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Evaluation Of Immunological Parameters Among Farmer's Workers Exposed Mixture of Different Pesticides Types in Different Durations

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Abstract

Pesticides are a first choice of farmers for usage against plant infections and despite their harmful impact on the environment and their body. The aim of this research to determine how pesticides affect the immune system of sprayer farmers and to determine the potential impacts of the long duration of the exposure. Ninety male farmers who exposed pesticides were considered participants after they answered on some question to be sure that they don't have any disease. They were split into two groups based on their exposure duration (<5years & ⩾5 years) and split into three age groups (<20, 20-40 and ⩾40). As well as, 30 healthy men made up as a control. Blood samples were collected and divided into two parts; on part were used to count the WBCs by CBC test and serums were separated from the second blood part to determine level for IL-1β, TNF-α and cortisol by using kits supplied by My BioSource, American. Our findings demonstrated significant increase in IL-1β and TNF-α levels although the number of WBCs was within the normal value, as well as, significant decrease in cortisol levels of farmers exposed pesticide serum. However, only TNF-α level significantly increased after ⩾5 years pesticide exposure duration than <5 years, but no significant difference between others as well as cortisol depended on the age groups of farmers exposed pesticide. While no significant correlation of IL-1β with the cortisol, age and duration, TNF-α has significant positive correlation with only cortisol levels. We could conclude that pesticide can have enduring consequences upon cytokine especially TNF-α that are thought resulting in neuro-immune and hormonal communication change and decrease in the cortisone that could have profound behavioral consequences.

Keywords

Pesticides, TNF- α, IL-1β, Cortisol and WBC.

The immune system is a complex network of anatomical areas and different types of specialized cells that aids in an organism's defense against dangerous infections and cancerous cells [1]. Cytokines, immune response modulators, such as interleukin-1β (IL-1β) and tumor necrosis factor alpha (TNF- α) and a sizeable portion of these substances have functional activity [2]. These cytokines protect against inflammatory events and control the immune system in the body [3].

Pesticides are chemicals used to attack living things that are considered detrimental to other living organisms. As a result, many individuals, including farmers and those employed in pesticide manufacturing plants, might be under a danger of occupational exposure to pesticides. However, extensive research on the long-term impacts of pesticide exposure is still limited [4].

According to experimental investigations, exposure to pesticides can have an adverse impact on the immune system [5]. Changes in humoral cytokine levels may have real effects on human health although these effects are yet unknown, particularly in the case of chronic disorders. The development of immunological disorders is reportedly dependent on the amount and duration of pesticide exposure, despite the fact that exposure to pesticides can influence the immune system's ability to function [6]. Exposure to the organochlorine compounds pentachlorophenol (PCP) & 4, 4'-Dichlorodiphenyltrichloroethane (DDT) can increase the release of pro-inflammatory cytokines from monocytes as well as peripheral blood cells, including interferon gamma (IFN), tumor necrosis factor alpha (TNF), and interleukin 1β (IL-1β) [7,8]. Other studies showed that all these compound-induced elevations in IL-1 secretion could be mediated by the ability of PCP and DDT to stimulate cellular production of IL-1 rather than by simply raising the release of already-existing IL-1 [9]. Additionally, to increase cellular synthesis of IL-1, PCP and DDT both used the p38 MAP kinase (MAPK) signaling pathway [9]. Another study that investigated effects of fenitrothion (MEP), glyphosate (GLP), and an organophosphorus insecticide, a herbicide that contains phosphorus, on the generation of cytokines by human peripheral blood mononuclear cells, MEP and GLP did not significantly decrease T cell proliferation or the generation of cytokines produced from T cells, however they did not have impact in a production of TNF- α and IL-1 β[10]. The long -term exposure to low concentration of the organothiophophate pesticide increased responses to Lipopolysaccharide activation for pro-inflammatory cytokines IFN-γ, TNF-α and IL-1β[11].

Cortisol, the end product of hypothalamus-pituitary- adrenal (HPA) axis, has a role in the regulation of blood pressure, an immune system, anti-inflammatory defenses within the body [13]. As a stress indicator, cortisol has been employed in numerous investigations [14,15]. Little research has been done up to now on the HPA axis cortisol levels in pesticide-exposed workers [16]. So, the purpose of this study is to investigate how pesticide spraying affects cytokines and WBCs and link this change with the stress hormone cortisol on conventional farming exposed the pesticide for different duration.

Material And Methods

The current study was done during March and April 2022 in a six field trips to the intensive vegetable cultivation regions in Iraq in Hilla, Kute, and Baghdad. In this study, 120 healthy adults between the ages of 15 and 45 took part after excluded the subjects that have any disease. Ninety male farmers who exposed pesticides were considered eligible participants; they were split into two groups based on their exposure duration (less than 5years & more than 5 years) and split into three age groups (less than 20, between 20 to 40 and more than 40 years). Thirty men made up the control group.

Ten milliliters of venous blood samples collected from patients and control by disposable syringe in two sterilized test tube; one with anticoagulant to be used immediately to determine the WBCs by complete blood count (CBC) test (Sweden), and the other without anticoagulant which were centrifuged for 15 min at 3000 rpm. The serums were separated and used to determine the level of cytokines include (IL-1β, TNF-α) by sandwich ELISA using the Kits commercially available from My BioSource/ USA and by Competitive enzyme-linked immunosorbent assay (ELISA) method for measuring cortisol levels, using the Kits commercially available from My BioSource/ USA.

Results were expressed in terms of mean ± SE or percentage (%) of case frequency. The data were examined for multiple comparisons after one-way analysis of variance (ANOVA), using the Fisher test or t-test. Regression analysis was then performed using analysis of combined variance (ANCOVA). Statview 5.0 was used to conduct all of the experiments. When $p < 0.05$ was reached, the differences have considered significant.

Result

The levels of Interleukins-1β (IL-1β), tumor necrosis factor- α (TNF- α), and Cortisol in the serum of farmers exposed pesticide and control groups are shown in table 1. TNF-α and IL-1β levels had significant increased (p<0001) while cortisol levels were significant decrease in farmers exposed pesticide serum (62.535 ± 3.602 pg/ml, 80.642 ± 4.523 pg/ml and 35.772 ± 1.012 ng/ml, respectively) compared to the control group (28.142 ± 2.071 pg/ml, 59.261 ± 5.778 pg/ml and 83.201 ± 4.114 ng/ml, respectively).

The statistical analysis results of this study also showed the differences in the cytokines and cortisol levels in farmers depend on the duration exposure ⩾5 years and <5 years. Table 2 demonstrate that there are no significantly different in the levels of IL-1β in farmers' serum after exposure duration of pesticide ⩾5 years compared to <5 years (64.813 ± 5.428 and 60.256 ± 4.784 pg/ml, respectively), but there was significantly increased (p>0.05) in TNF-α level after the ⩾5 years exposure duration than <5 years (93.251 ± 6.804 and 68.033 ± 5.257 pg/ml, respectively). However, the cortisol levels did not significantly differ after ⩾5 years exposure duration and <5 years (37.071 ± 1.286 and 34.473 ± 1.552 ng/ml, respectively).

Table 1: Levels of serum cytokines and cortisol in pesticide exposed farmers and controls.

Group	IL-1β (pg/ml) Mean ±SE	TNF- α (pg/ml) Mean± SE	Cortisol (ng/ml) Mean ±SE
Control	28.142 ± 2.071	59.261 ± 5.778	83.201 ± 4.114*
Exposed	62.535 ± 3.602*	80.642 ± 4.523*	35.772 ± 1.012
P-Value	<0001	<0001	<0001
* The significant difference between farmers exposed pesticide and control			

Table 2: Levels of serum cytokines and cortisol in farmers based on duration of exposure of pesticide.

Group	IL-1 β (pg /ml) mean ± SE	TNF-α (pg /ml) mean ± SE	Cortisol (ng /ml) mean ± SE
<5 years	60.256 ± 4.784	68.033 ± 5.257	34.473 ± 1.552
⩾ 5 years	64.813 ± 5.428	93.251 ± 6.804*	37.071 ± 1.286
P-Value	0.5309	0.0045	0.2016
* The significant difference between farmers exposed pesticide ⩾ 5 and <5 years			

Moreover, the differences between these parameters were also studied depend on the age of the farmers exposed pesticide. Table 3 shows no significant difference between all studied cytokines (IL-1β, TNF-α) and cortisol depended on age groups of farmers exposed pesticide; in 21-40 age group (64.902 ± 4.436 pg/ml, 83.950 ± 5.388 pg/ml and 36.075 ± 1.205 ng/ml, respectively), <21 (45.991 ± 2.8149 pg/ml, 57.551 ± 4.636 pg/ml and 31.523 ± 3.794 ng/ml, respectively) >40 (61.097 ± 7.696 pg/ml, 78.610 ± 12.068 pg/ml and 37.078 ± 1.374 ng/ml, respectively).

Table 3: Levels of serum cytokines based on the age of farmers exposed pesticide.

Group	IL-1 β (pg/ml) Mean ± SE	TNF- α (pg/ml) Mean ± SE	Cortisol (ng/ml) Mean ± SE
< 21	45.991 ± 2.814	57.551 ± 4.636	31.523 ± 3.794
21-40	64.902± 4.436	83.950 ±5.388	36.075 ± 1.205
> 40	61.097 ± 7.696	78.610 ± 12.068	37.078 ± 1.374
P.value	G1 vs. G2=0.1266 G2 vs. G3= 0.7176 G1 vs. G3=0.3181	G1 vs. G2=0.0888 G2 vs. G3= 0.6848 G1 vs. G3=0.2661	G1 vs. G2=0.1909 G2 vs. G3= 0.7352 G1 vs. G3=0.1937

The comparison of the white blood cell indices in the exposed group with control group including (the total white blood cell numbers (WBCs) and its types Lymphocytes, Monocytes, and Neutrophils) were shown in table 4. High tendency increase in the numbers of total WBCs and non-significantly differences in the number of lymphocytes and Monocytes in the serum of exposed group (7.118± 0.13, 2.733 ± 0.062 and 0.739± 0.029 x10^9cell/L, respectively) compared to control group (6.77 ± 0.249, 2.728 ± 0.115 and 0.739 ± 0.059 x10^9cell/L, respectively) while Granulocytes significantly increased in number in the exposed group's serum (3.714 ± 0.103 x10^9cell/L) compared to the control (3.239 ± 0.132 x10^9cell/L). All these number were within the normal value.

Table 4: The White blood cell indices (WBCs) count in the blood of exposed and control groups

	WBC indices (10^9cell/L) (Mean ±SD)			
Groups	Total WBC	Lymphocytes	Monocytes	Granulocytes
Control	6.77 ± 0.249	2.728 ± 0.115	0.739 ± 0.059	3.239 ± 0.132
Exposed	7.118± 0.13	2.733 ± 0.062	0.739 ± 0.029	3.714 ± 0.103*
P.value	0.064	0.968	0.99	0.0305
* The significant difference between farmers exposed pesticide and control				

The difference between WBCs indices of farmers exposed pesticide after ⩾5 years and <5 years groups were studied in the table 5. The total number of WBC and the number of monocytes have not changed significantly (p>0.05) ⩾5 years (7.136 ± 0.197 and 0.733 ± 0.039 x10^9cell/L, respectively) compared to <5 years (7.100 ± 0.182 and 0.744 ± 0.042 x10^9cell/L, respectively) while there were significant (P>0.05) decrease in the number of Lymphocytes and increase in the number of Granulocytes after ⩾5 years of exposure duration (2.567 ± 0.074 and 3.925 ± 0.158 x10^9cell/L, respectively), compared to <5 years (2.9 ± 0.093 and 3.503 ± 0.123 x10^9cell/L respectively). Although the changes in the lymphocyte and granulocyte numbers were found, their numbers still within the normal range.

Table 5: The white blood cell indices in farmers based on duration of exposure insecticide.

	WBCs indices (10^9cell/L) Mean± SE			
Groups	Total WBC	Lymphocytes	Monocytes	Granulocytes
<5 Years	7.100 ± 0.182	2.9 ± 0.093	0.744 ± 0.042	3.503 ± 0.123
⩾ 5 Years	7.136 ± 0.197	2.567 ± 0.074*	0.733 ± 0.039	3.925 ± 0.158*
P.value	0.893	0.0067	0.847	0.0386
* The significant difference between farmers exposed pesticide ⩾ 5 and <5 years.				

In the table 6, there are no significant difference between WBCs indices (total WBC, Lymph, Mono and Gran) depended on the age of farmers exposed pesticide; in 21-40 age group (7.125 ± 0.161, 2.716 ± 0.072, 0.707 ± 0.032 and 3.762 ± 0.121 x10^9cell/L, respectively), <21 (6.757 ± 0.421, 2.657 ± 0.173, 0.771 ± 0.092, 3.343 ± 0.336 x10^9cell/L, respectively), >40 (7.330 ± 0.223, 2.880 ± 0.181, 0.890 ± 0.074 and 3.710 ± 0.227 x10^9cell/L, respectively).

Table 6: The White blood cell indices (WBCs) counts based on age of farmers.

Group	WBCs indices (109cell/L) Mean± SE			
	Total WBC	Lymphocyte	Monocyte	Granulocyte
21-40	7.125 ± 0.161	2.716 ± 0.072	0.707 ± 0.032	3.762 ± 0.121
<21	6.757 ± 0.421	2.657 ± 0.173	0.771 ± 0.092	3.343 ± 0.336
> 40	7.330 ± 0.223	2.880 ± 0.181	0.890 ± 0.074	3.710 ± 0.227
P-Value	G1vs.G2=0.42 G1vs.G3= 0.60 G2vs.G3=0.31	G 1 vs. G 2=0.78 G 1 vs. G 3= 0.38 G 2 vs. G 3=0.39	G1 vs. G2=0.50 G1 vs. G3= 0.05 G2 vs. G3=0.32	G1 vs. G2=0.24 G1 vs. G3= 0.86 G2 vs. G3=0.39

Table 7 display that only Granulocytes has correlation with the duration of pesticides exposure as well as there was no correlation of WBCs indices with the age of farmers, cytokines and cortisol levels. On the other hand, the IL-1β has no significant correlation with the cortisol, age and duration while TNF-α has significant positive correlation with only cortisol levels.

However, cortisol has not significant correlation with the age and duration.

Table 7: correlation of serum cytokines, cortisol and WBC with age of farmers and duration of exposure.

	Total WBC (10^9cell/L)	Lymphocyte (10^9cell/L)	Monocyte (10^9cell/L	Granulocyte (10^9cell/L)	Age	Duration	cortisol
IL-1β (pg/ml)	r=0.151 p=0.1569	r=0.141 p=0.1832	r=1.888 p=0.8598	r=0.164 p=0.1217	r=0.08 p=0.49	r=-3.017 p=0.998	r=0.1 p=0.4
TNF-α (pg/ml)	r=0.054 p=0.607	r=0.077 p=0.4677	r=0.083 p=0.418	r=0.192 p=0.707	r=0.21 p=0.06	r=0.15 p=0.197	r=0.3 p=0.007
Cortisol ng/ml	r = -0.44 p=0.6545	r=2.191 p=0.9984	r=0.114 p=0.293	r=-0.16 p=0.1305	r=0.1 p=0.38	r=1.67 p=0.889	----
Age	r=0.083 p=0.4726	r=-0.14 p=0.22	r=-0.05 p=0.7447	r=0.0445 p=0.7088	-----	------	------
Duration	r=0.202 p=0.0882	r=-0.21 p=0.082	r=-1.07 p=0.9928	r=0.361 p=0.0018	------	-----	------

Discussion

Numerous studies have documented how pesticides can either stimulate or depress the immune system. In the current investigation, cytokine levels, which are produced by various types of WBCs, were measured in serum as immunological markers [17]. Data shown in the current study revealed significant increase of TNF-α and IL-1β secretion among pesticides-exposed farmers. This data agrees with Mecdad et al .,2011 found that workers exposed to insecticides had significantly higher TNF- α levels than the control group[18]. Other study examined the effects of early permethrin treatment on the progression of cardiotoxicity in rats showed increased in interleukin 1 (IL-1) level [19]. Other studies have revealed that pyrethroids like bifenthrin (BF) significantly boost the expression of TNF- α in microglia. TNF- α, IL-1 β, and IL-6 are produced by activated microglial cells and are important in controlling immunological responses in the central nervous system which may have negative effects [20]. The Type II pyrethroids have immunosuppressive effects since they can quickly bind to surface receptors on the T and B cells [17].

In our data, only TNF-α level increased depend on the long duration of pesticide exposure but not IL-1β although there was no correlation between these cytokines with the duration of pesticide exposure. On the other hand, these cytokines level were not affected depend on the age of exposed farmers and did not have any correlation with the age of exposed farmers. The TNF-α is time-dependently, increased with the time [21], which could explain the increase level of it in the exposure duration group more than 5 years compared to less than 5 years in current study unlikely IL-1β although there was no correlation between these cytokines with the duration of pesticide exposure which need more research to explain the reasons caused this difference between the time dependent of IL-1β, TNF-α that found in this work. However, TNF- and IL-1 levels did not significantly correlate with age according to our data, but some other researchs have found that older people have higher amounts of these cytokines than younger people [22,23,24]. whereas there was less significantly different in IL-6 levels between subjects and patients, the greatest levels of IL-6 were found in people over the age of 85[22]. Therefore, another explanation for the gap in results could be that there wasn't a large enough elderly population.

It is well established that alterations in the hormones of the hypothalamic-pituitary-adrenal (HPA) axis have been linked to a variety of long-term metabolic and cardiovascular health issues. The research on pesticides and cortisol levels in the HPA axis has mainly focused on laboratory studies of fish [25,26,27], Patients with organophosphate (OP) poisoning [28] and The cortisol levels of pregnant women who were found to be considerably higher that during OP pesticide spraying period than it was during [16]. In current study, the level of cortisol decreased in the farmers exposed pesticide and did not change depend on the exposure duration and farmer age. These finding suggest that environmental chemicals like pesticides can have long-lasting effects on cytokines, which are considered to contribute to a variety of pathological disorders, when they are exposed at important neuro-developmental stages. In particular, it would be anticipated that such protracted changes in the cytokine balance within the hypothalamus would favor noticeable changes in immune and hormonal communication, which could have significant behavioral consequences.

Occupational toxicity biomarkers were based on hematological characteristics [29,30]. Since the immune system depends on white blood cells and its components, any negative impacts on white blood cells may also affect the immune system. The findings of the WBCs count in those exposed to pesticides show a broad range. Many findings that agree with our data no variations in blood parameters about the farmers exposed to pesticides, including the WBCs indices [31,32,33,34]. The types of pesticides employed and the exposure situations may be responsible for this contradiction [34]. Interestingly, our results showed significant increase in total WBCs and granulocytes but not Monocytes and lymphocytes of pesticides-exposed compared to control groups although these values still within the normal range.

The number of lymphocytes and Granulocytes were significant higher in the farmer exposed the pesticide ⩾5 compared to <5 years although there didn't significant differences between WBCs & Monocytes in these groups. These results agreed with other research who found increase the lymphocytes and neutrophiles in the chronic exposed of insecticide which could explain the positive correlation between granulocytes with the exposure duration found in this study [35]. These results found in this study is agreed with other studies such as [36,37] who revealed Organophosphates' effects on the white blood cell account result in a lower level for cells, and it has been hypothesized that this is why lymphocytes respond reduced acutely to pesticides: 1) Adverse effects of endosulfan toxin on the thymus and lymph nodes. 2) Endosulfan toxicity impairs body defense mechanisms and eventually results in a reduction in lymphocytes, which impairs defense mechanisms and necessitates antibody intervention for protection. As a result, when oxidative stress is elevated, a decrease in neutrophils and lymphocytes can be observed in the acute clinical phase [38,39].

Conclusion

The environmental chemicals like pesticides can have long-lasting effects on cytokines, which are considered to contribute a variety of pathological disorders. In particular, it would be anticipated that such protracted changes in the cytokine balance within the hypothalamus would favor noticeable changes in immune and hormonal communication, which could have significant behavioral consequences.

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