

Journal of Ideas in Health



Journal homepage: www.jidhealth.com

Open Access

Original Article

Blood cholinesterase activities and oxidative stress status among farmworkers using pesticides in Duhok, KRG, Iraq

Simona Khamo Odisho¹, Fouad K. Mohammad^{2*}

Abstract

Background: The use of pesticides by farmworkers poses considerable health risks. This study was undertaken to examine plasma and erythrocyte cholinesterase activities, plasma oxidative biomarkers malondialdehyde (MDA), and total antioxidant status (TAS) among farmworkers using different pesticide products in Duhok, northern of Iraq.

Methods: This is a case-control study conducted between November 2021 to July 2022 on 92 male farmworkers who were exposed to pesticides in comparison with 44 non-exposed male subjects (control). The availability and uses of pesticides were obtained from 19 agrochemical shops and the farmworkers exposed to pesticides. Demographic data of pesticide-exposed farmworkers and their practice of pesticide applications were recorded. Plasma and erythrocyte cholinesterase activities and plasma MDA and TAS levels were determined in both groups.

Results: The farmworkers had a significant 10.0% increase in plasma MDA level, with no significant changes in blood cholinesterase activities or the TAS level. Odds and risk ratios of reduced plasma cholinesterase activity (20.0%) suggested an association of health risks in pesticide-exposed farmworkers. Most of the pesticide products (278) in use were insecticides (47.0%), which comprised mainly 26.0% pyrethroids and 3.0-7.0% anticholinesterase insecticides, among others. The majority of the farmworkers (51%) were merely aware of the general target use of the pesticide, and 75% had an exposure history of > 5 years. Pesticide application was mostly (50.0%) manual, and 54.0% used insufficient personal protection equipment; 32.0% ate and drank at work, 48.0% practiced disposal of empty pesticide containers by burning and/or burying them, whereas 25.0% dumped the containers indiscriminately, and 25% disposed them at garbage sites openly.

Conclusion: The farmworkers, with only a marginal increase in oxidative stress biomarker MDA, did not suffer from significant reductions in blood cholinesterase activities, although odds and risk ratios of reduced plasma cholinesterase activity suggested a health risk. Implementation of a national program is needed to measure pre-exposure blood cholinesterase activities in farmworkers.

Keywords: Farmers, Insecticides, Oxidative Biomarker, Plasma Cholinesterase, Iraq

Background

Pesticides are chemical substances used to control harmful organisms in agriculture, public health, and veterinary clinical practice [1-5]. They are generally classified as insecticides, herbicides, fungicides, biocides, rodenticides, molluscicides, nematicides, pediculicides, and plant growth regulators [1,2,4,5]. Pesticides are increasingly used worldwide to protect crops from infestations with pests and increase crop productivity that eventually poses health risks to the

*Correspondence: fouadmohammad@yahoo.com

A full list of author information is available at the end of the article



environment and users such as farmworkers [1,3,5-9]. Exposure of farmworkers to pesticides which gain entry to the body and systemic circulation usually occurs through dermal, oral, and respiratory routes as well as through the eyes and ears if proper protective measures are not taken [1,2,8-10]. This usually occurs during planting, spraying, harvesting, and packing activities in agriculture as well as through other activities related to direct or indirect pesticide applications [1,9,10].

The pesticide marketplace is undergoing a global expansion, especially in developing countries [3,7,11]. Pesticide products can be obtained easily by farmers, as they are sold as over-thecounter formulations in many countries, including Iraq [11-14], with the possibility of misuse and mishandling of the products [12]. Based on the wide usage of pesticides, studies universally

© The Author(s), 2022 Open Access This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (https://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article unless otherwise stated

²Department of Physiology, Biochemistry and Pharmacology, College of Veterinary Medicine, University of Mosul, Mosul, Iraq

indicated their environmental hazards and health risks in man and animals [2-5,8,15]. The availability of pesticide products in the local Iraqi market is not fully controlled or known. One study reported the types of pesticides used in greenhouses in Sulaimani, Iraq [14]. They were mainly insecticides and herbicides [14]. Studies have been conducted in Iraq for biomonitoring exposure of agricultural workers, farmers, and veterinarians to pesticides, and they reported variably low blood cholinesterase (ChE) activities but with limited information on the types of pesticides used, their frequency of application, and the extent of exposure [16-20]. A study conducted in Mosul found 21.0%-30.0% decreases in plasma (PChE) or erythrocytes (EChE) ChE activities in agriculture workers and veterinarians [17]. However, in another study performed in Erbil, PChE in agriculture workers and veterinarians exposed to pesticides for up to 19 years was below control levels by only 11.0% and 10.0%, respectively [18]. Subsequently, in a similar study in Kirkuk, the whole blood ChE activity of agriculture workers after six years of exposure was 22.0% below control values [16]. A single study conducted in Duhok found 14.0% and 4.0% reductions in PChE and EChE activities, respectively, in pesticide-exposed farmworkers in comparison to unexposed ones [20]. Pesticide exposure of agricultural workers and those involved in handling and dispensing pesticides can be monitored biologically by examining PChE or EChE activities [8,20-22], monitoring oxidative status such as plasma malondialdehyde (MDA) [8,23], as well as by analysis of concentrations of pesticides or their metabolites in human biological samples [24]. The studies which have been conducted in Iraq [16-18,20] suggested a marginal blood ChE inhibition, though important from a risk assessment point of view. This enzyme inhibition, however, is difficult to assess or interpret in the absence of pre-exposure blood ChE activities of the workers [5,25-28]. The aim of study was to examine blood ChE activities and the levels of the oxidative biomarkers plasma MDA and total antioxidant status in farmworkers in Duhok, KRG, northern of Iraq. The study also included surveying the availability and proper use of pesticides by farmworkers.

Methods

Study design and population

The present study was a case-control study conducted in Duhok, Iraq, between November 2021 to July 2022, where pesticideexposed farmworkers (case) were compared with non-exposed control subjects. The study recruited 98 pesticide-exposed male agricultural workers identified as farmworkers who were previously exposed to different types of pesticides during their handling of pesticides and routine work in farms of the Duhok province that included Zakho (11), Sumel (18), Mangesh (48), Shikhan (12) and Baadra (9). Of these, a total of 92 pesticideexposed subjects were finally eligible for the study and data analysis. The control group comprised 44 age-matched male subjects who were not exposed previously to pesticides.

Sample size

The samples size of the study was within the acceptable range for clinical research studies, as shown by the online tool (https://www.benchmarksixsigma.com/calculators/sample-sizecalculator-for-2-sample-t-test/), taking into consideration 95.0% confidence level, 80.0% power of the test, with 20.0% difference expected in blood ChE activities between the pesticide-exposed and control groups.

Inclusion and exclusion criteria

Inclusion criteria in the pesticide-exposed subjects of the study were any prior pesticide exposure, age > 15 years, and no recent major surgical procedures. Exclusion criteria included nonexposed workers, last exposure to pesticides was >10 years ago, or the presence of chronic diseases such as cancer, liver diseases, and advanced diabetes mellitus. The inclusion and exclusion criteria of the control subjects were the same as the case group, with the exception of the pesticide exposure status.

Data collection

Pesticide products in use

To examine the types of pesticides in use by farmworkers, a survey was conducted on 19 agrochemical shops in seven marketplace locations of the Duhok region, which included Duhok, Sumel, Zakho, Qedsh, Sarsing, Akre, and Shikhan. Farmers usually purchase their agrochemicals, including the pesticides needed, from these shops. Types of pesticides available in these agrochemical shops were identified according to the chemical nature of the active ingredient(s) and usage target [1,6], and whenever possible, they were classified as per WHO criteria [10]. The pesticide products were also tabulated according to their formulation as a single active ingredient or a combination of two or more pesticides in the product. Data were collected by visiting each agrochemical shop in person to record the types of pesticide products available for the farmers or agriculture workers to buy. The purpose of the study was clearly explained to every shop owner and/or attendant.

Demographic information

A structured questionnaire was designed to obtain the demographic information of the study subjects, which included age, gender, marital status, educational level, economic status, as well as any pesticide exposure information such as the type of pesticide used, years of working in farms, age of first exposure at the farm, home exposure if any and the practice of using personal protective equipment (PPE).

Laboratory investigations Blood samples

bioou samples

About five-ml heparinized venous blood samples were obtained from all study subjects by a qualified assistant. The plasma was separated from the erythrocyte by centrifugation at 3000 rpm for 15 min. and kept in deep freeze at -20 °C pending analysis within one month.

Determination of blood ChE activity

A modified electrometric method was used to determine PChE and EChE activities using 0.2 ml of plasma or erythrocytes aliquots, respectively [20,29,30]. The enzyme reaction mixture contained, in addition to the blood sample, 3 ml distilled water and 3 ml of pH 8.1 buffer consisting of 1.237 g sodium barbital, 0.163 g potassium dihydrogen phosphate, and 35.07 g sodium chloride/L of distilled water. The pH1 of the mixture was measured with the glass electrode of a pH meter (pH700, Eutech Instruments, Singapore) before the addition of 0.1 ml of 7.1% acetylcholine iodide substrate. After incubation in a water

bath at 37 °C for 20 min, the pH2 of the mixture was measured. Blood ChE activities of all the subjects were estimated [20,29,30] as follows:

PChE or EChE activity (Δ pH/20 min) = (pH1 - pH2) - Δ pH of blank (no blood sample).

Calculation of odds and risk ratios

Observed and expected cases of 20% reduction in PChE activity [28] among pesticide-exposed farmworkers and the control group were used to calculate odds and risk ratios of the association of having such a low PChE activity with applying pesticides in pesticide-exposed farmworkers. We derived the expected case numbers using a PChE activity (Δ pH/20 min) \leq 0.9. Accordingly, a table of odds ratios of pesticide-exposed farmworkers and their control counterparts was constructed [31].

Determination of plasma MDA level

A spectrophotometric method [32] was used to determine plasma MDA level as follows: In a dry test tube containing 0.25 ml of plasma aliquot, 2 ml of TBA reagent (0.375% w/v thiobarbituric acid, with 20% w/v trichloroacetic acid dissolved in 0.25N HCl) were added and mixed well. The test tubes with glass marbles on top were placed in a boiling water bath for 15 min. After cooling, the mixtures were centrifuged at 4000 rpm for 10 min. The absorbance of the supernatant solution was read by a spectrophotometer (Apel, PD-307, Japan) at 535 nm against the blank. Plasma MDA level (μ mol/L) was estimated as follows:

MDA (μ mol/L) = (absorbance of test – absorbance of blank) *106 / 156000

The calculation of plasma MDA level was also verified using an online tool (https://www.omnicalculator.com/chemistry/beer-lambert-law).

Determination of plasma TAS

The plasma TAS was determined using a colorimetric assay kit (Elabscience Biotechnology Inc., USA) and a microplate reader (ELx800, Biotek, USA) at 660 nm.

Statistical analysis

All data were statistically analyzed using the statistical software programPAST4.09:

(https://www.nhm.uio.no/english/research/resources/past/).

Categorical variables were expressed as frequencies and percentages. Unpaired Student's -t-test was applied for comparison between the means of the two groups. Odds ratios and risk ratios were estimated for cases of observed and expected low PChE activity. Whenever applicable, the Chi-squared test was applied to the frequencies, and the z score was calculated for two population proportions via the statistical tool at https://www.socscistatistics.com/tests/ztest/default.aspx. The level of statistical significance was p < 0.05.

Results

Descriptive and general characteristics of related factors

The mean (\pm SD) ages of pesticide-exposed farmworkers and their control counterparts were very close to each other (43 \pm 13.5 vs. 43.7 \pm 13.8 years). Primary school was the major education level of the farmworkers (40.0%), whereas 41.0% of

them did not have any formal education level (Table 1). Other demographic data of the participants (education level, cigarette smoking, alcohol consumption, marital status) are also shown in table 1.

| Table 1: Demographic data of male farmworkers and control |
|--|
| subjects who participated in the study from different regions of |
| Duhok, KRG, Northern of Iraq |

| Variables | Not exposed | Exposed to |
|-----------------------|---------------|---------------|
| | to pesticides | pesticides |
| | n= 44 | n= 92 |
| Age (mean ± SD years) | 43.7 ± 13.8 | 43 ± 13.5 |
| Education level | | |
| Primary school | 21 (47.7%) | 37 (40.2%) |
| High school | 10 (22.7%) | 8 (8.7%) |
| College | 11 (25.0%) | 9 (9.8%) |
| None | 2 (4.6%) | 38 (41.3%) |
| Smoking habit | 27 (61.4%) | 39 (42.4%) |
| Non-Smoking habit | 17 (38.6%) | 53 (57.6%) |
| Alcohol consumption | 17 (38.6%) | 16 (17.4%) |
| Non-alcohol | 27(61.4%) | 76(82.6%) |
| consumption | | |
| Married | 36 (81.2%) | 82 (89.1%) |
| Unmarried | 8 (18.8%) | 10 (10.9) |

According to the survey, which included 19 agricultural shops in the Duhok region, it was found that the total number of pesticide products for agricultural use by farmers was 278, of which 26.0% were pyrethroids, followed by 19.0% miscellaneous pesticides, 12.0% two or more pesticides in combination, 9.0% neonicotinoids, 7.0% glyphosate, 6.0% organophosphates, 6.0% avermectins and milbemycin, 5.0% phenoxy-propionates, 4.0% benzimidazoles 3.0% triazoles, and 3.0% carbamates (Table 2). Of the 278 types of pesticide products, insecticides comprised 47.0% of the pesticides in use, followed by fungicides 19.0%, herbicides 16.0%, combinations 14.0%, acaricides 3.0%, and miscellaneous 1.0% (Figure 1).

Table 2: Classification of commercial pesticides based on their chemical natures with corresponding percentages, which are available in the Duhok (KRG, Iraq) marketplace

| Pesticides type | Number of products | % |
|-------------------------|-----------------------|-----|
| Pyrethroids | 72 | 26 |
| Miscellaneous* | 53 | 19 |
| Combination** | 34 | 12 |
| Neonicotinoids | 26 | 9 |
| Organophosphate-like | | |
| compounds (glyphosate) | 20 | 7 |
| Organophosphates | 16 | 6 |
| Avermectins, Milbemycin | 16 | 6 |
| Aryloxy phenoxy- | | |
| propionates | 15 | 5 |
| Benzimidazole (Group 1) | 11 | 4 |
| Triazoles | 8 | 3 |
| Carbamates | 7 | 3 |
| Total | 278 | 100 |

*Miscellaneous (each < 2%).

**Various products contain 2 or more mixtures of pesticides.

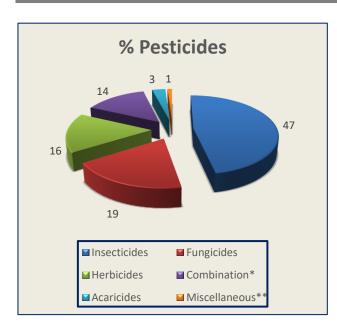


Figure 1: Percentages of classification and commercial types of pesticide products (total 278) available in the market place of Duhok (KRG, Iraq) for agricultural use by farmworkers.

*Various products containing 2 or more mixtures of pesticides [(insecticide, acaricide and miticide), (insecticide, acaricide, nematicide, metabolite, veterinary substance), (insecticide, acaricide), (insecticide, veterinary substance; insect growth regulator), (fungicide, bactericide), (fungicide, insecticide), (fungicide, nematicide)].

**Miscellaneous (<1 %) consisted of molluscicides and nematicide.

Upon meeting the farmers, they were all aware of the possible adverse effects of the pesticides; however, they did not know what to expect after the pesticide exposure, nor did they recognize the potential toxicity of such an exposure. This was reflected by their ignorance (49.0%) of the type (or the use) of pesticides they are dealing with (Table 3). The rest of them (51.0%) were merely aware of the general target use (e.g., insecticide or herbicide) of the pesticide. Most of the farmworkers (75.0%) had an exposure history of > 5 years, and 64.0% were exposed to pesticides within a year (Table 3). Pesticide application was mostly (50.0%) done manually or even openly (41.0%) using a tractor (Table 3). The use of proper PPE was also assessed, and only 54.0% of the farmworkers used some sort of protection, such as gloves, masks, and head covers, but still not a complete one (Table 3). The attitude and behavior of the farmworkers in the field reflected that 32% of them eat and drink at the work area; only 48% practiced the disposal of empty pesticide containers by burning and/or burying them in the field, and strangely enough, 25% of them dumped the containers indiscriminately and unattended in the field, whereas 25% disposed them at open garbage collection sites (Table 3).

Comparison of PChE and EChE activities, as well as plasma TAS levels between the pesticide-exposed farmworkers and the control group, did not reveal significant statistical differences (Tables 4 and 5). However, the plasma MDA level of the pesticide-exposed farmworkers significantly increased by 10% (p=0.022) in comparison with the control value (Table 5).

Table 3: The availability of information on the use of pesticides by farmworkers (n= 92) exposed to pesticide products in a different region of Duhok, KRG, northern of Iraq

| Variable | No. of farmworkers |
|--------------------------------------|--------------------|
| | (%) |
| Types of pesticides used | |
| Unknown | 45 (48.9) |
| Known (general target use) | 47 (51.1) |
| Years of exposure | |
| 1-5 | 23 (25.0) |
| > 5 | 69 (75.0) |
| Last exposure | |
| < 1 month | 30 (32.6) |
| Within a year | 59 (64.1) |
| > 1 year | 3 (3.3%) |
| Type of pesticide application | |
| Manual backpack sprayer | 46 (50.0) |
| Automatic backpack sprayer | 8 (8.7) |
| Using a tractor (open) | 38 (41.3) |
| Use of personal protection equipment | |
| None | 42 (45.7) |
| Some sort of protection | 50 (54.3) |
| Eat and drink in the field | |
| Yes | 29 (31.5) |
| No | 63 (68.5) |
| Disposal of empty containers by | |
| farmworkers | |
| Buried/ burned | 44 (47.8) |
| Dumped indiscriminately | 23 (25.0) |
| Garbage collection sites (open air) | 23 (25.0) |
| Did not know | 2 (2.2) |

Table 4: Comparison of plasma and erythrocyte cholinesterase (ChE) activities (Δ pH/20 min) of pesticide-exposed and agematched unexposed (control) male subjects

| Group | n | Plasma | % | Erythrocyte | % |
|---------|----|--------------|------------|----------------|------------|
| | | ChE | inhibition | ChE | inhibition |
| Control | 44 | $1.10 \pm$ | - | 1.38 ± 0.141 | - |
| | | 0.195 | | | |
| Exposed | 92 | 1.07 \pm | 3 | 1.39 ± 0.101 | 0 |
| _ | | 0.192 | | | |

Values are mean \pm SD

Table 5: Comparison of plasma malondialdehyde (MDA) level and total plasma antioxidant status (TAS) level in pesticideexposed male subjects and age-matched unexposed-controls

| Subjects | \mathbf{n}^{\dagger} | Plasma | p-value | TAS (mmol | p-value |
|----------|------------------------|-------------|---------|----------------|---------|
| | | MDA | | Trolox | |
| | | (µmol/L) | | Equiv/L) | |
| Control | 44 | 2.79 ± | | 1.34 ± 0.354 | |
| | | 0.540 | | | |
| Exposed | 92 | 3.07 ± | < 0.022 | 1.34 ± 0.326 | 0.94 |
| | | 0.677^{*} | | | |

Values are mean ± SD.

†One outlier value from each group was omitted from the statistical analysis.
*Significantly different from the corresponding control value, p < 0.05.

790

It was noticed that farmworkers who did not use any sort of PPE had significantly lower EChE activity by 6% in comparison to the control group (Table 6). The activity of PChE was not significantly different between the two groups (Table 6). When a reduction of 20% in PChE activity ($\leq 0.9 \Delta$ pH/20 min) was taken into consideration (cases) to construct the table of the odds ratio of farmworkers (Table 7), it was found that values of the odds ratio and the risk ratio of occurrence of such reduced PChE activities were 1.33 and 1.26, respectively (Table 8). These values indicated an association between having cases of low PChE activities with applying pesticides in pesticide-exposed farmworkers (Table 8).

Table 6: Comparison of plasma (PChE) and erythrocyte (EChE) cholinesterase activities (Δ pH/20 min) between farmworkers using a method for personal protection (PPE) compared to those not using any protection

| Use of PPE | n (%) | PChE | EChE |
|------------|------------|----------------|----------------------|
| | | | |
| Use PPE | 50 (54.3%) | 1.10 ± 0.189 | 1.43 ± 0.083 |
| | | | |
| None | 42 (45.7%) | 1.05 ± 0.194 | $1.35 \pm 0.099^{*}$ |

Values are mean \pm SD

*Significantly different from those with no protection, p < 0.05.

Table 7: Odds ratio table of the frequency of occurrence of plasma cholinesterase activity (PChE) $\leq 0.9 \Delta \text{ pH/20 min*}$ in pesticide-exposed and non-exposed control subjects

| Groups | $\leq 0.9 \Delta \text{ pH/20 min}$ | > 0.9 \Delta pH/20 min | Total |
|---------|-------------------------------------|------------------------|-------|
| Exposed | 21 (22.8%) | 71 (77.2%) | 92 |
| Control | 8 (18.2%) | 36 (81.8%) | 44 |

*A reduction of 20% in PChE activity was taken into consideration, according to Wilson et al. [28].

Table 8: Results of odds and risk ratios of 20% reduction in plasma cholinesterase (PChE) activity in pesticide-exposed and non-exposed control subjects.

| Parameters | Results |
|-------------------------|--------------|
| Odds ratio | 1.33 |
| 95% confidence interval | 0.537, 3.299 |
| p (ratio =1) | 0.54 |
| | |
| Risk ratio | 1.26 |
| 95% confidence interval | 0.605, 2.607 |
| p (ratio =1) | 0.54 |

Discussion

The present study reflects the diversity of pesticides available in the marketplace of the Duhok region (Iraq) for agricultural use by farmworkers. These pesticides, according to their target use, were mostly insecticides (47.0%). All pesticide products, including insecticides, were available on an over-the-counter basis without the supervision of health authorities or extension services. This condition predisposes users to the potential toxicity of pesticides [2,7,12,33].

The majority of the farmworkers (41.0%) had no formal education, whereas 40.0% had only a primary school education. This low level of formal education (or none at all) precludes the farmworkers from gaining proper information on pesticide

precautions, usage, and application, as well as disposal of pesticide containers [33]. Taking all these factors together, namely, uncontrolled purchase and use of pesticides, disposal practice of containers, and the low educational level, there would be a threat to human health, farm animals, and the environment [2,12,34,35]. Being within the middle age group $(43 \pm 13.5 \text{ years})$, it is strongly recommended to enroll farmworkers of the Duhok region in extension educational programs on the proper use and disposal of pesticides [36].

We noticed that 46% of farmworkers did not use PPE, and the rest did not use PPE properly. Furthermore, the method of pesticide application was manual (50.0%) or even applied openly via a tractor (41.0%). All these practices, together with improper disposal of pesticide containers and the habit of drinking and eating on the farm, unequivocally increase the risk of pesticide exposure through dermal contamination, inhalation, and ingestion of pesticides during the preparation, handling, and application of pesticide products [34,37,38]. It was speculated that the improper use of PPE may be associated with illiteracy or low education level and lack of training [39]. In support of the present notion about using PPE, we found that farmworkers who did not use any sort of PPE had lower EChE activity (6.0%) in comparison to the control group (Table 6). However, the adoption of precautionary measures in dealing with pesticide applications and proper disposal practices would reduce the potential health and environmental risks associated with pesticide use by farmworkers [34,38].

In the present study, PChE and EChE activities of pesticideexposed farmworkers were not significantly different from those of the control group (Table 4). Many studies have reported reduced blood ChE activity in farmers handling organophosphates pesticides, especially the [2.8.15-17,20,22,23,25,31,37,39]. The reason for this discrepancy could be related to the fact that pre-exposure PChE and EChE values of farmworkers of the present study were not known, as measuring pre-exposure ChE activity of farmworkers is not practiced in our region. A significant marginal decrease in PChE (14.0%), but no EChE activity was reported in Duhok region farmers [20]. However, in the present study, as in the previous one [20], the types of the ChE-inhibiting pesticides used (other than calling them insecticides) could not be verified. Within this context, the present study reported that theinhibiting insecticides organophosphates and carbamates comprised only 7.0% and 3.0% of the pesticides used, respectively (Table 2). Usually, reduced blood ChE activity is attributed to organophosphates since, contrary to carbamates, they are non-reversible ChE inhibitors [5,25-28]. Furthermore, in accordance with previous studies in Iraq [16-18,20], acupesticide intoxication of farmworkers was not reported in the present study. Therefore, we can deduct from these reports and ours that exposure of farmworkers to pesticides was limited in intensity and/or toxic doses involved.

According to the current situation involving the present interpretation of blood ChE activities, we strongly recommend establishing an Iraqi national plan such as that of California to assess the health status of farmworkers and prevent their overexposure to insecticides by recording their pre-exposure blood ChE activities for future post-exposure definitions and comparisons [28,37,40,41]. In support of such a national plan, the marginal reduction of EChE activity (6.0%) in farmworkers

not using any PPE in the present study could have been subjected to a different interpretation if the pre-exposure ChE activity (baseline) were available. Additional support was introduced by the finding of the present study that the assessment of odds and the risk ratios of occurrence of reduced PChE activity (20.0%) were 1.33 and 1.26, respectively, suggesting, in spite of the wide 95.0% CI, an association of having low PChE activity with pesticide application in pesticide-exposed farmworkers. A similar finding was reported when an association between blood ChE activity and organophosphate pesticide residues was examined in Thailand (Adjusted OR = 2.09, 95%CI: 0.63-6.99) [42]. Furthermore, a study conducted in Ethiopia on women farmers exposed to pesticides did not find significant changes in blood ChE activities in comparison to the non-exposed group [43]. Also, one study reported in a farm that active pesticide sprayers (4 out of 47), but not other pesticide handlers, had low PChE activity [44]. However, a cut point of 20% reduction in blood ChE activity was challenged by others, and it was found that a 15% reduction could be a better indicator for the expected significant change [45]. Nonetheless, according to the Washington State Department of Labor and Industries, the 20% reduction in an individual's blood ChE activity from baseline value suffices to initiate inquiries addressing the worker and workplace conditions [37].

A comparison of plasma MDA and TAS levels between the pesticide-exposed farmworkers and the control group revealed a significant increase in the MDA level (10%) with no changes in the TAS level (Table 5). These results corresponded to the nonsignificant changes we noticed in the PChE and EChE activities of the same farmworkers. However, farmworkers exposed to pesticides with reduced blood ChE activities were reported to show concomitantly oxidative stress, which was revealed as increases in oxidative stress biomarkers in the blood such as plasma MDA level, TAS, and total antioxidant capacity [8,23,46,47]. It is worth mentioning that nonsignificant changes in oxidative stress biomarkers could be expected concomitantly with no ChE inhibition [43]. According to the findings of the present study with regards to oxidative biomarkers, which showed a marginal increase in the plasma MDA level, it is recommended to further include other blood chemical and biochemical tests to evaluate the health status of farmworkers more properly. Indeed, we attempted this approach in our exposed subjects and found changes in serum creatinine and some electrolyte levels (MSc thesis of the present 1st author, unpublished data). Because of the potential occupational health risks of pesticides, several studies have also reported changes in biochemical blood variables other than ChE activity and oxidative stress biomarkers [48-50]. The present study suggests that regular monitoring of blood biochemical parameters and ChE activity as health risk assessments could be effective measures to control the exposure of farmworkers to pesticides. Last, we were unable to perform 30-day and long-term followup, which should be aimed at further studies.

Conclusion

Various types of pesticides are available for use by farmworkers, who do not abide completely by protection rules, and the most commonly used pesticides were pyrethroids, and ChE-inhibiting insecticides were of lower use and application. The farmworkers, with only a marginal increase in the oxidative stress biomarker MDA, did not suffer from significant reductions in blood ChE activities, although odds and risk ratios of reduced PChE activity suggested a health risk in pesticide-exposed ones. We strongly recommend the implementation of a national program to measure pre-exposure blood ChE activities and possibly other blood biochemical variables in farmworkers engaged in pesticide application and/or handling.

Abbreviation

ChE: Cholinesterase; EChE: Erythrocyte cholinesterase; MDA: malondialdehyde; PChE: Plasma cholinesterase; SD: Standard deviation; TAS: Total antioxidant status.

Declaration

Acknowledgment

The authors greatly acknowledge the cooperation of all study subjects involved in donating blood samples for the present research project. The authors thank the College of Pharmacy for the support and for providing facilities to conduct this research. This report represents a portion of a thesis to be submitted by the first author to the University of Duhok, KRG, northern of Iraq, in partial fulfillment of the requirements for an MSc degree in Toxicology.

Funding

The study was supported by the University of Duhok, Duhok, KRG, Iraq.

Availability of data and materials

Data will be available by emailing semona.91@gmail.com.

Authors' contributions

Simona Khamo Odisho (SKO) executed the survey and laboratory assays, acquired the data, conducted literature search and statistical analyses, and shared in drafting the manuscript. Fouad K. Mohammad (FKM) conceptualized and supervised the study, shared in literature search and statistical analyses, and drafted the manuscript. Both authors have read and approved the final version of the manuscript. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

We conducted the research following the Declaration of Helsinki. The protocol of the study was approved by the Research Ethics Committee, Duhok Directorate General of Health, Ministry of Health, KRG, Iraq (No. 10112021-11-2 on November 10, 2021) as well as by the Committee of Post Graduate Studies in the College of Pharmacy, University of Duhok, KRG, Iraq (No. 535, October 28, 2021) with the approval of the University of Duhok (No. 8813, October 31, 2021). Written consent consented from individuals participate who d in the study.

Consent for publication

Not applicable

Competing interest

The authors declare that they have no competing interests.

Open Access

This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article unless otherwise stated.

Author Details

¹Department of Pharmacology, College of Pharmacy, University of Duhok, KRG, Iraq.

²Department of Physiology, Biochemistry and Pharmacology, College of Veterinary Medicine, University of Mosul, Mosul, Iraq.

Article Info

Received: 21 November 2022 Accepted: 27 December 2022 Published: 29 December 2022

References

- Pandya IY. Pesticides and their applications in agriculture. Asian J Appl Sci Technol. 2018;2(2):894-900.
- Rashid S, Rashid W, Tulcan RXS, Huang H. Use, exposure, and environmental impacts of pesticides in Pakistan: a critical review. Environ Sci Pollut Res Int. 2022;29(29):43675-43689. doi: 10.1007/s11356-022-20164-7.
- Tudi M, Daniel Ruan H, Wang L, Lyu J, Sadler R, Connell D, Chu C, Phung DT. Agriculture development, pesticide application and its impact on the environment. Int J Environ Res Public Health. 2021;18(3):1112. doi: 10.3390/ijerph18031112.
- Baynes RE. Ectoparasiticides. In: Riviere JE, Papich MG (Eds). Veterinary pharmacology and therapeutics. 10th edition. Hoboken, NJ, USA: Wiley Blackwell & Son, Inc. 2018; 1166-1187.
- Wilson BW. Cholinesterase inhibition. In: Wexler P (Ed). Encyclopedia of toxicology. 3rd ed. Amesterdam: Elsevier 2014; 942-951.
- WHO. WHO recommended classification of pesticides by hazard and guidelines to classification, 2019 edition. Geneva: World Health Organization; 2020.
- WHO. Global situation of pesticide management in agriculture and public health. Geneva: World Health Organization and Food and Agriculture Organization of the United Nations; 2019.
- Samareh A, Asadikaram G, MojtabaAbbasi-Jorjandi, Abdollahdokht D, Abolhassani M, Khanjani N, Nematollahi MH. Occupational exposure to pesticides in farmworkers and the oxidative markers. Toxicol Ind Health. 2022;38(8):455-469. doi: 10.1177/07482337221106754.
- Rajmohan KS, Chandrasekaran R, Varjani S. A review on occurrence of pesticides in environment and current technologies for their remediation and management. Indian J Microbiol. 2020;60(2):125-138. doi: 10.1007/s12088-019-00841-x.
- Saravi SSS, Shokrzadeh M. Role of pesticides in human life in the modern age: a review. In: Stoytcheva M, editor. Pesticides in the modern world - risks and benefits Rijeka, Croatia: In Tech; 2011.
- Ecobichon DJ. Pesticide use in developing countries. Toxicology. 2001;160(1-3):27-33. doi: 10.1016/s0300-483x(00)00452-2.
- Ben Khadda Z, Fagroud M, El Karmoudi Y, Ezrari S, Berni I, De Broe M, Behl T, Bungau SG, Sqalli Houssaini T. Farmers' knowledge, attitudes, and perceptions regarding carcinogenic pesticides in Fez Meknes Region (Morocco). Int J Environ Res Pub Health. 2021;18(20):10879. doi: 10.3390/ijerph182010879.
- Jongerden J, Wolters W, Dijkxhoorn Y. Explorative study agricultural development in Iraq and the Federal Kurdistan Autonomous Region; 2018. https://www.government.nl/documents/reports/2018/11/09/explor ative-study-agricultural-development-in-iraq-and-the-federalkurdistan-autonomous-region (accessed 7.8.2021).
- Ahmed ZFG, Majeed BK. A survey study of pesticide application pattern in selected plastic houses in Sulaimani governorate/Iraq. J Z Sulaimani. (University of Sulaimani). 2020;2: 63-76.
- Lopes-Ferreira M, Maleski ALA, Balan-Lima L, Bernardo JTG, Hipolito LM, Seni-Silva AC, Batista-Filho J, Falcao MAP, Lima C. Impact of pesticides on human health in the last six years in Brazil. Int J Environ Res Pub Health. 2022;19(6):3198. doi: 10.3390/ijerph19063198.

- Ahmed AS. Evaluation of acetylcholine esterase activity in the blood of workers exposed to organophosphate and carbamate insecticides by an electrometric method. Kirkuk J Sci Stud. (University of Kirkuk). 2013;3(3): 26-33.
- Ahmed OAH, Mohammad FK. Electrometric determination of blood cholinesterase activities in workers exposed to insecticides in Mosul, Iraq. R J Env. Toxicol. 2007;1: 144-148. https://dx.doi.org/10.3923/rjet.2007.144.148
- Al-Haseni ANA, Yahya BM. Measurement of plasma cholinesterase activity in field workers. Iraqi J Pharm. 2012;12(1):41-47.
- Othman BA, Kakey ES. Environmental pesticides residues and health biomarkers among farmers from greenhouses of Erbil cucumber crops. Iraqi J Agric Sci. 2020;51(5):1357-1366.
- Mohammed AA, Mohammad FK. Monitoring blood cholinesterase activity of farmworkers: in vitro inhibition by diphenhydramine and carbaryl. Malaysian Appl Biol. 2022;51:23-32.
- Cotton J, Edwards J, Rahman MA, Brumby S. Cholinesterase research outreach project (CROP): point of care cholinesterase measurement in an Australian agricultural community. Environ Health. 2018;17(1):31. doi: 10.1186/s12940-018-0374-1.
- Assis CRD, Linhares AG, Cabrera MP, Oliveira VM, Silva KCC, Marcuschi M, Maciel Carvalho EVM, Bezerra RS, Carvalho LB Jr. Erythrocyte acetylcholinesterase as biomarker of pesticide exposure: new and forgotten insights. Environ Sci Pollut Res Int. 2018;25(19):18364-18376. doi: 10.1007/s11356-018-2303-9.
- Abbasi-Jorjandi M, Asadikaram G, Abolhassani M, Fallah H, Abdollahdokht D, Salimi F, Faramarz S, Pournamdari M. Pesticide exposure and related health problems among family members of farmworkers in southeast Iran. A case-control study. Environ Pollut. 2020; 267:115424. doi: 10.1016/j.envpol.2020.115424.
- Kapka-Skrzypczak L, Cyranka M, Skrzypczak M, Kruszewski M. Biomonitoring and biomarkers of organophosphate pesticides exposure - state of the art. Ann Agric Environ Med. 2011;18(2):294-303. PMID: 22216802.
- Jaga K. Dharmani C. Sources of exposure to and public health implications of organophosphate pesticides. Revista Panamericana de Salud Publica. (Pan Am J Pub Health). 2002;14(3):171-185.
- Vale A, Lotti M. Organophosphorus and carbamate insecticide poisoning. Handb Clin Neurol. 2015;131:149-68. doi: 10.1016/B978-0-444-62627-1.00010-X.
- Wilson, BW. Cholinesterase inhibition. In: Wexler P (Ed). Encyclopedia of toxicology. 3rd ed, Amesterdam: Elsevier; 2014. pp. 942-951.
- Wilson BW, Arrieta DE, Henderson JD. Monitoring cholinesterases to detect pesticide exposure. Chemico-biol Interac. 2005 Dec;157-158:253-256. DOI: 10.1016/j.cbi.2005.10.043. PMID: 16298353.
- Mohammad FK, Alias AS, Ahmed OA. Electrometric measurement of plasma, erythrocyte, and whole blood cholinesterase activities in healthy human volunteers. J Med Toxicol. 2007;3(1):25-30. doi: 10.1007/BF03161035.
- Mohammad FK. Clarifying an electrometric method for determining blood cholinesterase activity: a scientific letter. Asia Pac J Med Toxicol. 2022;11(1):30-32. doi: 10.22038/apjmt.2022.19924
- Ciesielski S, Loomis DP, Mims SR, Auer A. Pesticide exposures, cholinesterase depression, and symptoms among North Carolina migrant farmworkers. Am J Public Health. 1994;84(3):446-51. doi: 10.2105/ajph.84.3.446.
- Buege JA, Aust SD. Microsomal lipid peroxidation. Methods Enzymol. 1978; 52:302-10. doi: 10.1016/s0076-6879(78)52032-6.
- 33. doi: 10.2105/ajph.84.3.446. PMID: 8129063; PMCID: PMC1614838.
- Imoro ZA, Larbi J, Duwiejuah AB. Pesticide Availability and Usage by Farmers in the Northern Region of Ghana. J Health Pollut. 2019 Aug 6;9(23):190906. doi: 10.5696/2156-9614-9.23.190906.
- Buczyńska A, Szadkowska-Stańczyk I. Identification of health hazards to rural population living near pesticide dump sites in

Poland. Int J Occup Med Environ Health. 2005;18(4):331-9. PMID: 16617849.

- Atreya K, Sitaula BK, Bajracharya RM. Pesticide use in agriculture: the philosophy, complexities and opportunities. Sci Res Essays. 2012;7(25):2168–73.
- Remoundou K, Brennan M, Hart A, Frewer LJ. Pesticide risk perceptions, knowledge, and attitudes of operators, workers, and residents: a review of the literature. Hum Ecol Risk Assess. 2014;20(4):1113–38. doi: 10.1080/10807039.2013.799405.
- Environmental Protection Agency. Pesticides: health and safety. National Assessment of the Worker Protection Workshop #3. Washington DC, USA: EPA; 2007.
- Sharma N, Singhvi R. Effects of chemical fertilizers and pesticides on human health and environment: a review. Int J Agric Environ Biotechnol. 2017;10(6):675–9.
- Neupane D, Jørs E, Brandt L. Pesticide use, erythrocyte acetylcholinesterase level and self-reported acute intoxication symptoms among vegetable farmers in Nepal: a cross-sectional study. Environ Health. 2014; 13:98. doi: 10.1186/1476-069X-13-98.
- Lessenger JE. Fifteen years of experience in cholinesterase monitoring of insecticide applicators. J Agromedicine. 2005;10(3):49-56. doi: 10.1300/j096v10n03_06.
- Lessenger JE, Reese BE. Rational use of cholinesterase activity testing in pesticide poisoning. J Am Board Fam Pract. 1999;12(4):307-14. doi: 10.3122/jabfm.12.4.307.
- 43. Nganchamung T, Robson MG, Siriwong W. Association between blood cholinesterase activity, organophosphate pesticide residues on hands, and health effects among chili farmers in Ubon Ratchathani Province, northeastern Thailand. Rocz Panstw Zakl Hig. 2017;68(2):175-183. PMID: 28646835.
- 44. Martin-Reina J, Casanova AG, Dahiri B, Fernández I, Fernández-Palacín A, Bautista J, Morales AI, Moreno I. Adverse health effects in women farmers indirectly exposed to pesticides. Int J

Environ Res Public Health. 2021; 18(11):5909. https://doi.org/10.3390/ijerph18115909

- Mekonnen Y, Ejigu D. Plasma cholinesterase level of Ethiopian farm workers exposed to chemical pesticide. Occup Med (Lond). 2005;55(6):504-5. doi: 10.1093/occmed/kqi088.
- 46. Quandt SA, Chen H, Grzywacz JG, Vallejos QM, Galvan L, Arcury TA. Cholinesterase depression and its association with pesticide exposure across the agricultural season among Latino farmworkers in North Carolina. Environ Health Perspect. 2010;118(5):635-9. doi: 10.1289/ehp.0901492.
- 47. Abdollahdokht D, Asadikaram G, Abolhassani M, Pourghadamyari H, Abbasi-Jorjandi M, Faramarz S, Nematollahi MH. Pesticide exposure and related health problems among farmworkers' children: a case-control study in southeast Iran. Environ Sci Pollut Res Int. 2021;28(40):57216-57231. doi: 10.1007/s11356-021-14319-1.
- Curl CL, Spivak M, Phinney R, Montrose L. Synthetic Pesticides and Health in Vulnerable Populations: Agricultural Workers. Curr Environ Health Rep. 2020;7(1):13-29. doi: 10.1007/s40572-020-00266-5.
- Dereumeaux C, Mercier F, Soulard P, Hulin M, Oleko A, Pecheux M, Fillol C, Denys S, Quenel P. Identification of pesticides exposure biomarkers for residents living close to vineyards in France. Environ Int. 2022; 159:107013. doi: 10.1016/j.envint.2021.107013.
- Soulard P, Dereumeaux C, Mercier F. Determination of biomarkers of exposure to boscalid, captan, folpel, mancozeb, and tebuconazole in urine and hair samples. MethodsX. 2022; 9:101671. doi: 10.1016/j.mex.2022.101671.
- Hayat K, Afzal M, Aqueel MA, Ali S, Saeed MF, Qureshi AK, Ullah MI, Khan QM, Naseem MT, Ashfaq U, Damalas CA. Insecticide toxic effects and blood biochemical alterations in occupationally exposed individuals in Punjab, Pakistan. Sci Total Environ. 2019; 655:102-111. doi: 10.1016/j.scitotenv.2018.11.175.