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A study on oxidative stress biomarkers and immunomodulatory effects of pesticides in pesticide-sprayers

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Abstract This work was conducted on 95 adult males from Al-Salheya Algadeeda-Sharkeya governorate. They were classified according to pesticides residues into control group (30 unexposed healthy adult males living in the same area), insecticides exposed workers group (55 adult males exposed to organophosphate and carbamates) and fungicides exposed workers group (10 adult males exposed to fungicides). The study was designed to investigate and compare the oxidative stress and immunomodulatory effects of pesticides exposure among agricultural workers according the level of pesticide residues in their blood which was measured by HPLC. The oxidative stress status has been evaluated by assessment of (SH-protein), glutathione-S-transferase (GST), glutathione reductase (GR), total antioxidant capacity and malondialdehyde (MDA). In addition, the acetylcholinesterase (AchE) activity was measured as a biomarker of toxicity. We used IgG, IgM, as immunological biomarkers to test the humoral immune function as well as TNFα as a biomarker of cellular immune function. Our result revealed statistically significant reduction of the activity of (AchE), antioxidant defense enzymes, total antioxidant capacity, IgM and IgG while, MDA and TNF levels showed significant elevations in insecticides-exposed workers versus control. Results of fungicides exposed workers revealed non-significant reduction of the activity of (AchE), antiox-

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idant defense enzymes, IgM, IgG and TNFα levels while there was significant elevation of MDA level and significant reduction of total antioxidant capacity level.

1. Introduction

During the last decades the use of pesticides has increased steadily in developing countries in an effort to increase food production and control vector-borne diseases. At present, there are more than 65,000 chemicals that are classified as pesticides. Because large amounts of these chemicals are released into the environment daily and many of them affect non-target organisms unfortunately, this has resulted in some negative side effects on human health and the environment.[1,2]

Occupational exposures to theses pesticides occur from skin absorption and inhalations. The exposure of pesticides mainly occurs in the mixing and loading of the equipment, in the spraying of insecticide and improper handling.[3] The farmers who use pesticides have only little or no access to information about proper use or the precautions needed when handling pesticides. Therefore, they often do not use even the simplest hygienic and protective measures.[4,5]

Biological monitoring of pesticide exposure can be carried out by determining intact compounds or their metabolites in the blood serum, plasma or urine.[6] Much work has been done on pesticide residues and its cumulative effect on human beings in the developed countries.[3,7]

Among the pesticides, organophosphorous (OPs) insecticides and carbamates have been widely used, as these compounds are non-persistent in the environment.[8] OPs, carbamates and fungicide are widely sprayed in plantations of Al-Salheya-Zakazeek. There are strong associations between exposure to aerial pesticides and neurological symptoms, as the cholinesterase activity is significantly reduced in the exposed pesticide sprayers. Exposure to low-level of pesticides is known to produce a variety of biochemical changes, some of which may be responsible for the adverse biological effects reported in human and experimental studies.[9–11]

Oxidative stress can also be induced by pesticides, either by overproduction of free radicals or by alteration in antioxidant defense mechanisms, including detoxification and scavenging enzymes.[12] Oxidative stress has been reported to play an important role in the toxicity of various pesticides, including organochlorines, (OPs),[13,14] carbamates and pyrethroids.[15]

There is increasing evidence that environmental factors play important roles in the regulation of the immune system. Because of the wide use of pesticides for domestic and industrial purposes, the evaluation of their immunotoxic effects is of major concern for public health.[16]

Data from animal studies and human case studies suggest that many pesticides are immunomodulatory. Consequences may include immune enhancement, such as hypersensitivity and autoimmunity, or immune suppression, which may increase the incidence of infectious disease or neoplastic transformation. Additional consequences could occur through interactions and feedback mechanisms associated with the endocrine and central nervous systems.[17,18]

It was therefore, worthwhile to investigate oxidative stress, and derangement of the antioxidant defense system in erythrocytes stemming from the pesticide exposure in agricultural workers. In addition, the immunomodulatory effects following exposure to these pesticides were also evaluated.

2. Subjects and methods

2.1. Subjects selection

The present study was performed on 65 workers occupationally directly exposed to mixtures of pesticide in an intensive agricultural area (Al-Salheya farms-Zakazeek). They were divided into two groups according to the result of pesticides residues analysis (insecticides-mixture group and fungicide group). The control group consists of 30 unexposed healthy males of similar mean age distribution (38.94 ± 8.46) and living in the same area.

All studied groups were surveyed by an interviewer administration questionnaire covering standard demographic questions including personal history with special concern to age and special habits (drugs, tobacco), as well as medical and family history with special emphasis on the immuno-modulatory diseases, occupational history job title, duration of application of the pesticides, kind of pesticides and personal protective equipment (PPE) used. All individuals were offered health examinations consisting of a detailed physical examination and laboratory tests for evaluation of hepatic functions which were normal.

2.1.1. Exclusion criteria

Based on the questionnaire, individuals with history of diabetes mellitus, hypertension, liver dysfunction, cancer, other chronic disease, and use of steroids, non-steroidal anti-inflammatory drug, and chemotherapy were excluded from the study.

2.2. Sample collection

Venous blood samples (10 ml) were collected from all study subjects by venipuncture, the blood samples were collected in non-heparinized vacutainers to get serum. The blood sample was centrifuged at 3500 rpm for 15 min in a refrigerated centrifuge to separate serum. Serum was divided into two parts and kept frozen at −20 °C and −70 °C for further biochemical assays.

2.3. Biochemical assays

Pesticide residues were determined using HPLC (high performance liquid chromatography) analysis.[19] Acetylcholinesterase (AchE) activity was determined by the method of Ellman.[20] Total thiol proteins were determined in serum[21] the method based on the development of a yellow color when 5,5-dithiobis (2-nitrobenzoic acid) DTNB is added to plasma. The glutathione-S-transferase (GST) activity was determined in serum[22] by 1-chloro-2,4-dinitrobenzene (CDNB)–GSH conjugate formed at 37 °C which was spectrophotometrically assayed at 340 nm. Glutathione reductase (GR) was measured

in plasma.[23] Lipid peroxidation was estimated in serum as malondialdehyde (MDA) level[24] after incubation at 95 °C with thiobarbituric acid in aerobic conditions (pH 3.4). The pink color produced by these reactions was measured spectrophotometrically at 532 nm to measure malondialdehyde (MDA) level. The total antioxidant capacity (TAC) is determined in serum.[25] Immunoglobulin G (IgG) and immunoglobulin M (IgM) titers are determined using single radial immuno diffusion technique.[26] Tumor necrosis factor alpha (TNFα) level was measured in serum by human TNFα ELISA kits.[27]

2.4. Statistical analysis

Statistical analysis was based on comparing the values of control group as compared to the exposed groups. The results are expressed as means ± SD. The statistical significance of the data has been determined using one way analysis of variance (ANOVA-LSD) using SPSS statistical software package version 15. Pearson correlation test was used to determine the significant correlations between variables. The level of significance was set at $p < 0.05$.

3. Results

3.1. Pesticides residues analysis

The number and percentage of workers exposed to pesticides that had high level of pesticide residues above the acceptable daily intake (ADI) in their blood are taken into consideration. Most of workers had organophosphate residues consisting of ethion, profenfos and chloropyrofos residues followed by carbamates in the form of methomyle and fungicides (abamectin and mancozeb) residues above the ADI as shown in Table 1.

Table 1 Quantitative analysis of pesticide residues in the blood of exposed workers, level above the acceptable daily intake (ADI) (μg/kg).

Parameters	ADI	Workers no. and (%)	Pesticides residue (M ± SD)
Ethion	0.002	11 (20%)	0.199 ± 0.726
Profenfos	0.010	3 (5.5%)	0.299 ± 0.990
Methomyle	0.030	4 (7%)	0.273 ± 1.022
Chloropyrofos	0.010	4 (7%)	0.601 ± 1.446
Abamectin	0.001	4 (40%)	0.033 ± 0.056
Mancozeb	0.006	3 (30%)	0.124 ± 0.351
Diniconazol	0.057	0 (0%)	ND

3.2. Socio-demographic criteria of the study group

Analysis of questionnaires revealed that the duration of exposure in (insecticides-exposed workers) ranged from 4 to 30 years with a mean of 10.96 ± 6.64 years. Duration of exposure in (fungicide-exposed workers) ranged from 3 to 30 years with a mean of 11.60 ± 5.71 years. It also revealed that 29% of the insecticides-exposed workers wear personal protective equipment (PPE) during pesticides application (gloves and boots) and 20% of the fungicide exposed workers wear (PPE). Furthermore, 14.4% of group 2 had past history of acute intoxication while none of group 3 had past history of acute intoxication.

3.3. Biochemical status of exposed workers

Biochemical changes in blood of exposed workers reflect the degree of hazards induced as a result of pesticide exposure. The present study was designed to evaluate oxidative stress and immunomodulatory effects induced by pesticide exposure. As expected, the exposed individuals recorded reduction in AchE level. A significant inhibition in the activity of AchE of Insecticides Exposed workers group versus control at ($^{*}p < 0.001$) was demonstrated in Table 2. The blood MDA level, as an end product of lipid peroxidation was significantly higher in the two groups of exposed workers than those of the controls ($^{*}p < 0.001$). However, SH-proteins content, an antioxidant molecule showed reduction among exposed groups which was significant between insecticides exposed group versus control at ($^{*}p < 0.001$).

As depicted in Table 2, detoxifying enzyme biomarkers glutathione-S-transferase (GST) and glutathione reductase (GR) in serum of insecticides exposed group recorded inhibition in the activity versus control at ($^{*}p < 0.001$). Moreover, total antioxidant capacity (TAC) recorded significant reduction in both exposed groups versus control at ($^{*}p < 0.05$).

Data shown in Table 2 also declared that there was a significant reduction in (IGG and IGM) level of Insecticides exposed workers group versus control ($^{*}p < 0.001$). While the reduction of (IgG and IgM) in fungicide exposed workers group was insignificant versus control. In contrast, TNFα gave

Table 2 Changes in oxidative stress biomarkers and immunological parameters in the serum of pesticide exposed workers and controls.

Parameters	Control	Insecticides exposed workers	Fungicides exposed workers
AchE (U/ml)	1768.8 ± 446.02	1284.2 ± 245[a]	1743 ± 232.31[b]
SH-proteins (μmol/dl)	3711.6 ± 419.35	2918.9 ± 568.4[a]	3266.91 ± 398.32
MDA (μmol/dl	2.25 ± 1.56	7.16 ± 3.41[a]	5.40 ± 0.93[a]
GST (μmol/min/ml)	344.23 ± 70.03	239.89 ± 69.51[a]	294.96 ± 57.32
GR (U/l)	20.64 ± 6.39	15.52 ± 4.60[a]	18.87 ± 2.99
TAC (mm/l)	2.56 ± 0.84	1.18 ± 0.49[a,b]	1.96 ± 0.72[a,b]
IgG (mg/dl)	901.88 ± 144.7	585.73 ± 222.2[a]	892.5 ± 154.73[b]
IgM (mg/dl)	121.38 ± 47.31	71.25 ± 16.52[a]	116.82 ± 34.7[b]
TNFα (pg/ml)	4.319 ± 2.42	7.31 ± 4.72[a]	2.77 ± 1.4[b]

[a] Significant differences versus control at $p < 0.05$.
[b] Significant differences versus insecticides exposed workers group at $p < 0.05$.

Table 3 Overall correlation among studied parameters insecticides exposed workers.

	AchE	SH-protein	GST	GR	TAC	MDA	IgG	IgM	TNFα
AchE	–	–	–	–	0.337*	–	–	0.282*	–
SH-protein	–	–	–	–	–	–	–	–	–
GST	–	–	–	–	–	–	–	–	–
GR	–	–	–	–	–	–	–	–	–
TAC	0.337*	–	–	–	–	–	–	–	–
MDA	–	–	–	–	–	–	0.321*	0.436**	–
IgG	–	–	–	–	–	0.321*	–	–	–
IgM	–	–	–	–	–	0.436**	–	–	–
TNFα	–	–	–	–	–	–	–	–	–
Duration of exposure	–	–	–	–	–	–	0.392**	0.457**	–
Residues	–	–	–	–	–	–	0.390**	0.527**	–

* Correlation is significant at $p < 0.05$.
** Correlation is significant at $p < 0.01$.

Table 4 Overall correlation among studied parameters fungicides exposed workers.

	AchE	SH-protein	GST	GR	TAC	MDA	IgG	IgM	TNFα
AchE	–	–	–	–		–	–		–
SH-protein	–	–	–	–	–	–	–	–	–
GST	–	–	–	–	0.771**	–	–	–	–
GR	–	–	–	–	–	–	–	–	–
TAC		–	0.771**	–	–	–	–	–	–
MDA	–	–	–	–	–	–	–	0.723*	–
IgG	–	–	–	–	–		–	–	–
IgM	–	–	–	–	–	0.723*	–	–	–
TNFα	–	–	–	–	–	–	–	–	–
Duration of exposure	–	–	–	–	–	–	–	–	–
Residues	–	–	–	–	–	–	–	–	–

* Correlation is significant at $p < 0.05$.
** Correlation is significant at $p < 0.01$.

rise to a highly significant increase in sera of insecticides exposed group versus control at ($^{*}p < 0.001$). However, insignificant reduction in TNFα level in fungicides exposed workers group versus control group.

On the other hand, the Pearson correlation analysis showed significant correlation between most of the studied parameters as depicted in Table 3. Where AchE correlated positively and significantly with total antioxidant capacity and IgM; MDA correlated negatively and significantly with IgG and IgM; also duration of exposure correlated negatively and significantly with IgG and IgM among insecticides exposed workers. While, Table 4 showed a significant negative correlation between duration of exposure and each of total antioxidant capacity, GST, IgM and TNFα. Also a negative correlation between MDA and IgM among fungicides exposed workers. Regarding pesticides residues, there is a significant positive correlation between the average organophosphates residual level and duration of exposure, but significant negative correlations between average organophosphates residual level and both of IgG and IgM.

4. Discussion

There is a jump in the total number of used pesticides in Egypt, of which the increase was mainly attributed to the increase in the number of fungicides and herbicides. The increasing demand on fungicides may refer to expansion of greenhouse cultivation in Egypt, which is specifically vulnerable to fungal disease.[28]

The pesticides residues analysis revealed that 22 out of group 2 (insecticides-exposed group, $n = 55$) involved in this study had residues of ethion, profenfos, chloropyrofos, methomyle and 7 out of group 3 (fungicide-exposed group) involved in this study had residues of abamectin and mancozeb in their blood. No residue could be detected for diazonin or diniconazole. The aforementioned insecticides and fungicides are the chemicals used by the farmers for mired control on their farms. This is indicative of occupational exposure.

Blood cholinesterases have been widely used for monitoring exposure to organophosphorus and carbamate pesticides. There are strong associations between exposure to pesticides and cholinesterase activity.[29–31] The present study revealed that there was a classical inhibitory effect on (AchE) activity. This inhibitory effect is of significant value in insecticides exposed workers (organophosphate and carbamates). These results were consistent with those of previous studies.[32–34] It must be noted here that the inhibition in significantly (AchE) correlated with the reduction (TAC) and IgM.

Previous studies have reported that exposure to different categories of pesticides (organophosphates, carbamates and fungicides) leads to oxidative stress in pesticides applicators.[35] Accumulation of oxygen free radicals in erythrocytes and other cells, leading to tissue damage as a result of oxidative binding of key intracellular molecules containing thiol groups like GSH and lipid peroxidation of biological membranes which

might be of greatest importance in the cytotoxicity of pesticides and can be eventually responsible for cellular death.[36]

Our study revealed marked disruption in the oxidative stress markers as evidenced by a significant decrease in the total thiol protein level in insecticides exposed workers versus control group ($^{*}p < 0.001$). However, estimation of SH-containing proteins level, in fungicides exposed workers, showed non-significant reduction versus control. These findings run in parallel, with previous studies that showed significant reduction in the glutathione-S-transferase activity in insecticides exposed workers together with a non-significant reduction in fungicides exposed workers in comparison to the control group.[13,37] This decrease in GST activity could be attributed to the direct binding of pesticide with GST.[38,39] Moreover, these findings may be attributed to slight significant decrease in the activity of glutathione reductase activity (GR).[10,40] GR is the key enzyme for the regeneration of reduced glutathione from its oxidized form (GSSG), it was not surprising that a decrease in GR activity should challenge the compensatory mechanism for replenishing the GSH concentration inside the erythrocytes. It is possible that chronic exposure to pesticide may exert direct effect on GR.[36] In the present study, total antioxidant capacity showed statistical significant lower level in both (insecticides-exposed workers) and (fungicide-exposed workers) versus control group.

The explanation for these findings was attributed to the long-lasting exposure to OPs which leads to generation of reactive oxygen species which simply consume and exhaust antioxidant agents present in the body.[13,41] Moreover, the present data revealed significant increase in malondialdehyde (MDA) level, the biomarker of lipid peroxidation, in both groups in comparison to the control group. Our results were supported by previous findings.[42,34]

In the last decades, evidence supporting an immunomodulatory effect of fungicides has been collected. Sodium diethyldithiocarbamate (mancozeb) has been demonstrated to be a potent in vivo immunomodulator, influencing maturation and activation of T cells, NK cells, IgG secretion.[43]

In the present study, we selected IgG and IgM as markers of humoral immunity, and tumor necrosis factor alpha as a marker of cell-mediated immunity. The results showed statistical significant lower levels in immunoglobulin G and immunoglobulin M levels in insecticides-exposed workers compared to control group, while in fungicide-exposed workers data revealed non-significant reduction in the level of immunoglobulin. These findings run in parallel with those previously obtained.[18,44] The immunoglobulin level in fungicide-exposed workers.[43]

Evaluating the cell mediated immunity, data shown in the current study revealed significant increase of TNFα secretion among insecticides-exposed workers, whereas there was non-significant inhibition of TNFα release in fungicide-exposed workers.[33] Enhancement of TNFα release was explained by the fact that pesticides modulate immune response via different mechanisms: Th1-like immune response was enhanced with the release of cytokines (IL-2 and TNFα) affecting B-cell maturation and immunoglobulin production and, The IL-2 and TNFα increase may result from a mechanism to compensate for the decrease in IFN-gamma after pesticide exposure.[45] TNFα is also associated with the activation of repair mechanisms following xenobiotic damage.[46]

In the present study, there was a positive correlation between the total pesticide residues and the decrease of immunoglobulin G and immunoglobulin M levels ($r = 0.39$, $^{*}p < 0.05$) and ($r = 0.52$, $^{*}p < 0.05$), respectively. The higher the pesticide residue the more frequent infection and immunological abnormalities in the form of decreased immunoglobulin and cytokines production.[47]

The results of the present study clarified some correlations in insecticides-exposed workers. There is a significant positive correlation between (AchE) and both IgM[48] and total antioxidant capacity ($r = 0.427$, $^{*}p < 0.05$).[13] A negative correlation between MDA and both IgG ($r = -0.321$, $^{*}p < 0.05$) and IgM ($r = -0.436$, $^{*}p < 0.05$) as MDA is the biomarker of the oxidative stress damage produced by pesticides exposure which in turn had deleterious effect on humoral immune response.[10] Concerning the correlations of fungicide-exposed workers, data shown in the present work revealed a positive correlation between GST and total antioxidant capacity ($r = 0.77$, $^{*}p < 0.05$), a negative correlation between MDA and IgM ($r = -0.724$, $^{*}p < 0.05$).

5. Conclusions and recommendations

In conclusion, the results of this study clearly stressed that pesticides exposure in pesticides applicators for prolonged duration leads to accumulation of pesticides residues in their blood, significant oxidative damage, compromised antioxidant status and modulation of the immune system involving impairment of humoral and cellular immune functions. These changes are more prominent and marked in insecticides-exposed workers than in fungicides-exposed workers, it is thus concluded that it is important to educate these workers about harmful (toxic) effects of synthetic pesticides. Moreover, they should know the importance of the use of protective measures during spraying as well as the disadvantages (pollution, residual effects and resistance in insects) of the conventional pesticides. Rural workers and public health authorities must become aware of the importance of protective equipment, periodic health examinations and reduced environmental pollution in order to lessen occupational risk of field workers and promote improved conditions of life for the population at large scale. They should also be educated for the use of phytopesticides and other IPM techniques, which will be beneficial for human health.

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