ORIGINAL PAPER

International Journal of Occupational Medicine and Environmental Health 2017;30(4):603–615
https://doi.org/10.13075/ijomeh.1896.00810

IMMUNOLOGICAL AND GENOTOXIC EFFECTS OF OCCUPATIONAL EXPOSURE TO α-CYPERMETHRIN PESTICIDE

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Abstract

Objectives: The aim of this work has been to find out the occupational oxidative stress, immunological and genotoxic health hazards among α-cypermethrin (CYP) pesticide-exposed workers. **Material and Methods:** A cross-sectional study was performed including 200 workers divided into 3 groups according to the level of exposure: highly exposed group (50 workers), moderately exposed group (50 workers) and unexposed group (100 workers). All workers were subjected to detailed laboratory investigation for gene P53 mutations, immunological parameters as a cluster of differentiation into 3 percentage (CD3%), CD4% and CD8% in addition to peripheral blood total leukocytic and platelet counts that were measured. Spectrophotometer technique was used for detection of superoxide dismutase (SOD), catalase (CAT), glutathione (GSH) and glutathione peroxidase (GPx). Air samples were collected with a High Volume Small Surface Sampler for measurement of α-cypermethrin level. **Results:** A highly exposed group to the α-cypermethrin had lower CD4/CD8 as compared to an unexposed group with statistically significant difference. As regards gene mutation, exons 5a and 6 were more frequent among the highly exposed group as compared to no mutation among moderately exposed and unexposed groups with significant difference. As regards antioxidants; SOD, CAT, GSH and GPx were higher among the unexposed group as compared to the highly and moderately exposed group with statistically significant difference. Significant negative correlation was found between working years and antioxidant parameters. **Conclusions:** Repeated exposure to α-CYP may lead to gene mutations, immunological disturbances and oxidative stress. Strict safety precautions are required not only for workers but also for public users. Int J Occup Med Environ Health 2017;30(4):603–615

Key words:
P53, Pyrethroids, Immunological, Genotoxic, Cypermethrin, Oxidative stress

INTRODUCTION

α-Cypermethrin pesticide (CYP) is a widely used synthetic pyrethroid II insecticide worldwide. It is mainly used in agriculture and home pest control [1]. Occupational exposure to CYP may occur during preparation, mixing and transportation of the product. It may also occur during application of the product in farms for pest control [2].

α-Cypermethrin pesticide may be absorbed through inhalation, ingestion or skin contact. Accordingly, strict safety precautions and work hygiene should be enforced to control health hazards [3]. In addition, application of local

Received: October 4, 2015. Accepted: May 19, 2016.
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laws and regulations in the workplace is very important. However, this is extremely difficult in absence of a known permissible exposure level for CYP [4].

According to the World Health Organization (WHO) safety report, CYP has been classified as grade II or moderate toxicity chemical [1]. Acute symptoms may occur especially with poor work conditions like skin, eye or respiratory system irritation. Research has confirmed that CYP is a complete carcinogenic agent that may lead to either tumor initiation or promotion in mammals [5]. Genotoxic effect of CYP has also been reported. Recent studies have found that α-cypermethrin itself could form deoxyribonucleic acid (DNA) adducts and when metabolized through the cytochrome P450 microsomal system, it may lead to DNA cross links and oxidative stress [6,7]. However, the exact mechanism of CYP genotoxicity is still not well known. In addition, Raina et al. [8] in 2009 have studied the impact of the CYP chronic exposure on the occurrence of oxidative stress in experimental rat brain and blood. They have marked reduction of glutathione (GSH) and lipid peroxidation [8]. Reproductive effects of CYP have been investigated in many experimental research projects that have concluded possible disturbance and direct effect of CYP on the reproductive system [9].

Egypt is considered to be one of the highest countries in importing and using pesticides. More than 8600 t of pesticides, with 148 different including pyrethroids are available in the Egyptian market per year [10]. In spite of this high availability and usage of pesticides which are associated with high occupational and non-occupational exposure, few epidemiological research projects studying health effects of pyrethroids, and in particular CYP, are available. Accordingly, the current study may be essential to highlight the health effects associated with long-term exposure to CYP products among working population.

The aim of this study is to investigate oxidative stress, immunological and genotoxic effects of CYP among exposed workers.

MATERIAL AND METHODS

Study design, timing, population and setting

A comparative cross-sectional study was conducted from February to August 2014. The study included workers at different departments of a CYP production factory in Egypt. The sample size (N) was calculated according to the following formula [11]:

$$N = \frac{t^2 \times p(1-2)}{m^2} \quad (1)$$

where:

t – confidence level at 95% (standard value of 1.96),

p – estimated prevalence of pesticides induced hazards,

m – margin of error at 5% (standard value of 0.05).

Accordingly, the total sample was estimated to be 200 workers divided into 3 groups according to their level of exposure:

- Group 1 (highly exposed) included 50 workers working in the raw materials weighing and production departments. They were highly exposed to α-CYP and not exposed to any other pesticide or solvent.
- Group 2 (moderately exposed) included 50 workers working in the packaging and storage departments. They had moderate exposure to α-CYP and not exposed to any other pesticide or solvent.
- Group 3 (unexposed) included 100 workers working in administrative departments. They were not exposed to α-CYP or any other pesticide or solvent.

All workers were pooled in a common frame by the identification (ID) number and simple random selection was computed to allocate workers to be included.

Study methods

An interviewed questionnaire was used for collecting data about socio-demographic data, smoking and occupational history, use of protective equipment and relevant medical history of all participating workers.

Environmental monitoring of the residual indoor air CYP concentrations was done. Air samples were collected by means of a High Volume Small Surface Sampler (HVS3) from different random sites covering all facility areas:

– area I: raw materials weighing and production departments,
– area II: packaging and storage departments.

Sampling method

Collected dust particles containing CYP residues were manually sieved to obtain the fine fractions (< 150 μm) which are more likely to adhere to human skin, followed by ultrasonication extraction. Finally, the concentrated extracts were analyzed with an electron impact gas chromatography mass spectrometer in the multiple ion detection modes (Phenomenex company ZB-35 column, 30 m × 0.25 mm, 0.25 μm film) with temperatures adjusted from 130–340°C at 6°C/min.

Sample analysis

Working range standards were prepared by diluting stock solutions with desorbing solution. Stock and dilute standards were stored in a freezer. The glass fiber filter of 13-mm and the 270-mg sampling section of the tube were then transferred to a 4-ml vial. The first foam plug and the 140-mg backup section were placed in a separate vial. The instrument was adjusted according to the following program: injector temperature at 25°C, detector temperature at 300°C, column temperature at 230°C, hydrogen flow rate at 11:1, and the injection volume was 1 ug. Detector response was measured using electronic integration. A calibration curve was constructed by plotting concentration of analyte per 1 ml vs. response of standard concentration in μg/ml of α-cypermethrin samples with prepared analytical standards over a range of concentrations. The pg/ml of α-cypermethrin in both sections of each sample was determined and blanked from the calibration curve. α-Cypermethrin found on the backup section was added to the amount found on the front section. Blank corrections were performed before adding the results together.

Evaluation of oxidative stress

Blood samples were collected from all participating workers for determining the enzymatic activities of the superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), and glutathione peroxidase (GPx). Collected blood samples were centrifuged after clotting at 3000 rpm for 15 min using a cooling centrifuge to separate the corresponding sera samples.

Determining the enzymatic activities of SOD

The collected sera samples were divided and transferred into small vials. The phenazine methosulfate (PM) was diluted 100 times immediately with distilled water before use (0.1 ml + 9.9 ml distilled water). Sera samples were also diluted to give an inhibition between 30–60%. Test tubes were prepared for each serum sample vial by adding 1 ml of buffer (pH 8.5) to 0.1 ml of nitroblue tetrazolium and 0.1 ml of nicotinamide adenine dinucleotide (NADH), then 0.05 ml of each serum sample was dispensed to the corresponded test tube and mixed very well using the mechanical vortex. The reaction was initiated by adding 0.01 ml of phenazine methosulfate to each test tube. A control tube was also prepared for comparison results. A part of each test tube, either the samples or control, was poured into 1 cm path length cuvette, and the increase in absorbance was measured using the ultraviolet (UV) spectrophotometer [12].

Determining the enzymatic activities of CAT

The chromogen buffer was prepared by mixing 100 mM/l of phosphate buffer (pH 7.0) with 1 mM/l of detergent using mechanical vortex and H_2O_2 was diluted 1000 times with distilled water immediately before use (10 ul + 10 ml distilled water). Then 0.5 ml of the prepared chromogen

buffer was added to the sample test tube and mixed very well using mechanical vortex. The sample test tube was put in the electric incubator at 25°C for exactly 1 min. 0.1 ml of catalase inhibitor was added to the sample test tube then 0.5 ml of peroxidase enzyme was mixed using mechanical vortex. The sample test tube was put in the electric incubator at 37°C for 10 min. Finally, the sample test content was poured into 1 cm path length cuvette to be read using the UV spectrophotometer [13].

Determining the enzymatic activities of GSH

Glutathione was measured using enzyme-linked immunosorbent assay (ELISA) method – 270 µl ice-cold 5% metaphosphoric acid (MPA) was added to the serum sample tube and mechanical vortex was used briefly (dilution factor = 4) then centrifuged at 1000×g at 4°C for 10 min. Then 50 µl of the supernatant was added to 700 µl assay buffer in a new micro-centrifuge tube (dilution factor = 15, to make the final dilution factor = 60). The diluted extract was placed on ice before use and the GSH sample (50 µl) was thawed and mixed immediately. Then 350 µl ice cold 5% MPA was added to the micro-centrifuge tube and mechanical vortex was used briefly (dilution factor = 8) then centrifuged at 1000×g at 4°C for 10 min. Then 25 µl of the supernatant was added to 1.5 ml assay buffer in a new micro-centrifuge tube (dilution factor = 61, to make the final dilution factor = 488). The diluted extract was placed on ice before use. The standard curve was prepared by labeling dilution tubes and dispensing the indicated volumes of the assay buffer.

Determining the enzymatic activities of GPx

Glutathione peroxidase enzymatic activity was determined using ELISA method. Assay reagents were prepared by adding 500 µl assay phosphate buffer to glutathione peroxidase positive control tube, then mixed well by mechanical vortex and kept it into ice. The caliber was mixed with 188 µl of distilled water (equivalent to 6 mM nicotinamide adenine dinucleotide phosphate – NADPH) and 10 µl of the diluted caliber were transferred into wells of a clear flat-bottom 96-well plate, then 190 µl of assay phosphate buffer were added to all wells. Then 10 µl of sample serum and 10 µl of reconstituted GPx positive control were then transferred into separate wells of the 96-well plate, including a background control that only contained 10 µl assay buffer. Efficiently working reagents for samples and controls wells were prepared by mixing 85 µl assay buffer, 2 µl glutathione, 2 µl 35 mM NADPH and 8 µl of glutathione reductase enzyme for each well. Using a multi-channel pipette, 100 µl of 0.35 mM H_2O_2 reagent was added to all samples and control wells [14].

Detection of P53 gene mutations

Step 1 – DNA extraction from blood

Ethylenediaminetetraacetic acid (EDTA)-blood samples of 200 µl in volume were mixed very well with 800 µl of cell lysis buffer in a sterile test tube. The lysed EDTA-blood samples were incubated in ice bath for 10 min. The mixture in the sterile test tube was centrifuged at 5000 rpm for 15 min by using of a cooling centrifuge. Then 600 µl of nuclei lysis buffer (NLB) was dispensed to the mixture in the sterile test tube. The sediment (the lysed cells) were re-suspended, 2.16 µl of 20% sodium dodecyl sulfate (SDS) and 5 µl of proteinase-K (PK) were added to the lysed cells in the test tube and were incubated for 1 h at 55°C. After incubation, 200 µl of sodium chloride was dispensed to the test tube content and was mixed using electric vortex for 15 s. The whole of test tube content was centrifuged at 5000 rpm for 15 min at 4°C. The supernatant was transferred into a new sterile test tube at which a double volume of 10% ethanol was dispended and mixed very well with the supernatant. The new mixture was centrifuged at 10 000 rpm for 20 min at 4°C. The resulted pellets were washed with 70% ethanol and then centrifuged at 9500 rpm for 5 min at 4°C. The washed pellets were

dried after discarding of the supernatant and re-suspended in 50 μl of Tris-EDTA (TE).

Step 2 – Polymerase chain reaction (PCR)

Each specific primer sequences for P53 tumor suppressor gene of 1 μl in volume were mixed well with 1 μl of isolated DNA (treated pellets) from the last step in PCR-tubes, then the volume was completed to 20 μl of water for injection (WFI). The thermal cycler was adjusted according to special program parameters; the incubation condition was at 94°C for 10 min to fix the linearity of DNA and numbers of cycles were 30 cycles. Each cycle included 3 steps: denaturation step (94°C for 30 s) at which the DNA base pairs were broken and the single stranded DNA was released to act as a template, annealing step (55°C for 30 s) at which the primers were hyperized, one to each complementary strand of the DNA so that each primer was attached to its complementary templates, and an extension step (72°C for 30 s) at which DNA was synthesized by Taq-polymerase. At the same time another DNA synthesis (*de-novo* synthesis) was accelerated to a maximal velocity by using a Taq-polymerase and the specified nucleotides. During this *de-novo* DNA synthesis, another DNA strand was created complementarily to the original DNA strand.

Step 3 – Single stranded conformational polymorphism (SSCP) staining

The target of this step was detecting the amplified fragment of the DNA by using 2% agarose gel electrophoresis. This step was performed in 2 stages: loading and running stage and silver staining of the gel.

Finally, evaluation of P53 gene exons mutations was done by comparing the resulted scanned bands with certain standard known bands submitted with the primers sequences for P53 tumor suppressor gene, the evaluation was available to judge on the exons (5a, 5b, 6, 7, 8) if there were possibilities for mutations occurrence [15].

Hematological parameters

Total leukocyte count (TLC)

EDTA-blood samples of 20 μl in volume were mixed well with 0.38 ml of lymphocytes diluting buffer in a test tube for 2 min using a mechanical vortex. Diluted EDTA-blood samples were dispensed into the chambers of the hemocytometer slide with its cover glass in position. The diluted blood samples were left for 2 min to settle. The white blood cells were counted in the four large squares on both sides of the hemocytometer slide chamber by using a binocular microscope and the counting was based on the area measured and the dilution factor used [16].

Platelets count

EDTA-blood samples of 20 μl in volume were mixed well with 0.38 ml of platelets diluting buffer in a test tube for 2 min using a mechanical vortex. The diluted EDTA-blood samples were dispensed into the hemocytometer chambers and covered with a Petri dish for 10 to 20 min before examination to allow the platelets to be settled. A piece of wet cotton was left in the petri dish to prevent evaporation. The platelets were counted by using a binocular microscope in the large squares of 1 mm of the hemocytometer slide. Platelets counting was applied in many squares to reach granular structure.

Immune phenotyping assessment of CD3, CD4 and CD8 using flowcytometry technique

EDTA-blood samples of 50 μl in volume containing up to 10 000 cells were mixed with specific monoclonal antibodies in a test tube. This mixture was shacked gently, and incubated at a dark place for 30 min. Then 1–2 ml of erythrocytes lysing reagent was added to each test tube and was mixed gently. The mixture included lysed erythrocytes and was centrifuged at 1500 rpm for 5 min using a cooling centrifuge. The supernatant was aspirated leaving about 50 μl of solution. The phosphate buffer saline of 3 ml in volume was added to the remaining solution and the new

mixture was shacked gently and centrifuged at 1500 rpm for 5 min. The supernatant was aspirated leaving about 50 µl of the solution. At the same time, there was another test tube (control tube) without monoclonal antibodies adjusted in order to obtain basic histogram showing the main cells population and to adjust the auto-fluorescence region.

The argon laser (488 nm) of the flowcytometry machine; Fluorescence activated cell sorter (FACSCalibur immunocytometry systems) was warmed up for 30 min before use and a full alignment method was performed using the standard flow-check alignment fluorospheres for adjusting forward scatter. Other tubes were introduced to the machine, at which about 10 000 cells were passed in front of the laser for each case. From these cells, only the lymphocytes were selectively gated for the immunophenotyping analysis of the studied markers cluster of differentiation into 3 (CD3), CD4 and CD8, either total percentage or its absolute count in cells/cm according to their forward angle scatter (FSC) and side angle light scatter (SSC) [17].

Ethical considerations

Written consent was signed by workers after explanation of the aim of the study and confidentiality of information was guaranteed.

Statistical analysis

The analysis of data was done by means of an IBM computer using SPSS v. 16. Description of quantitative variables as mean (M), standard deviation (SD) and description of qualitative variables as the number and percentage share; Chi^2 test was used for comparing qualitative variables between groups. One way ANOVA-test was used for comparing quantitative variables having more than 2 groups with *post hoc* least significant difference (LSD) test [18].

RESULTS

The participating workers included 200 workers with average age of 37±10.7 years old, the majority of them were males 175 (87.5%) and 25 (12.5%) of them were females. The average work duration was 8.3±3.6 years. More than 95% of workers lived in Greater Cairo, while 5% lived outside Cairo. There is no statistically significant difference between the studied groups as regards their age, gender, residence, body mass index (BMI), smoking habit, working duration and use of personal protective equipment (PPE) ($p > 0.05$) (Table 1).

The mean residual air CYP concentrations in the area I (high exposure area) and area II (moderate exposure area) were 1.3±0.5 µg/m^3 and 0.8±0.40 µg/m^3, respec-

Table 1. Socio-demographic and occupational data of the workers exposed to α-cypermethrin pesticide and control group

Characteristics	Group 1 (N = 50)	Group 2 (N = 50)	Group 3 (N = 100)	p*
Age [years] (M±SD)	38±8.6	37±7.3	36.7±7.9	0.37
Gender [n (%)]				0.53
male	42 (84)	43 (86)	90 (90)	
female	8 (16)	7 (14)	10 (10)	
Residence [n (%)]				0.39
Greater Cairo	48 (96)	50 (100)	97 (97)	
outside Cairo	2 (4)	0	3 (3)	
Smoking [n (%)]	36 (72)	40 (80)	75 (75)	0.39
Body mass index [kg/m^2] (M±SD)	28.0±4.3	29.1±5.0	28.5±3.9	0.56

Table 1. Socio-demographic and occupational data of the workers exposed to α-cypermethrin pesticide and control group – cont.

Characteristics	Group 1 (N = 50)	Group 2 (N = 50)	Group 3 (N = 100)	p*
Use of personal protective equipment [n (%)]				0.54
no	31 (62)	26 (52)	60 (60)	
yes	19 (38)	24 (48)	40 (40)	
Seniority [years] (M±SD)	9.3±3.0	8.1±4.0	8.9±4.3	0.23

Group 1 (highly exposed) – working in the raw materials weighing and production departments, highly exposed to α-CYP and not exposed to any other pesticide or solvent.
Group 2 (moderately exposed) – working in the packaging and storage departments, moderately exposed to α-CYP and not exposed to any other pesticide or solvent.
Group 3 (unexposed) – working in administrative departments, not exposed to α-CYP or any other pesticide or solvent.
M – mean; SD – standard deviation.
* Significant difference at $p \leq 0.05$.

Table 2. Residual air α-cypermethrin concentration in different workplace areas

Area	α-Cypermethrin concentration [μg/m^3]		
	M±SD	min.	max
I	1.3±0.5	0.20	2.20
II	0.8±0.4	< LOD	2.00

Area I – raw materials weighing and production departments.
Area II – packaging and storage departments.
min. – minimal value; max – maximal value.
LOD – limit of detection ≤ 0.014 μg/m^3.
Other abbreviations as in Table 1.

tively (Table 2). Group 1 (high CYP exposure) had lower CD4/CD8 as compared to the unexposed group with statistically significant difference ($p = 0.04$). On the other hand, there was no statistically significant difference between the studied groups as regards CD3%, CD4%, CD8%, TLC and platelet count (Table 3).

As far as gene mutation is concerned; exon 5a was detected only among the highly exposed group (24%). Moreover, exon 6 and 7 were detected among moderately and highly exposed groups and not detected in the unexposed group with significant difference between them ($p = 0.0001$)

Table 3. Comparison between the blood parameters of the studied workers exposed to α-cypermethrin pesticide and control group

Laboratory tests	Respondents' blood			p
	group 1 (N = 50)	group 2 (N = 50)	group 3 (N = 100)	
Platelets [n/cmm] (M±SD)	343.0±72.0	358.0±62.0	329.0±43.0	0.23
Total leukocyte count (TLC) [n/cmm] (M±SD)	6.2±2.0	6.5±1.4	5.1±1.6	0.14
CD3% (M±SD)	54.0±6.8	66.8±1.3	65.2±10.5	0.35
CD4% (M±SD)	34.0±11.0	42.0±8.9	41.3±7.0	0.66
CD8% (M±SD)	25.2±7.0	27.3±7.0	23.0±7.3	0.41
CD4/CD8%[a] (M±SD)	1.2±0.3	1.4±0.2	2.0±0.7	0.002*

CD – cluster of differentiation.
Groups and other abbreviations as in Table 1.
[a] Least significant difference (LSD): group 1 vs. group 3 ($p = 0.04$)*, group 1 vs. group 2 ($p = 0.23$), group 2 vs. group 3 ($p = 0.14$).
* Significant difference at $p \leq 0.05$.

Table 4. Comparison of prevalence of P53 gene mutational exons in the studied workers exposed to α-cypermethrin pesticide and control group

P53 gene mutational exon	Respondents [n (%)]			p
	group 1 (N = 50)	group 2 (N = 50)	group 3 (N = 100)	
Exon 5a	12 (24)	0	0	0.0001*
Exon 5b	0	0	0	–
Exon 6	3 (6)	8 (16)	0	0.0001*
Exon 7	3 (6)	8 (16)	0	0.0001*
Exon 8	0	0	0	–

Groups as in Table 1.
* Significant difference at $p \leq 0.05$.

Table 5. Comparison of blood antioxidant parameters in the studied workers exposed to α-cypermethrin pesticide and control group

Laboratory tests	Respondents' blood			p	LSD		
	group 1 (N = 50)	group 2 (N = 50)	group 3 (N = 100)		group 1 vs. group 3	group 2 vs. group 3	group 1 vs. group 2
Superoxide dismutase (SOD) [U/ml] (M±SD)	62.1±17.0	75.0±3.0	116.0±18.2	0.0001*	0.0001	0.001	0.03
Catalase (CAT) [U/l] (M±SD)	17.2±8.8	24.0±11.0	33.7±8.4	0.0001*	0.0002	0.03	0.13
Glutathione (GSH) [μM] (M±SD)	2.25±0.19	2.5±0.2	2.8±0.09	0.0001*	0.03	0.11	0.39
Glutathione peroxidase (GPx) [U/l] (M±SD)	0.78±0.21	1.08±0.17	1.24±0.12	0.002*	0.002	0.20	0.03

Groups and abbreviations as in Tables 1 and 3.
* Significant difference at $p \leq 0.05$.

(Table 4). As regards antioxidants: SOD, CAT, GSH and GPx were significantly higher among the unexposed group as compared to the moderately exposed group and highly exposed group, which had the lowest levels ($p < 0.001$) (Table 5).

Correlation between the measured immunological and hematological parameters and the age of exposed workers (highly and moderately exposed groups) and their duration of work revealed that there was negative correlation between age and SOD ($p = 0.01$). On the other hand there were no statistically significant correlations between age and other laboratory findings. Results also revealed that the duration of work was negatively correlated to SOD, CAT, GSH and GPx ($p < 0.01$) (Table 6).

In respect of the relation between age of exposed workers and their duration of work versus P53 gene mutation, there were no statistically significant differences either in the age of exposed workers or in their duration of work between workers with or without exon expression (Table 7).

Furthermore, there were no statistically significant differences in antioxidant levels and other immunological and hematological parameters of the exposed groups in relation to their gender or smoking habit (Table 8).

Table 6. Correlation between seniority and age vs. laboratory parameters among studied workers exposed to α-cypermethrin pesticide (N = 100)

Laboratory tests	Seniority		Age	
	r	p	r	p
Superoxide dismutase (SOD)	–0.77	0.0001*	–0.45	0.01*
Catalase (CAT)	–0.68	0.0001*	–0.11	0.45
Glutathione (GSH)	–0.48	0.0001*	–0.09	0.67
Glutathione peroxidase (GPx)	–0.44	0.001*	0.02	0.81
Platelets	0.18	0.22	0.04	0.85
Total leukocyte count (TLC)	0.02	0.55	0.10	0.41
CD3%	–0.09	0.60	0.11	0.35
CD8%	0.12	0.33	–0.18	0.40
CD4%	0.13	0.21	0.01	0.84
CD4/CD8%	0.10	0.38	0.19	0.12

CD – cluster of differentiation.
* Significant difference at $p \leq 0.05$.

Table 7. Relation between the prevalence of P53 gene mutational exons vs. seniority and age of studied workers exposed to α-cypermethrin pesticide (N = 100)

P53 gene mutational exon and its prevalence	Seniority		Age	
	years (M±SD)	p	years (M±SD)	p
Exon 5a		0.30		0.49
no	8.2±2.0		38.3±8.0	
yes	8.5±3.1		37.4±7.3	
Exon 6		0.72		0.32
no	8.4±3.2		37.1±6.5	
yes	8.7±3.0		38.0±7.0	
Exon 7		0.12		0.27
no	8.3±2.9		37.5±7.0	
yes	9.0±3.0		38.2±5.5	

Abbreviations as in Table 1.
* Significant difference at $p \leq 0.05$.

DISCUSSION

α-Cypermethrin pesticide, as one of the pyrethroids, has been widely used for agricultural and domestic purposes. However, the limited number of studies evaluated acute or chronic health effects on humans. The majority of available research projects were either *in vivo* or *in vitro* animal studies. In the current study, study groups were classified based on the occupational exposures to CYP. Except for the level of exposure, no significant difference between the study groups as regards different socio-demographic

Table 8. Relation between smoking and gender vs. laboratory parameters among studied workers exposed to α-cypermethrin pesticide (N = 100)

Laboratory tests	Respondents' blood					
	non smokers	smokers	p	males	females	p
Superoxide dismutase (SOD) [U/ml] (M±SD)	69.1±10.0	67.8±12.6	0.78	70.0±18.0	68.3±11.9	0.45
Catalase (CAT) [U/l] (M±SD)	19.2±8.1	17.5±8.5	0.80	18.4±9.1	20.1±9.6	0.31
Glutathione (GSH) [μM] (M±SD)	2.1±0.6	2.4±0.7	0.13	2.7±0.9	2.09±0.5	0.40
Glutathione peroxidase (GPx) [U/l] (M±SD)	1.0±0.2	0.79±0.3	0.26	0.78±0.3	0.80±0.4	0.83
Platelets [n/cmm] (M±SD)	348.0±80.0	361.0±76.0	0.87	351.0±70.0	355.0±69.0	0.89
Total leukocyte count (TLC) [n/cmm] (M±SD)	6.2±2.0	6.0±1.3	0.76	6.3±1.5	6.4±1.1	0.68
CD3% (M±SD)	62.0±10.0	54.0±7.3	0.22	61.0±9.3	59.0±7.5	0.77
CD8% (M±SD)	36.0±8.0	34.5±10.0	0.90	39.0±7.0	40.1±8.2	0.73
CD4% (M±SD)	24.6±8.0	23.0±6.7	0.82	24.1±5.0	25.1±7.0	0.86
CD4/CD8% (M±SD)	1.4±0.4	1.2±0.3	0.56	1.3±0.4	1.2±0.2	0.47

Abbreviations as in Tables 1 and 3.
* Significant difference at $p \leq 0.05$.

and occupational characteristics was detected. Monitoring of the air level of CYP in different departments of the factory was investigated in the current study and was the base for dividing the workers according to their level of exposure. The mean residual air CYP concentration was higher in the area I (raw materials weighing and production departments) than in the area II (packaging and storage departments). The lack of available information on the permissible exposure level of workplace CYP did not allow for judgment of the safe level [19,20]. Accordingly, workers were divided into highly exposed, moderately exposed and unexposed groups.

The finding that the highly exposed group of workers had lower CD4/CD8 as compared to the unexposed group was in agreement with a cross-sectional study of CYP occupational exposures, which found that exposed workers had lower level of CD4% and CD4/CD8% as compared to other less or not exposed groups [21]. Although the reference range for normal value was not exceeded in both studies but the possibility of immune system imbalance was in place with prolonged exposure or higher levels of exposures may occur. Other studies investigated the harmful effect of pesticides on the immune system as a European wide study which described minimal changes of the immune markers as detected in the current study [22]. However, this study was not studying specifically CYP but included other pesticides as well.

As regards gene mutation, the current study revealed that CYP effect extended to involve tumor suppressor gene of P53 mutation as shown in exon 5a in which nitrogenous base was replaced with adenine, which was more frequent among the highly exposed group as compared to the moderately exposed and unexposed groups. Moreover, exons 6 and 7 mutations were more frequent among the moderately exposed group as compared to the other 2 groups in which cytosine was replaced by adenine base. These results are consistent with Hewehy et al. [21], as their study included 4 groups according to the method of CYP exposure. It was concluded that genotoxic effects of CYP was not related to the dose of exposure especially

in the household and environmental exposures, and not only in occupational settings. The difference between both studies was in the site of mutation [21]. α-Cypermethrin-induced DNA damage was investigated also in the case of the big list of *in vitro* studies on human extracted elements like sperm or lymphocytes. Marked DNA damage at different exons was detected for the exposed sample in one of the *in vitro* studies on human spermatozoa. Dramatic improvement was also observed after treatment by antioxidants (vitamins E and C), which makes the researchers recommend daily antioxidant supplementation for exposed workers [23]. Although this research had different methodology from the current study, it could support the same hypothesis of genotoxic possibility of CYP alone even on low doses. Other researchers found gene mutations through experimental studies *in vivo* and *in vitro*, one of these research projects tested α-CYP mixture with another pesticide on human peripheral lymphocyte and concluded that mitotic index of lymphocytes was inhibited compared to controls [24]. Another *in vitro* human study detected DNA damage in workers' lymphocytes after adding α-CYP at 200 μg dose [25].

Different mechanisms of possible carcinogenicity and chronic effect of pyrethrins exist, one of which is oxidative stress. The current study concluded that SOD, CAT, GSH and GPx antioxidants were higher for the unexposed group as compared to the highly-exposed workers indicating the presence of oxidative stress in the latter group represented as low level of antioxidant enzymes. These findings are in agreement with the results of Abou El-Magd et al. [26] who studied the toxic effects of pyrethroids, including CYP, on some hormonal profile and biochemical markers among workers in an insecticide production factory. Oxidative stress and lipid peroxidation were evaluated in their study and significant decline of the total antioxidants among the exposed group compared to the controls was detected [26].

Moreover, a recent study, although on rats, has drawn the attention to the possibility of occurrence of chronic diseases as Parkinsonism as a result of oxidative stress caused by CYP exposure [27].

The current study investigated the effect of socio-demographic and occupational factors on oxidative stress, genotoxicity and immunological disturbances among exposed workers. It was found that all measured antioxidant levels decreased by increasing the duration of work. Moreover, SOD level was found to be lower for older workers. These findings confirm that longer duration of exposure to CYP may have a direct effect on increasing the oxidative stress among exposed workers. Other factors as gender, smoking, use of personal protective equipment during work, residence and BMI were not significantly related to oxidative stress, genotoxicity or immunological disturbances among exposed workers. Accordingly, these factors were not considered as confounding factors in the current study. These findings are consistent with the results of Abou El-Magd et al. [26] who studied the chronic toxic effects of synthetic pyrethroids among workers in a pyrethroid insecticides company in Egypt. Their results showed that there were no statistically significant differences between the studied groups as regards general factors and occupational factors [26].

CONCLUSIONS

Repeated exposure to α-cypermethrin pesticide may lead to gene mutations in addition to possible disturbance of the immunological balance. Further research is required to firmly confirm these effects. Strict safety precautions are required not only for workers but also for public users.

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