Figure 2. Body composition characteristics in vegetable farmers

The results of the correlation test with the Rank Spearman Test are shown in Table 3. AChE levels had no correlation with all components of the exposure history, but had a significant correlation with body composition parameters with p value <0,05, that is, body weight, BMI, percent fat, percent visceral fat, and resting metabolism with weak correlation (R 0,311-0,346). The resulting correlation coefficient has a positive correlation, meaning that the association between the two variables will move in the same direction. For example, when one variable increases, the other variable will also increases. In this study, an increase in AChE level was also followed by changes in body composition, including increases in body weight, body mass index, total fat, visceral fat, and resting metabolism. Another finding in this study was a significant correlation between AChE level and history of exposure, especially for other types of pesticides used by farmers, with a p-value of 0,015 (<0,05) and a positive correlation (R 0,361). This means that an increase in the type of pesticides used by farmers other than the four groups (organophosphate, carbamate, pyrethroid, and organochlorine) will also increase the serum AChE level. However, the results of weak correlations may not be strong enough to reach significant conclusions regarding the relationship between AChE and body composition or AChE and history of exposure. Consequently, despite the results being statistically significant, their impact may not be clinically significant.

Similarly, a study in male gave similar results including serum cholinesterase had a significant association and a very weak correlation with BMI (Oda, 2015). Farmers exposed to these pesticides show significantly lower AChE levels, which correlate with various self-reported symptoms such as muscle weakness (Manyilizu et al., 2019). On the other hand, studies in agricultural communities showed better results with a significant association between AChE levels and metabolic health including BMI, skeletal muscle mass, body fat mass and visceral fat (p<0,05 dan koefisien korelasi kuat R>0,6)(How et al., 2020).

Table 2. Correlation between cholinesterase levels with history of exposure and potential body composition alteration

variable	R	p value
Body Composition Alterations		
Body Weight	0,329*	0,027
Body Mass Index	0,346*	0,020
Total Fat	0,311*	0,038
Visceral Fat	0,325*	0,030
Resting Metabolism	0,312*	0,037
Body Age	0,279	0,063
Subcutaneous Fat	0,194	0,201
Skeletal Muscle	-0,152	0,319
Time Living	-0,018	0,904
Total of Vegetables	0,112	0,464
History of Exposure		
Amount of Pesticide	0,041	0,788
Insecticide	0,016	0,917
Fungicide	0,153	0,315
Herbicide	-0,162	0,289
Adhesive	0,129	0,398
Organophosphate	-0,004	0,980
Pyrethroid	0,083	0,587
Carbamate	-0,072	0,638
Organochlorine	0,025	0,869
Others	0,361*	0,015

Pearson Correlation *significant (p<0,05)

The alteration in body composition had a correlation with AChE levels. Excess ACh stimulates nicotinic and muscarinic receptors, resulting in an increase in intracellular Ca^{2+}, thus activating nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) (Cestonaro et al., 2022; Liu et al., 2017). The activation of NF-κB leads to disruption of cytokine production. The levels of pro-inflammatory factor cytokines such as interleukin 6 (IL-6) and Tumor Necrosis Factor α (TNFα) increase, and this is a marker inflammation in the human body (Liu et al., 2017). Inflammation also occurs in the adipose tissue, especially in the white adipose tissue (WAT) of visceral fat. WAT is an important endocrine organ that produces adipokines and cytokines (Lopez-Yus et al., 2024), which play an important role in lipid metabolism, energy storageand body weight homeostasis (Nunn et al., 2022) Visceral fat undergoes hypertrophy and hyperplasia, which is characterized by an increase in fatty acids (triglycerides) stored in various organs. Hypertrophy and hyperplasia contribute to adipose tissue dysfunction (Lopez-Yus et al., 2024). This leads to obesity, with a preceding increase in visceral fat, total body fat, body weight, and body mass index.

In this study, as a limitation of the study, other factors that may affect AChE levels and body composition were not controlled either by design or by analysis, including diet, physical activity, or socioeconomic index, although gender was controlled by design. On the other hand, the small sample size and narrowness of the study location may not be good for generalising the results of the study. Limited data analysis by not controlling for confounding variables either through multivariate analysis or sensitivity tests and data stratification.

Conclusion

Long-term pesticide exposure (through serum AChE measurement) may contribute to metabolic alterations in body composition, but in contrast, AChE levels had no correlation with all components of the exposure history, especially pesticide types other than the four groups (organophosphates, pyrethroids, carbamates, and organochlorines). Changes in body composition may occur due to the disruption of lipid metabolism.

The results of this study provide important information The results indicate a weak correlation between AChE levels and metabolic alterations in body composition, suggesting that further studies are needed to confirm AChE as a reliable biomarker. Cross sectional studies have not been able to determine causal relationships, therefore research needs to be developed with other better research designs to examine causal relationships. Potential bias in self-reported history of exposure needs to be strengthened by direct observation and further clinical diagnosis using biomarkers of pesticide metabolites.

It is recommended to implement better pesticide management practices, promote the use of protective equipment, and establish regular health monitoring programs for farmers to assess pesticide exposure and metabolic health.

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