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Research Article

Association between butyrylcholinesterase enzyme activity and HbA1c Levels in diabetic patients exposed to pesticides residues through fruit and vegetable consumption

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ABSTRACT

Exposure to pesticide residues in fruits and vegetables has been associated with alterations in butyrylcholinesterase (BChE) enzyme activity, a potential biomarker of chemical exposure. This cross-sectional study investigated the association between BChE enzyme activity and glycated hemoglobin (HbA1c) levels among diabetic patients in Khun Si Subdistrict, Sai Noi District, Nonthaburi Province. A total of 126 diabetic individuals from eight villages participated in the study. Data on fruit and vegetable consumption behaviors and adherence to antidiabetic medication were collected using structured questionnaires. BChE enzyme activity was measured using cholinesterase reactive paper kits from capillary blood samples, and HbA1c levels were obtained from patient medical records. Statistical analyses, including Chi-square and Fisher's Exact tests, were used to examine associations among variables. The results showed no statistically significant association between fruit and vegetable consumption behaviors and BChE enzyme activity ($p > 0.05$). Likewise, BChE activity was not significantly associated with HbA1c levels ($p > 0.05$), and no significant relationship was found between types or patterns of antidiabetic medication use and glycemic control. Given the limited sample size (n=126) and cross-sectional design, these preliminary findings suggest that BChE enzyme activity was not found to be a reliable indicator of glycemic control in this specific population. However, the data may not be sufficient to draw definitive conclusions about the broader utility of BChE as a glycemic control biomarker. Further research using larger sample sizes, longitudinal design, and biochemical exposure markers is needed to clarify potential relationships and long-term effects.

Keywords:
Glycemic control; Pesticide exposure; Dietary intake assessment; Cholinesterase; Agrochemical

1. INTRODUCTION

Thailand has seen a substantial rise in pesticide use, with imports reaching 140,861 tons in 2023, valued at over 23.91 trillion baht. The country ranked fifth globally in insecticide use and fourth in herbicide consumption in 2022, reflecting heavy reliance on pesticides for crop production. With 19.1% of the population working in agriculture and nearly half of the land used for farming, pesticide application remains a common practice to enhance yield and income[1,2]. However, this widespread use has raised concerns about food safety. Surveys have shown high levels of pesticide residues in fruits and vegetables, with some exceeding

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maximum residue limits (MRLs), particularly in kale, tomatoes, chili, and oranges[3-5]. Such exposure poses health risks ranging from acute symptoms (nausea, respiratory distress) to chronic effects including endocrine disruption, neurotoxicity, and cancer [6,7]. Diabetic patients are especially vulnerable, as certain pesticides (e.g., dimethoate, omethoate) may impair insulin regulation and worsen glycemic control[8].

Agrochemicals, a term that encompasses pesticides, fertilizers, and growth enhancers, play a crucial role in modern agriculture by increasing productivity but also contribute to environmental pollution and health risks due to their persistence and bioaccumulation [9]. Conventional pesticide formulations often suffer from problems such as poor dispersibility, leaching, and nonbiodegradability, which has motivated the development of novel approaches such as nanotechnology-based agrochemicals[10,11]. These innovations include nanopesticides, nanofertilizers, and encapsulation techniques that improve efficiency, reduce toxicity, and enable controlled release of active ingredients, although their large-scale application remains limited by cost [12].

Many pesticides, especially organophosphates and carbamates, act by inhibiting cholinesterase enzymes, including butyrylcholinesterase (BChE), which is an established biomarker of pesticide exposure[13,14]. Although pyrethroids and neonicotinoids are less acutely toxic, they too have been linked to chronic neurotoxic and endocrine effects[15,16]. Of particular interest is the potential relationship between BChE enzyme activity and diabetic control. Since diabetes involves hormonal regulation—especially of insulin—pesticide-induced endocrine disruption may aggravate insulin resistance and glucose metabolism [8]. Given that BChE activity may influence metabolic regulation, its potential association with glycated hemoglobin (HbA1c) levels in diabetic patients warrants investigation.

Therefore, this study aimed to evaluate the risk of pesticide exposure from fruit and vegetable consumption by assessing its association with BChE enzyme levels and exploring the relationship between BChE activity and HbA1c levels in diabetic patients.

2. MATERIALS AND METHODS

2.1 Study design

This study employed a descriptive cross-sectional design to investigate the association between fruit and vegetable consumption behavior, BChE enzyme activity, and HbA1c levels among diabetic patients. Data were collected at a single point in time, which was appropriate given the limited timeframe available for the study. This design allowed for efficient data collection and facilitated the comparison between dietary exposure and relevant biomarkers without the need for long-term follow-up.

Ethical approval was obtained from the Mahidol University Central Institutional Review Board (MU-CIRB), and written informed consent was obtained from all participants prior to data collection.

2.2 Study area

The study was conducted in Khun Si Subdistrict, Sai Noi District, Nonthaburi Province, Thailand. This area is semi-urban and comprises eight villages, where both agricultural practices and chronic disease management, including diabetes care, are prominent. Khun Si Subdistrict was selected due to its accessibility to a defined population of diabetic patients under the care of a local primary healthcare facility. The location represents a typical Thai agricultural community, where concerns regarding pesticide contamination in fruits and vegetables are relevant to daily life and public health.

2.3 Study population

The study population included individuals diagnosed with any type of diabetes mellitus and registered a total of 346 persons at the primary healthcare facility in Khun Si Subdistrict, Sai Noi District, Nonthaburi Province [17,18]. Eligible participants were aged 18 years or older, had a confirmed medical diagnosis of diabetes, resided in the study area, were able to communicate effectively, and had recent records of glycated hemoglobin (HbA1c) levels, which reflect average blood glucose levels over the previous 1–3 months. Individuals who were pregnant or breastfeeding were excluded. Participants were included regardless of their fruit and vegetable consumption. In this study, all participants reported consuming fruits and vegetables to some extent; no participant reported zero consumption. HbA1c values were categorized into three risk levels: normal (<6.0%), moderate risk (6.0–6.4%), and high risk (≥6.5%), consistent with national diabetes care guidelines[19]. It should be noted that HbA1c levels represent a longer-term average, whereas BChE enzyme activity reflects current exposure, which may result in temporal differences between the two measures. Individuals who were pregnant or breastfeeding were excluded from participation.

The sample size was calculated using the formula proposed by Wayne W. Daniel [20], assuming a standard deviation of 0.25, a 95% confidence level (Z = 1.96), and a margin of error of 0.05. The minimum required sample size was 113 participants. To account for a potential 10% non-response or dropout rate, the final sample size was increased to 126 participants.

Participants were selected using systematic random sampling from the diabetic patient registry. The registry was stratified by village to ensure proportional representation from each of the eight villages within the subdistrict. A systematic sampling technique with a fixed interval was employed to evenly select individuals from the list of eligible diabetic patients.

2.4 Instruments

This study utilized both quantitative and biological instruments to assess the consumption behavior of fruits and vegetables, pesticide exposure, and health biomarkers in diabetic patients. The primary instrument was a structured questionnaire, which was developed based on relevant literature and expert recommendations. It consisted of six sections: (1) demographic characteristics; (2) health information; (3) fruit and vegetable consumption behavior; (4) types of consumed fruits and vegetables; (5) agricultural chemical usage behavior; and (6) biochemical indicators including butyrylcholinesterase enzyme levels and HbA1c.

To ensure content validity, the questionnaire was evaluated by three experts in evaluation and health research. The Index of Item-Objective Congruence (IOC) was used to assess the alignment of each item with the study objectives. Each item was rated on a 3-point scale (+1 = relevant, 0 = uncertain, −1 = not relevant), and items with IOC scores below 0.50 were revised or excluded. After refinement, all 56 items achieved IOC scores between 0.50 and 1.00, with an average IOC of 0.899, indicating excellent content validity. Reliability testing was conducted through a pilot study with 30 diabetic patients from a similar demographic group in Khao Thong Subdistrict, Phayuha Khiri District, Nakhon Sawan Province. Internal consistency was assessed using Cronbach's Alpha Coefficient. The results were acceptable across all sections: 0.789 for fruit and vegetable consumption behavior, 0.707 for food type selection, and 0.922 for pesticide use behavior, indicating high reliability.

Scoring of the fruit and vegetable consumption behavior section was based on 32 items, each rated on a five-point Likert scale ranging from 1 (never) to 5 (always). The possible total score therefore ranged from 32 to 160. To classify the behavior levels, the scores range was divided into five categories using the formula (maximum score – minimum score)/5 = 25.6 as ≈ 26 points per level. Accordingly, the interpretation was as follows: very low (32–57), low (58–83), moderate (84–109), good (110–135), and very good (136–160). The questionnaire allowed both quantitative analysis (frequencies, percentages, standard deviations) and correlation analysis with biochemical outcomes.

Medication adherence behavior was also assessed using the questionnaire. Participants were asked about their frequency of antidiabetic drug use. Those who reported regular use as prescribed were classified as adherent, while those reporting never taking or irregular use were classified as non-adherent. This classification was later used in statistical analyses to examine the relationship between adherence and glycemic control.

To assess pesticide exposure, the study employed a Cholinesterase Reactive Paper Test, which is widely used in community-based screenings for exposure to organophosphate and carbamate pesticides. This colorimetric test provides semi-quantitative results based on blood sample reactivity and is appropriate for field conditions. The test results were interpreted using a four-color scale: yellow (normal, ≥100 U/mL), yellow-green (safe, 87.5–99.9 U/mL), green (at risk, 75–87.4 U/mL), and dark green (unsafe, <75 U/mL) [21]. The test kits were regularly checked for expiration dates, physical integrity, and responsiveness using control reagents. Only kits with stable baseline color and immediate color reaction were considered valid.

HbA1c levels were obtained from medical records or measured at participating health facilities. As HbA1c reflects cumulative blood glucose over the preceding 1–3 months, it does not necessarily coincide with the exact time of BChE measurement. This temporal difference is acknowledged as a limitation when interpreting the association between BChE enzyme activity and glycemic control. The values were classified into three risk levels: normal (<6.0%), moderate risk (6.0–6.4%), and high risk (≥6.5%), consistent with national diabetes care guidelines [22].

The combination of validated questionnaires and biochemical measurements allowed for a comprehensive analysis of dietary behaviors, pesticide exposure, and health outcomes among diabetic patients in the study area.

2.5 Procedures

The data collection process was carried out after obtaining ethical approval from the CIRB-MU, and permission from the Nonthaburi Provincial Public Health Office and local administrative authorities. Prior to fieldwork, the researcher coordinated with health officers at two primary health care centers: Wat Yod Phra Phimon Health Promoting Hospital (responsible for villages 1–5) and Ban Khlong Sib Sok Health Promoting Hospital (responsible for villages 6–8) in Khun Si Subdistrict, Sai Noi District, Nonthaburi Province. The purpose and detailed procedures of the study were explained to ensure transparency and cooperation at the local level. The researcher then visited the target communities to recruit eligible diabetic patients from eight villages using a systematic random sampling method. Each participant was informed about the study's objectives, procedures, confidentiality of personal data, and their right to voluntarily participate or withdraw at any time. Informed consent was obtained prior to data collection.

Data were collected between January and March 2025. The process included administering a structured questionnaire and collecting capillary blood samples. Each day, approximately 30–40 participants were enrolled. Participants completed the questionnaire, which assessed demographics, health status, consumption behavior of fruits and vegetables, and pesticide exposure. The questionnaire session took approximately 20–30 minutes, depending on individual comprehension.

After the questionnaire, blood samples (75 µL) were collected by a licensed nurse using sterile techniques. Blood samples were allowed to settle for 1–2 hours for serum separation. The serum was then tested for butyrylcholinesterase enzyme levels using cholinesterase reactive paper (produced by the Government Pharmaceutical Organization, Thailand). The interpretation followed guidelines issued by the Bureau of Occupational and Environmental Diseases, Ministry of Public Health. The test's sensitivity, specificity, and accuracy had previously been validated against the Bigg's method, showing no statistically significant difference ($p < 0.01$) with high levels of sensitivity (89.89%), specificity (95.65%), and positive predictive value (94.59%) in laboratory settings, and adequate reliability in field conditions (sensitivity 77.04%, specificity 90.01%, PPV 90.38%). Serum drops were applied to the reactive paper, and results were interpreted based on standardized color comparison charts according to room temperature. Yellow indicated normal enzyme levels (≥100 U/mL), yellow-green indicated safe levels (87.5–99.9 U/mL), green indicated risk (75–87.4 U/mL), and dark green indicated unsafe levels (<75 U/mL). Participants were later contacted by phone to receive their results along with guidance on how to reduce pesticide exposure, particularly through safer fruit and vegetable consumption practices.

Finally, the researcher reviewed all completed questionnaires for completeness and accuracy. Any missing or inconsistent data were resolved through follow-up contact with participants. All hardcopy questionnaires were securely stored and will be retained for one year post-study completion, after which they will be confidentially destroyed to protect participant privacy.

2.6 Statistical analysis

Descriptive statistics were used to summarize general characteristics of the participants, including demographic data, health status, fruit and vegetable consumption behaviors, types of produce consumed, pesticide use behaviors, butyrylcholinesterase enzyme levels, and glycated hemoglobin (HbA1c) levels. The results were presented using frequencies, percentages, means, and standard deviations, as appropriate. Inferential statistics were applied to assess the associations between key variables. The relationship between fruit and vegetable consumption behavior and butyrylcholinesterase enzyme levels was analyzed using the Chi-square test or Fisher's Exact Test, depending on cell sizes. These tests were used to determine whether the consumption behaviors (categorized as practiced vs. not practiced) were significantly associated with categorized enzyme levels. HbA1c levels were initially classified into three risk categories (normal <6.0%, moderate risk 6.0–6.4%, high risk ≥6.5%). However, for the purpose of statistical analysis, HbA1c categories were collapsed into two groups (normal vs. elevated ≥6.0%) to ensure sufficient cell counts for valid Chi-square testing. All statistical analyses were conducted using IBM SPSS software version 29 (Armonk, NY). A p-value of less than 0.05 was considered statistically significant.

3. RESULTS

3.1 Demographic and health characteristics of the participants (n=126)

Among the 126 diabetic patients, two-thirds were female (66.7%) and most were aged ≥60 years (61.1%; mean age 61.5 ± 11.4 years, range 22-86). Over one-third were unemployed (35.7%), followed by agricultural workers (28.6%) and general laborers (17.5%). The majority had primary education (86.5%) and were covered under the Universal Coverage Scheme (91.3%). All demographic data are presented in **Table 1**.

3.2 Health conditions and diabetes treatment regimens of all participants (n=126)

The mean systolic and diastolic blood pressures were 139 ± 10.9 mmHg and 79.9 ± 9.9 mmHg, respectively. Most participants were non-smokers (88.9%) and non-drinkers (91.3%). Over half had diabetes for <10 years (57.0%; mean duration 9.8 ± 7.7 years). Hypertension (84.9%) and hyperlipidemia (76.2%) were the most common comorbidities. Almost all participants received oral antidiabetic drugs (95.2%), mainly metformin (87.3%), followed by glipizide (30.2%) and pioglitazone (14.3%). Most patients reported good adherence to prescribed regimens (92.9%). Complete health characteristics are shown in **Table 2**.

Table 1. Descriptive statistics of demographics among diabetic patients (n=126).

Variables	n (%)
Sex	
Men	42 (33.3)
Women	84 (66.7)
Age (Year)	
20 – 29	1 (0.8)
30 – 39	4 (3.2)
40 – 49	13 (10.3)
50 – 59	31 (24.6)
≥ 60	77 (61.1)
Mean (SD.) = 61.54 (11.36) Min = 22 Max = 86	
Occupation	
Unemployed	45 (35.7)
Farmer	36 (28.6)
Government officer	1 (0.8)
General laborer	22 (17.5)
Self-employed/Business owner	22 (17.5)
Education level	
No formal education	1 (0.8)
Primary education	109 (86.5)
Lower secondary education	5 (4)
Lower secondary education /Vocational certificate	6 (4.8)
Diploma/Associate degree	3 (2.4)
Bachelor's degree	2 (1.6)
Healthcare coverage/insurance scheme	
Universal coverage scheme (UCS)/Gold card	115 (91.3)
Social security scheme (SSS)	6 (4.8)
Civil servant medical benefit scheme (CSMBS)	5 (4.0)

Table 2. Descriptive statistics of health charactoristic among diabetic patients (n=126).

Variables	n (%)
Blood pressure	
Systolic blood pressure (SBP)	
< 120 mmHg	4 (3.2)
120 – 129 mmHg	15 (11.9)
130 – 139 mmHg	52 (41.3)
140 – 159 mmHg	50 (39.7)
≥ 160 mmHg	5 (4)
Mean (SD.) = 139 (10.85) Min = 115 Max = 184	
Diastolic blood pressure (DBP)	
< 80 mmHg	59 (46.8)
80 – 84 mmHg	27 (21.4)
85 – 89 mmHg	20 (15.9)
90 – 99 mmHg	17 (13.5)
≥ 100 mmHg	3 (2.4)
Smoking behavior	
Non-smoker	112 (88.9)
Current smoker	11 (8.7)
Former smoker	3 (2.4)
Alcohol consumption behavior	
Non-drinker	115 (91.3)
Daily drinker	4 (3.2)
Occasional drinker (1-2 times/week)	2 (1.6)
Infrequent drinker (1-2 times/month)	1 (0.8)
Former drinker	4 (3.2)

Table 2. Descriptive statistics of health charactoristic among diabetic patients (n=126) (Continued).

Variables	n (%)
Duration of diabetes since diagnosis (Year)	
< 10	72 (57)
10 – 14	26 (20.6)
15 – 19	6 (4.8)
20 – 24	16 (12.7)
25 – 29	1 (0.8)
≥ 30	5 (4)
Mean (SD.) = 9.78 (7.645) Min = 1 Max = 40	
Herbal medicine use behavior	
Non-user	118 (93.7)
Current user	6 (4.8)
Former user	2 (1.6)
Medication status (Current diabetes medication use)	
No	6 (4.8)
Yes	120 (95.2)
Comorbidities (excluding diabetes)	
Hypertension	107 (84.9)
Chronic kidney disease (CKD)/Nephropathy	5 (4)
Cerebrovascular disease/Stroke	3 (2.4)
Dyslipidemia/Hyperlipidemia	96 (76.2)
Thyroid disorder	1 (0.8)
Colorectal cancer/Bowel cancer	2 (1.6)
Gout	1 (0.8)
Heart disease/Cardiac disease	1 (0.8)
Coronary artery disease (CAD)/Cardiovascular disease	1 (0.8)

3.3 Fruit and vegetable consumption behavior among diabetic patients of all participants (n=126)

Most participants (89.7%) reported moderate consumption levels. When purchasing produce, 39.7% rarely bought from general markets and 89.7% rarely used supermarkets. Only 40.5% occasionally grew their own vegetables. A significant portion preferred seasonal fresh produce (36.5%) but rarely chose organic options (93.7%).

Regarding consumption patterns, 38.1% occasionally consumed high-sugar fruits while 38.9% consumed low-sugar fruits at similar frequency. Over half (54.8%) regularly consumed vegetables recommended for diabetic patients, yet 86.5% never practiced portion control. In terms of hygiene, 84.1% consistently washed produce before consumption, primarily using plain water (79.4%). Most consumed less than the recommended 400 grams daily: 59.5% never met vegetable intake targets and 60.3% fell short of fruit recommendations. All data are presented in **Table 3**.

3.4 Commonly consumed fruits and vegetables (n=126)

The most frequently consumed vegetables were chilies (88.9%), holy basil (84.1%), coriander (81.7%), eggplant (70.6%), yardlong beans (69.0%), and kale (65.9%). Among fruits, oranges were most common (88.9%), followed by guava (60.3%), apples (38.1%), dragon fruit (30.2%), bananas (20.6%), and grapes (19.8%). All data are shown in **Table 4**.

Table 3. Fruit and vegetable consumption behaviors among diabetic patients (n = 126).

Fruit and Vegetable Consumption Behavior	n (%)				
	Never	Rarely	Sometimes	Often	Always
1. You purchase fruits and vegetables from general market vendors	4 (3.2)	50 (39.7)	43 (34.1)	14 (11.1)	15 (11.9)
2. You purchase fruits and vegetables from supermarkets/department stores	113 (89.7)	9 (7.1)	2 (1.6)	1 (0.8)	1 (0.8)
3. You grow fruits and vegetables at home for personal consumption	25 (19.8)	51 (40.5)	37 (29.4)	8 (6.3)	5 (4.0)
4. You typically select fresh seasonal fruits and vegetables for consumption	11 (8.7)	2 (1.6)	25 (19.8)	46 (36.5)	42 (33.3)
5. You purchase organic or pesticide-free fruits and vegetables	118 (93.7)	4 (3.2)	3 (2.4)	0 (0)	1 (0.8)
6. You consider pesticide contamination when selecting fruits and vegetables	16 (12.7)	11 (8.7)	35 (27.8)	31 (24.6)	33 (26.2)

Table 3. Fruit and vegetable consumption behaviors among diabetic patients (n = 126) (Continued).

Fruit and Vegetable Consumption Behavior	n (%)				
	Never	Rarely	Sometimes	Often	Always
7. You consume high-sugar fruits (e.g., banana, grape, cherry, mango, pineapple, orange, longan, watermelon, custard apple, jackfruit)	0 (0)	36 (28.6)	48 (38.1)	23 (18.3)	19 (15.1)
8. You consume low-sugar fruits (e.g., dragon fruit, guava, rose apple, avocado)	0 (0)	38 (30.2)	49 (38.9)	27 (21.4)	12 (9.5)
9. You consume vegetables suitable for diabetic patients (e.g., long beans, Chinese kale, Chinese cabbage, napa cabbage, tomato)	1 (0.8)	7 (5.6)	17 (13.5)	32 (25.4)	69 (54.8)
10. You control portion sizes at each meal (e.g., consuming ½ cup or approximately 80-100 grams of rice per meal)	109 (86.5)	7 (5.6)	7 (5.6)	3 (2.4)	0 (0)
11. You peel fruits and vegetables before consumption	0 (0)	3 (2.4)	34 (27)	60 (47.6)	29 (23)
12. You use a knife to peel fruits before consumption	2 (1.6)	3 (2.4)	31 (24.6)	59 (46.8)	31 (24.6)
13. You bite fruit peels directly with your teeth when eating	114 (90.5)	7 (5.6)	4 (3.2)	0 (0)	1 (0.8)
14. You peel fruits with your hands and handle food without washing hands first	52 (41.3)	23 (18.3)	36 (28.6)	10 (7.9)	5 (4.0)
15. You wash fruits and vegetables before consumption	0 (0)	0 (0)	5 (4)	15 (11.9)	106 (84.1)
16. You clean fruits and vegetables by washing with regular clean water	0 (0)	0 (0)	6 (4.8)	20 (15.9)	100 (79.4)
17. You clean fruits and vegetables by running water over them for at least 2 minutes	3 (2.4)	4 (3.2)	31 (24.6)	55 (43.7)	33 (26.2)
18. You clean fruits and vegetables by soaking in vinegar solution (3-5%) for 15-30 minutes, then rinsing with clean water	119 (94.4)	1 (0.8)	2 (1.6)	0 (0)	4 (3.2)
19. You clean fruits and vegetables using sodium bicarbonate (baking soda), soaking for 15 minutes, then rinsing with clean water	120 (95.2)	0 (0)	0 (0)	0 (0)	6 (4.8)
20. You clean fruits and vegetables by soaking in baking powder solution (1 tablespoon per 20 liters of water) for 15 minutes	126 (100)	0 (0)	0 (0)	0 (0)	0 (0)
21. You clean fruits and vegetables by soaking in potassium permanganate solution (20-30 crystals per 4 liters of water) for 10 minutes	121 (96)	2 (1.6)	1 (0.8)	2 (1.6)	0 (0)
22. You clean fruits and vegetables using salt water (1-2%) for 15-30 minutes	63 (50)	14 (11.1)	23 (18.3)	1 (8.7)	15 (11.9)
23. For leafy vegetables, you separate leaves before washing and cleaning	56 (44.4)	12 (9.5)	34 (27)	22 (17.5)	2 (1.6)
24. You consume vegetables without cooking or any heat treatment	18 (14.3)	46 (36.5)	36 (28.6)	21 (16.7)	5 (4)
25. You consume vegetables after cooking or heat treatment	0 (0)	2 (1.6)	10 (7.9)	51 (40.5)	63 (50)
26. You consume processed fruits (e.g., fried banana, candied mango)	100 (79.4)	22 (17.5)	4 (3.2)	0 (0)	0 (0)
27. You purchase pre-cooked vegetables from the market	125 (99.2)	1 (0.8)	0 (0)	0 (0)	0 (0)
28. You avoid consuming fruits and vegetables known to contain chemical residues	36 (28.6)	5 (4)	26 (20.6)	28 (22.2)	31 (24.6)
29. You consume more than 400 grams of vegetables per day	75 (59.5)	30 (23.8)	8 (6.3)	6 (4.8)	7 (5.6)
30. You consume less than 400 grams of vegetables per day	2 (1.6)	26 (20.6)	12 (9.5)	44 (34.9)	42 (33.3)
31. You consume more than 400 grams of fruits per day	76 (60.3)	28 (22.2)	13 (10.3)	4 (3.2)	5 (4)
32. You consume less than 400 grams of fruits per day	6 (4.8)	34 (27)	48 (38.1)	25 (19.8)	13 (10.3)

Table 4. Consumption patterns of specific fruits and vegetables among diabetic patients (n = 126).

Vegetables	n (%)	Fruits	n (%)
Chinese broccoli	83 (65.9)	Grape	25 (19.8)
Coriander/Cilantro	103 (81.7)	Chinese dates/Jujube	5 (4)
Cabbage	34 (27)	Orange	112 (88.9)
Bok choy/Chinese white cabbage	49 (38.9)	Guava	60 (47.6)
Cauliflower	69 (54.8)	Dragon fruit	38 (30.2)
Chili pepper	112 (88.9)	Custard apple/Sugar apple	3 (2.4)
Eggplant	89 (70.6)	Longan	5 (4)
Cucumber	73 (57.9)	Apple	48 (38.1)
Long bean/Yard long bean	87 (69)	Mangosteen	7 (5.6)
Holy basil	106 (84.1)	Watermelon	76 (60.3)
Napa cabbage/ Chinese cabbage	28 (22.2)	Banana	26 (20.6)
Sesbania flower	1 (0.8)	Rose apple/Water apple	1 (0.8)
Angled loofah/Ridged gourd	1 (0.8)	Mango	15 (11.9)
Water spinach	1 (0.8)	Papaya	2 (1.6)
Morning glory	1 (0.8)	Pineapple	4 (3.2)
		Pear	2 (1.6)

Note: Each diabetic patient could select more than one type of vegetable and fruit.

3.5 Pesticide use behavior (n=126)

Most participants (77%) did not use pesticides at home, while 23% did. Among users, family members most commonly applied pesticides (11.9%), followed by self-application (9.5%). For agricultural use, 72.2% did not use pesticides while 27.8% did. Among organophosphate users (28.6%), chlorpyrifos was most common (26.2%), followed by diazinon (16.7%) and dimethoate (11.9%). For carbamate users (27.8%), carbaryl was most common (21.4%), followed by carbosulfan (16.7%). Complete pesticide use data are shown in **Table 5**.

Table 5. Chemical use behaviors among diabetic patients (n = 126).

Chemical use behavior	Used n (%)	Not used n (%)
Pesticide application for home-grown vegetables and fruits	29 (23.0)	97 (77.0)
Self-application	12 (9.5)	114 (90.5)
Family member application	15 (11.9)	111 (88.1)
Hired labor application	2 (1.6)	124 (98.4)
Use of pesticides in agricultural practices	35 (27.8)	91 (72.2)
Self-application	13 (10.3)	113 (89.7)
Family member application	4 (3.2)	112 (96.8)
Hired labor application	18 (14.3)	108 (85.7)
Organophosphate pesticide use in agricultural activities	36 (28.6)	90 (71.4)
Cadusafos	1 (0.8)	125 (99.2)
Chlorpyrifos	34 (26.2)	92 (73.0)
Diazinon	21 (16.7)	105 (83.3)
Dichlorvos	1 (0.8)	125 (99.2)
Dimethoate	15 (11.9)	111 (88.1)
Omethoate	3 (2.4)	123 (97.6)
Malathion	1 (0.8)	125 (99.2)
Carbamate pesticide use in agricultural activities	35 (27.8)	91 (72.2)
Benfuracarb	7 (5.6)	119 (94.4)
Carbaryl	27 (21.4)	99 (78.6)
Carbosulfan	21 (16.7)	105 (83.3)
Fenobucarb	1 (0.8)	125 (99.2)

Note: Each diabetic patient could select more than one type of agrochemical exposed.

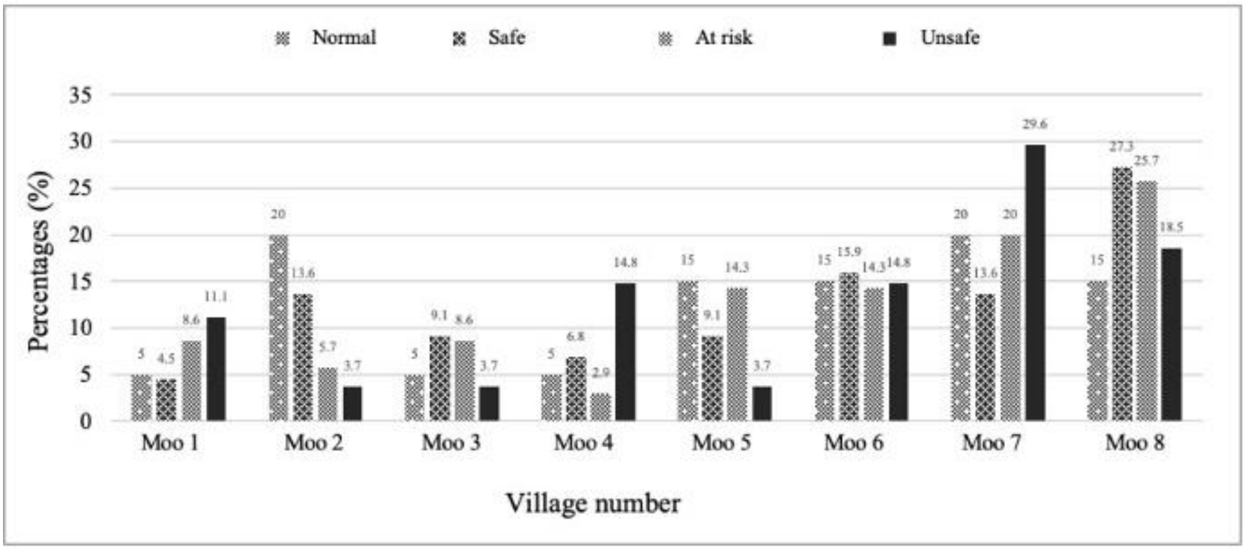

Figure 1. Distribution of butyrylcholinesterase (BChE) enzyme levels by village

3.6 Cholinesterase enzyme levels and blood sugar levels (n=126)

BChE enzyme levels showed 34.9% of participants in the "safe" range, 27.8% "at-risk," 21.4% "unsafe," and 15.9% "normal." Village-level variations are illustrated in **Figure 1**. For glycemic control, 75.4% exhibited high HbA1c levels, 15.9% were moderately high, and only 8.7% were within normal range.

3.7 Statistical analysis of factors associated with BChE levels

No statistically significant association was found between general fruit and vegetable consumption behaviors and BChE enzyme levels (Fisher's Exact Test, $p > 0.05$). However, participants who consumed grapes had significantly different BChE levels compared to non-consumers (Mann-Whitney U test = 829.5, p = 0.006) (**Table 6**).

Table 6. Butyrylcholinesterase levels by consumption status of specific fruits and vegetables among diabetic patients (n = 126).

Type of vegetables/Fruits	n (%)	Mean Rank of BChE	Mann-Whitney U test	*P* -value
Chinese broccoli				
Did not consume	43 (34.1)	66.00	1677.000	0.565
Consumed	83 (65.9)	62.20		
Coriander/Cilantro				
Did not consume	23 (18.3)	58.20	1062.500	0.422
Consumed	103 (81.7)	64.68		
Cabbage				
Did not consume	92 (73)	65.07	1419.500	0.408
Consumed	34 (27)	59.25		
Bok choy/Chinese white cabbage				
Did not consume	77 (61.1)	66.51	1654.500	0.227
Consumed	49 (38.9)	58.77		
Cauliflower				
Did not consume	57 (45.2)	62.82	1927.500	0.842
Consumed	69 (54.8)	64.07		
Chili pepper				
Did not consume	14 (11.1)	62.64	772.000	0.923
Consumed	112 (88.9)	63.61		
Eggplant				
Did not consume	37 (29.4)	65.39	1576.500	0.696
Consumed	89 (70.6)	62.71		

Table 6. Butyrylcholinesterase levels by consumption status of specific fruits and vegetables among diabetic patients (n = 126) (Continued).

Type of vegetables/Fruits	n (%)	Mean Rank of BChE	Mann-Whitney U test	P -value
Cucumber				
Did not consume	53 (42.1)	70.01	1589.500	0.076
Consumed	73 (57.9)	58.77		
Long bean/Yard-long bean				
Did not consume	39 (31)	61.62	1623.000	0.686
Consumed	87 (69)	64.34		
Holy basil				
Did not consume	20 (15.9)	76.68	796.500	0.067
Consumed	106 (84.1)	61.01		
Napa cabbage/Chinese cabbage				
Did not consume	97 (77)	66.64	1102.000	0.066
Consumed	29 (23)	53.00		
Grape				
Did not consume	101 (80.2)	59.21	829.500	0.006*
Consumed	25 (19.8)	80.82		
Chinese dates/Jujube				
Did not consume	121 (96)	64.51	180.500	0.112
Consumed	5 (4)	39.10		
Orange				
Did not consume	14 (11.1)	60.36	740.000	0.722
Consumed	112 (88.9)	63.89		
Guava				
Did not consume	66 (52.4)	65.33	1859.000	0.538
Consumed	60 (47.6)	61.48		
Dragon fruit				
Did not consume	87 (69)	61.10	1488.000	0.252
Consumed	39 (31)	68.85		
Custard apple/Sugar apple				
Did not consume	123 (97.6)	63.70	160.000	0.683
Consumed	3 (2.4)	55.33		
Longan				
Did not consume	122 (96.8)	63.30	219.000	0.717
Consumed	4 (3.2)	69.75		
Apple				
Did not consume	78 (61.9)	61.48	1714.500	0.410
Consumed	48 (38.1)	66.78		
Mangosteen				
Did not consume	119 (94.4)	64.03	354.000	0.488
Consumed	7 (5.6)	54.57		
Watermelon				
Did not consume	50 (39.7)	64.78	1836.000	0.740
Consumed	76 (60.3)	62.66		
Mango				
Did not consume	111 (88.1)	64.58	713.000	0.349
Consumed	15 (11.9)	55.53		
Banana				
Did not consume	100 (79.4)	60.48	998.000	0.058
Consumed	26 (20.6)	75.12		

Note: *Statistical significantly ($P < 0.05$).

Among demographic variables (**Table 7**), age was significantly related to BChE levels, with participants ≥60 years more likely to have unsafe BChE levels ($p < 0.05$). Participants who self-applied agricultural pesticides had significantly higher rates of unsafe BChE levels ($p < 0.05$) shown in **Table 8**, while other pesticide exposure methods showed no significant associations.

Table 7. Analysis of the association between demographic variable and butyrylcholinesterase enzyme (BChE) levels among diabetic patients (n = 126).

Age	BChE Level (n=126)		Pearson Chi-Square	*P* - value
	Normal (n=64) n (%)	Elevated (n=62) n (%)		
< 60 Years old	32 (50)	17 (27.4)	6.757	0.009*
≥ 60 Years old	32 (50)	45 (72.6)		

Note: Statistical analysis was performed using Chi-Square Test. BChE levels were categorized as safe (≥87.5 U/mL) and unsafe (<87.5 U/mL), where reduced enzyme activity suggests potential pesticide exposure and associated health risks.

Table 8. Association between agricultural pesticide application practices and butyrylcholinesterase emzyme (BChE) levels among diabetic patients, stratified by self-application versus non-application.

BChE Level	Pesticides applied behavior (n=35)		*P* - value
	Self-application (n=13) n (%)	Non-self-application (n=22) n (%)	
Safe (Normal)	2 (15.4)	14 (63.6)	0.006*
Unsafe (At risk)	11 (84.6)	8 (36.4)	

Note: Statistical analysis by Fisher's exact Test. BChE levels were categorized as safe (≥87.5 U/mL) and unsafe (<87.5 U/mL), where reduced enzyme activity suggests potential pesticide exposure and associated health risks.

No statistically significant association was found between BChE levels and HbA1c ($p > 0.05$) (**Table 9**). Analysis of antidiabetic medications showed no significant relationships with HbA1c levels ($p > 0.05$ for all medications) (**Table 10**). Similarly, medication adherence showed no significant association with HbA1c levels (**Table 11**).

4. DISCUSSION

4.1 Demographic characteristics

The demographic profile of diabetic patients in Khun Si Subdistrict revealed a predominantly female (66.7%) and older population (85.7% aged ≥50 years), consistent with previous studies such as Jantasuwan and Jareprapa (2018)[23], who reported similar trends in gender and age distribution among populations exposed to pesticide residues.

Most participants had completed only primary education (86.5%), aligning with rural demographic patterns reflected in the study by Jantasuwan and Jaraeprapal^19^. The high proportion of unemployed participants (35.7%) contrasts with Juntarawijit and Juntarawijit [24], who observed higher percentages of agricultural workers. This discrepancy likely stems from the current study's inclusive sampling and older participant age, many of whom had retired.

4.2 Health conditions and comorbidities

The high prevalence of hypertension (84.9%) among diabetic participants is consistent with findings by Jantasuwan and Jareprapa (2018)[23]. This comorbidity is clinically significant, as hypertension exacerbates complications in diabetic patients.

Compared to a previous study by Pongrut Kairprom[25], where only 12.7% had comorbid hypertension, the elevated prevalence in this study may be attributed to differences in age distribution and selection criteria. Health behaviors such as low smoking (88.9%) and alcohol consumption (91.3%) rates were consistent with prior community-based research in Thailand.

4.3 Fruit and vegetable consumption patterns

Most participants (89.7%) reported moderate levels of fruit and vegetable consumption behavior, aligning with findings from Jantasuwan and Jaraeprapal [23], who found similar behavior regarding dietary practices aimed at reducing pesticide exposure.

Table 9. Correlation between demographic factors and butyrylcholinesterase levels among diabetic patients (n = 126).

BChE Level	HbA1c Level (n=126)		*P* - value
	Normal (n=12) n (%)	Elevated (n=114) n (%)	
Safe (Normal)	7 (58.3)	57 (50)	0.583
Unsafe (At risk)	5 (41.7)	57 (50)	

Note: Statistical analysis was performed using Chi-Square Test. HbA1c levels were originally classified into three categories: normal (<6.0%), moderate risk (6.0–6.4%), and high risk (≥6.5%), consistent with national diabetes care guidelines. However, for statistical testing, HbA1c was collapsed into two groups: normal (<6.0%) and elevated (≥6.0%) to ensure adequate cell size for analysis. BChE levels were categorized as safe (≥87.5 U/mL) and unsafe (<87.5 U/mL), where reduced enzyme activity suggests potential pesticide exposure and associated health risks.

Table 10. Association between use of specific antidiabetic medications and glycated hemoglobin (HbA1c) levels among diabetic patients.

Medication status	HbA1c Level (n=126)		*P* - value
	Normal (n=12) n (%)	Elevated (n=114) n (%)	
1. Glipizide 5 mg			
Medicated	5 (41.7)	33 (28.9)	0.509[b]
Non-medicated	7 (58.3)	81 (71.1)	
2. Metformin 425 mg			
Medicated	0 (0)	5 (4.4)	1.000[b]
Non-medicated	12 (100)	109 (95.6)	
3. Metformin 850 mg			
Medicated	9 (75)	96 (84.2)	0.420[b]
Non-medicated	3 (25)	18 (15.8)	
4. Pioglitazone 15 mg			
Medicated	0 (0)	11 (9.6)	0.598[b]
Non-medicated	12 (100)	103 (90.4)	
5. Pioglitazone 30 mg			
Medicated	0 (0)	7 (6.1)	1.000[b]
Non-medicated	12 (100)	107 (93.9)	

Note: [a]Statistical analysis by Chi-Square Test, [b]Statistical analysis by Fisher's exact Test.
HbA1c elevated level (>6.0%): Poor glycemic control or uncontrolled diabetes. HbA1c Level (<6.0%): Normal.

Table 11. Correlation between diabetes medication adherence and HbA1c levels among diabetic patients (n = 126).

Medication adherence behavior	HbA1c Level (n=126)		*P* - value
	Normal (n=12) n (%)	Elevated (n=114) n (%)	
Non-adherent	1 (8.3)	8 (7)	1.000[b]
Adherent	11 (91.7)	106 (93)	

Note: [a]Statistical analysis by Chi-Square Test, [b]Statistical analysis by Fisher's exact Test.
Non-adherent: Never taking/irregular medication use, Adherent: Regular medication use as prescribed
HbA1c elevated level (>6.0%): Poor glycemic control or uncontrolled diabetes. HbA1c Level (<6.0%): Normal.

The significant association between grape consumption and altered BChE levels supports reports by the Thailand Consumers Council [29] and Thai-PAN [3] which reported frequent pesticide residues—particularly chlorpyrifos—on imported grapes, especially Shine Muscat varieties. This suggests that certain fruits may pose higher chemical exposure risks despite general safe eating habits. Age-related BChE decline (participants ≥60 years) is consistent with findings from Munfiah et al. (2023)[30] showing age-related declines in cholinesterase levels. Furthermore, participants who personally applied pesticides had significantly lower BChE levels compared to those who hired others, confirming earlier evidence by Chuenchom [31] about the risks of direct chemical exposure.

4.4 Metabolic control and associations

The absence of significant associations between BChE enzyme activity and HbA1c levels contrasts with the study by Katoh et al. (2014)[32], which reported significant associations between cholinesterase levels, liver fat, and HbA1c among 5,384 hospital-based patients.

The discrepancy may be due to several factors: smaller sample size in this study (n=126), reliance on community-based data collection, absence of laboratory-confirmed pesticide levels, and differences in sample stratification and lab precision. These factors likely limited the sensitivity of detecting subtle metabolic associations in this population. The elevated HbA1c levels (75.4%) were consistent with results from similar community studies [33], ndicating poor glycemic control across the sample.

5. LIMITATIONS AND RECCOMMENDATIONS

This cross-sectional study prevents causal relationship establishment between BChE and glycemic control. The sample size (n=126) and geographic limitation may affect generalizability and statistical power. Self-reported consumption data may introduce recall bias, and lack of biochemical pesticide residue confirmation limits exposure assessment accuracy.

Temporal differences between HbA1c (2-3 month average) and single-point BChE measurements may limit association interpretation. Future research should employ longitudinal designs with regular biomarker monitoring, larger diverse samples, and biochemical pesticide residue testing.

6. CONCLUSIONS

This study found no significant associations between fruit and vegetable consumption behaviors and BChE enzyme levels, nor between BChE levels and glycemic control in diabetic patients. While grape consumption and direct pesticide application were associated with altered BChE activity, current evidence is insufficient to establish definitive links between pesticide exposure and diabetes outcomes in this population.

Therefore, while this study provides preliminary insight into the possible interplay between dietary exposure to agrochemicals and metabolic health in diabetic patients, the findings highlight the need for further research. Future studies should adopt a longitudinal design with regular monthly monitoring of both BChE and HbA1c levels over a one-year period to better understand potential long-term relationships. In addition, biochemical confirmation of pesticide residues in consumed produce and more precise exposure assessment methods are recommended to strengthen causal inferences.

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Author contributions

NN: Conceptualization, Investigation, Data curation, Writing-original draft. **RT:** Formal analysis, Writing–review & editing. **DW:** Writing–review & editing. **PP:** Writing–review & editing. **TN:** Writing–review & editing. **TB:** Conceptualization, writing–original draft, writing–review & editing. All the authors read and approved the final manuscript.

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Conflict of interest

The authors declare that they have no competing interests.

Ethics approval

This study was conducted in accordance with the Declaration of Helsinki. Ethical approval was granted by the Mahidol University Central Institutional Review Board (MU-CIRB) (COA No. MU-CIRB 2024/321.1712). Written informed consent was obtained from all participants after they had received information about the study procedures, risks, and benefits.

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