Ain Shams Journal of Forensic Medicine and Clinical Toxicology
July 2016, 27: 37- 48

Some Clinical and Biochemical Effects Associated With Acute Malathion-Induced Immunotoxicity in Minia City, Egypt

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Abstract Pesticides have been implicated in increasing the prevalence of diseases associated with alterations of the immune response. The purpose of this review was to demonstrate some clinical effects and to investigate some biochemical changes that occur with acute malathion pesticide intoxication regarding immune system. During a period of six months that started from 1st of November 2014 till 30th of April2015, 43 subjects were chosen to be involved in this study. They were divided into 2 groups: *group A* ***(control):*** 10 apparently healthy subjects and *group B:* consists of 33 patients acutely intoxicated by malathion. Pseudocholine estrase enzyme level, interleukin 2 (IL-2), and cluster of differentiation cells (CD4, CD8 and CD19) were investigated for all subjects. The results of the current study revealed significant increase in male intoxicated cases andingestion was the most common route of intoxication. The mean ages of patients was 30.1±8.5 years old. Suicidal intent was the common mode of toxicity.The mean of delay interval between intoxication and hospital admission was 8.3±5.8 hours. Significant statistical differences in all vital signs, local and some systemic manifestation of the patient. Pseudocholine esterase level was reduced in all cases. Significant decrease in CD4, CD8 and CD19 cell count, while significant increase in IL-2 levels in group 2 patients as compared to control subjects. Significant positive correlation was evident between IL-2 and CD4 count, while significant negative correlation between IL-2 and CD19 count appeared. Insignificant correlations between pseudocholine esterase level and (CD 19, CD8 & CD4) and between IL-2 and (CD8 & Pseudocholine esterase level) were noticed. Critical cases represent 27.3% of patients.Hospital stay duration mean was 8.6±4.8 days. The study concluded that acute malathion toxicity can carry the risk of immunotoxicity. There is demand to continue to improve immunotoxicological studies. A concerted effort is required to organize and standardize a study protocol.

Keywords immunotoxicological, malathione, interleukin 2 (IL-2), cluster of differentiation(CD4, CD8, CD19)

Introduction

Pesticides are substances used to kill, repel, or control certain forms of plant or animal life that are considered to be pests. They include for instance, insecticides, herbicides and fungicides. It is estimated that approximately 7000 metric ton (Mt) of herbicides, 8000 Mt of insecticides and 9000 Mt of fungicides are used worldwide every year (Corsini et al., 2013).

Exposure to xenobiotics has the capability of producing any combination of the following recognized adverse outcomes: 1) focused or more extensive immunosuppression, 2) increased propensity for allergic disease, including atopy, food allergies and asthma, 3) hypersensitivity reactions directed at the chemical itself, 4) increased risk of autoimmune disease and 5) dysfunctional inflammatory responses of innate immune cells, producing tissue or organ damage or dysfunction (IPCS, 2012).

The immune system may be a target of several pesticides. (Corsini et al., 2008). Hoppin et al. (2007) and Slager et al. (2010), showed that specific pesticides, including diazinon, chlorpyrifos, dichlorvos, malathion, carbaryl, permethrin, may contribute to elevated rhinitis episodes in farmers. Several studies observed increased infections in human (Sunyer et al., 2010), in occupationally exposed workers, that may be explained by pesticides- induced immunosuppression.

Epidemiological studies raise the possibility that exposures to some pesticides may be involved in

the pathogenesis of autoimmune diseases, such as rheumatoid arthritis especially in females (Corsini et al., 2013).

The potential of pesticides induced-carcinogenicity in human is still a matter of debate. Cancer is a multifactorial disease with contributions from genetic, environmental, and life style factors (McDuffie, 2005). Hemopoietic cancers have been associated with pesticides, and cancers of the prostate, pancreas, liver, and other body systems (Jaga and Dharmani, 2005; Orsi et al., 2009 ; Persson et al., 2012).

Most of evidences of immunotoxic effects werederived from experimental studies. Also, the available data regarding the current evidence of pesticides-induced immunotoxicity in human are incomplete (the majority of the data are relative to old pesticides and only few immune parameters have been investigated) and the results are contradictory or difficult to interpret. In addition, most human epidemiological studies on pesticide-induced immunotoxicity were for chronic or subchronic toxicity.So, this study was carried out to demonstrate some clinical effects and to investigate some biochemicalchanges in the immune system after acute malathion intoxication.

Our clinical study depends on Corsini et al., (2008) who reported that the first phase of immunotoxicity studies should include the determination of CD3, CD4, CD8 (CD4/ CD8 ratio), CD19 and CD16/56 positive cells. The results obtained from this simple test panel are sufficient to display the balance between lymphocyte subpopulations and highlight possible alterations.

Subjects and Methods

Subjects

Our study involved 43 subjects of both sexes.

Clinical protocol

This study was a cross sectional descriptive study that was conducted prospectively in the period from 1st November 2014 till 30th April2015 inPoison Control Center (P.C.C) of Minia University Hospital.The subjects were divided into 2 groups:

Group A: 10 apparently healthy adults with no systemic diseases.

GroupB: 33 patients from the patients admitted at P.C.C of Minia University Hospital, with history of acute malathion organophosphorus insecticide intoxication (suicidal intention and accidental exposure). Both group A and B were matched in age.

The subjects were diagnosed as malathion intoxication by history, presence of specificmalathion residue, clinical manifestations and assay of pseudocholine esterase enzyme level. The cases received antidotal therapy atropine sulphate and oximes (toxogonin which is obidoxime chloride and is a product of Merck-Serono Company)

This clinical study included demographic data collected from the *history* (sex and age distribution, mode of toxicity, route of intoxication&delay time of presentation), *clinical manifestations* (vital signs, local and systemic manifestations), *laboratory investigation*s (pseudocholine esterase enzyme levelenzyme linked immunoassay (ELISA) kits, IL-2 by (ELISA), CD4, CD8, CD19 by flowcytometry), *grading of cases* (critical and non-critical)*antidotetreatment* (atropine dose, oximes dose)*duration of hospital stay*and*mortality rate and cause*. Critical case was definedaccording to the severity of clinical manifestations (presence of marked muscle weakness, chest wheezes & crepitation, cyanosis) and the laboratory investigations (decrease of pseudocholine esterase enzyme level below 1000 IU/ml). These data were tabulated and analyzed.

Inclusion and Exclusion criteria

Only patients acutely intoxicated with malathion were involved in this study as it is the most common organophosphorus insecticide used in Minia governorate. Subjects within age group 18-45 years were involved in our research. All subjects with a history of systemic diseases, or concomitant use of any medications were excluded from this study. Any patient with previous treatment trial with atropine or oximes were also excluded.

Human rights

All procedures followed were in accordance with the ethical standards of the Medical Ethical Committee at National Research Centre, Cairo, Egypt and with the Helsinki Declaration of 1975, as revised in 2008. Informed consent was obtained from all patients included in this study. Confidentiality of records is saved by keeping the records anonymous.

Blood collection and separation

Four ml of venous blood were drawn from the both patients and control subjects. Two ml of blood were taken on EDTA containing tube for immunophenotyping (measurement of CD4,CD8 and CD19). Another two ml of blood were evacuated into a plain tube, allowed to clot for 30 minutes after collection then centrifuged at 3000 rpm for 15 minutes to separate the serum, then the serum was kept frozen at -70^{0}C for determination of interleukin-2 level (Chan and perlstein, 1987).

Serum Biochemical Analysis

CD4, CD8 and CD19 were determined by flow cytometer(FACSCalibur BD bioscience, USA).

Interleukin 2:Human IL-2 ELISA kit KOMA BIOTECH INC.Catalog No. K0331193 contains all the necessary reagents required for performing quantitative measurement of Human IL-2 levels from samples including serum, plasma, culture medium or other biological fluids in a sandwich ELISA format.

pseudocholine esterase pseenzyme level ELISA kits: the kits were obtained from diamond diagnostcs company.

Statistical analysis

The collected data of clinical and laboratory results were organized, tabulated and statistically analyzed using SPSS software statistical computer package version 19. Data were expressed as Mean $\pm$ Standard Deviation (SD). Independent sample-*t* test

was used to differentiate between two means where probability (P); P < 0.05: was considered significant and if P < 0.01 was considered highly significant. Qui-square test was used to test the statistical significance of differences in a classification system i.e. determine whether there is a significant association between two variables. The Correlation coefficient (r)isa correlation that describes the degree of relationship between two variables. Correlations were estimated using Pearson's test where probability (P): P < 0.05 was considered significant.A positive correlation coefficient means that as the value of one variable increases, the value of the other variable increases; as one decreases the other decreases. A negative correlation coefficient indicates that as one variable increases, the other decreases, and vice-versa.

Results

Demographic data from history (tables 1, 2, 3 & 4)

A significant statistical increase in number of male cases (72.7%) than female (23.3%) cases was observed. The mean age of patients was 30.1±8.5 years as shown in table (17). The cases in age group (18-27 years)were (42.4%), those in age group (28-37years) were (33.3%), while those in age group (38-45 years) were (24.2%).Number of suicidal cases was (69.7%), while that of accidental cases was (30.3%) of cases. There was a highly significant statistical increase in cases intoxicated by ingestion route (81.8%) than other routes i.e. dermal and inhalation (12.1%& 6.1% respectively).The mean interval between malathion ingestion and patient arrival to P.C.C was 8.3±5.8 hours as shown in table (17). Significant statistical difference in delay time of admission of malathion intoxicated patients as cases came after a delay time 5-12 hours were (54.5%), those came after a delay ≤ 4 hours were (27.3%) and those came after a delay 12-24 hours were (18.2%).

Clinical picture results (tables 5, 6)

Regarding vital signs, a significant statistical change in cases presented with malathion intoxication where tachycardia (66.7%), normal blood pressure (51.5%), normal temperature (63.6%) and normal respiratory rate (78.8%) were the predominant signs. Local manifestations showed highly significant statistical change aslacrimation, salivation and sweating occurred in (78.8%) of cases. As regards systemic manifestations, a highly significant statistical difference was found that miosis (84.8%), urinary incontinence (78.8%), vomiting and diarrhea (87.9%), chest crepitations and wheezes (81.8%) occurred in these cases. Significant statistical difference regarding muscle twitches (72.7%) and muscle weakness (69.7%). Cases with intact sensorium were 48.5% of all patients. Coma presented in 33.3% of case, while convulsion presented in 18.2% of cases.

Laboratory investigation results (tables 7, 8, 9, 10, 11& figs.1, 2, 3, 4)

Group Bsubjects showed statistically significant decrease in CD4 cellcount (P <0.001) after acute malathion toxicitywhen compared with group A controls.The reduced CD8 cellcount was found to be with statistically significant reduction(P <0.001) in group B subjects after acute malathion toxicityas compared to controls. CD19 cell level wasfound to be of highly significant decrease (P <0.001) in group B subjects after acute malathion toxicitywhen compared to control. On the other hand, a significant increase was observed in the interleukin IL-2 levels ($P \leq 0.05$) of group B subjects after acute malathion toxicitywhen compared with control (Table 2& Fig. 4).Pseudocholine esterase mean was 1257.6±603.7 IU/l as shown in table (17) and itslevel showed statistically significant difference between its subgroups as level 1000-1400 IU/ml was the most predominant level (45.4%).Significant positive correlation was observed between IL-2 & CD4 i.e. when IL-2 level increases CD4 count increases. Insignificant statistical correlation between IL-2 and CD8 was seen. Highly significant statistical negative correlation between IL-2 and CD19 was noticed i.e. when IL-2 level increases CD19 count decreases. No significant statistical correlation between pseudocholine esterase level and (CD 19, CD8 & CD4)

Treatment results (tables 12, 13, 14, 15)

Out of malathion intoxicated patients (27.3%) of cases were admitted at ICU as critical cases. The mean value of atropine ampoules was 11.6±4.3 ampoules as shown in table (17). Using 10-15 ampoules of atropine was the most common used dose in this study (45.5%) with significant statistical difference when compared with other doses. The mean value of toxogonin ampoules was 7.5±3.2 ampoules as shown in table (17). Administration of 5-9 toxogonin ampoules represented (54.5%) of toxogonin doses and showed significant statistical difference when compared with other doses. The mean duration of patients' hospital stay was 8.6±4.8 days as shown in table (17). Admission for 4-7 days was the most common hospital stay duration (36.4%).

Mortality rate and cause results (table 16)

Out of 33 cases, 3 cases died (9.1%) as a result of adult respiratory distress syndrome (ARDS).

Table (1): Sex distribution of malathion intoxicated cases.

Sex	Cases	
	N	%
Male	24	72.7
Female	9	27.3
Chi-square	5.9	
P value	0.014*	

N (number of cases), P-value calculated by Chi-square *test, *=significant (P-value < 0.05)*

Table (2): Age distribution of malathion intoxicated cases.

Age group (years)	Cases	
	N	%
18-27	14	42.4
28-37	11	33.3
38-45	8	24.3
Chi-square	1.6	
P value	0.441	

N (number of cases), P-value calculated by Chi-square *test, P-value > 0.05 (non-significant)*

Table (3): Mode of toxicity in malathion intoxicated cases.

Mode of toxicity	Cases	
	N	%
accidental	10	30.3
Suicidal	23	69.7
Chi-square	4.4	
P value	0.036*	

N (number of cases), P-value calculated by Chi-square *test,*= significant (P-value < 0.05)*

Table (4): Route of toxicity in malathion intoxicated cases.

Route of intoxication	Cases	
	N	%
Ingestion	27	81.8
Dermal	4	12.1
inhalation	2	6.1
Chi-square	35.1	
P value	<0.001**	

N (number of cases), P-value calculated by Chi-square *test, **= P-value < 0.01 (highly significant)*

Table (5): **Delay time in patient presentation of malathion intoxicated cases.**

Delay time (hours)	Cases	
	N	%
≤ 4	9	27.3
5-12	18	54.5
12 -24	6	18.2
Chi-square	7.1	
P value	0.028*	

N (number of cases), P-value calculated by Chi-square *test, *: P-value < 0.05 (significant)*

Table (6): Clinical picture in malathion intoxicated cases.

Clinical manifestation		N	%	Chi-square	P value
Vital signs					
pulse	Tachycardia	22	66.7	16.9	0.002**
	Bradycardia	7	21.2		
	Normal	4	12.1		
Blood pressure	Normal	17	51.5	6.5	0.037*
	Hypertensive	5	15.2		
	Hypotensive	11	33.3		
Temperature	Normal	26	78.8	31.1	<0.001**
	Hypothermia	5	15.2		
	Hyperthermia	2	6.1		
Respiratory rate	Normal	21	63.6	14.4	<0.001**
	Tachypnea	8	24.2		
	Bradypnea	4	12.1		
Local manifestations					
Skin, mucous membrane, eye	Lacrimation, sweating and salivation	26	78.8	9.8	0.001**
Systemic manifestations					
CNS	Convulsions	6	18.2	4.5	0.103
	Coma	11	33.3		
	Intact sensorium	16	48.5		
Eye	Miosis	28	84.8	147	<0.001**
	Normal	5	15.2		
GIT	Vomiting and diarrhea	29	87.9	17.4	<0.001**
Urinary	Incontinence	26	78.8	9.8	0.001**
Respiratory	Chest crepitation and wheezes	27	81.8	12.1	<0.001**
Musculoskeletal	Muscle twitches	24	72.7	5.9	0.014*
	Muscle weakness	23	69.7	4.4	0.036*

N (number of cases), P-value calculated by Chi-square *test, ***:** P-value < 0.05 (significant), ****:** P-value < 0.01 (highly significant), P-value > 0.05 (non-significant).*

Table (7): Independent sample t-test showing CD4,CD8, CD19 cell count (cell/cc^3)of group A and group B subjects after acute malathion toxicity.

Parameter	Control	Patient	P-value
CD4 (cell/cc^3)	41.59 ± 4.05	25.27±3.38	< 0.001**
CD8 (cell/cc^3)	32.07 ± 3.91	14.82±2.40	< 0.001**
CD 19 (cell/cc^3)	19.21 ± 4.52	9.55±1.37	< 0.001**

*****:** p value < 0.01 (highly significant). Values were expressed as mean± standard deviation, cc^3 = cubic centimeter.*

Table (8): Independent sample t-test of IL2 levelws (pg/ ml) of group A subjects (control) and group B (patients) after acute malathione toxicity.

parameter	Control	Patient	*P*-value
IL-2 (pg/ml)	20.30±4.22	78.19±16.74	< 0.001**

***: p value < 0.01 (highly significant).Values were expressed as mean± standard deviation*

Table (9): Pseudocholine esterase level in malathion intoxicated cases.

Pseudocholine esterase level (IU/ml)	Cases	
	N	%
>2600	2	6.1
1400-2600	7	21.2
1000-1400	15	45.4
≤ 1000	9	27.3
Chi-square	8.6	
P value	0.035*	

N (number of cases), P-value calculated by Chi-square *test, *: P-value < 0.05 (Significant)*

Table (10): Correlation between IL-2 and some parameters (CD 19, CD8 and CD4)

parameter	*IL-2*		Sig.
	r	P value	
CD4	0.3	0.043*	S
CD8	0.2	0.199	NS
CD 19	-0.5	0.002**	HS
Pseudocholine esterase level	0.2	0.199	NS

*r = Correlation coefficient,P-value calculated by Pearson's correlation, S or ***:** P-value < 0.05 (significant), HS or ****:** P-value < 0.01 (highly significant),NS: P-value > 0.05 (non-significant).*

Table (11): Correlation between pseudocholine esterase level and (CD 19, CD8 & CD4)

	Pseudocholine esterase level		Sig.
	r	P value	
CD 19	-0.2	0.253	NS
CD8	0.1	0.433	NS
CD4	0.2	0.405	NS

*r= Correlation coefficient,P-value calculated by Pearson's correlation, ***:** P-value < 0.05 (significant),****:** P-value < 0.01 (highly significant), P-value > 0.05 (non-significant).*

Table (12): Grading of malathion intoxicated cases.

Grading of cases	N	%
Critical cases	9	27.3
Non critical cases	24	72.7
Chi-squared	5.9	
P value	0.014*	

N (number of cases), P-value calculated by Chi-square *test, *: P-value < 0.05 (significant)*

Table (13): Atropine dose in malathion intoxicated cases.

No. of vials	Cases	
	N	%
≤ 4	3	9.1
5-9	7	21.2
10-15	15	45.5
>15	8	24.2
Chi-square	9.1	
P value	0.028*	

N (number of cases), P-value calculated by Chi-square *test, P-value > 0.05 (non-significant)*

Table (14): Toxogonin dose in malathion intoxicated cases.

No. of vials	Cases	
	N	%
≤ 4	9	27.3
5-9	18	54.5
10-12	6	18.2
Chi-square	7.1	
P value	0.028*	

N (number of cases), P-value calculated by Chi-square *test, P-value > 0.05 (non-significant)*

Table (15): Hospital stay duration (days) in malathion intoxicated cases.

Hospital stay duration (days)	Cases	
	N	%
≤ 3	4	12.1
4 - 7	12	36.4
8-14	11	33.3
≥ 15	6	18.2
Chi-square	5.4	
P value	0.143	

N (number of cases), P-value calculated by Chi-square *test, P-value > 0.05 (non-significant)*

Table (16): Mortality ratein malathion intoxicated cases.

cases	N	%
Died cases	3	9.1
Live cases	30	90.9
Chi-squared	20.4	
P value	<0.001**	

N (number of cases), P-value calculated by Chi-square *tes,****: P-value < 0.01 (highly significant), ARDS =adult respiratory distress syndrome.*

Table (17): Mean values of some parametersin malathion intoxicated cases.

parameter	Mean± S.D
Age (Year)	30.1±8.5
Delay time (hours)	8.3±5.8
Atropine dose	11.6±4.3
Oxime dose	7.5±3.2
Hospital stay duration (days)	8.6±4.8
Pseudocholine esterase level	1257.6±603.7

S.D = standard deviation

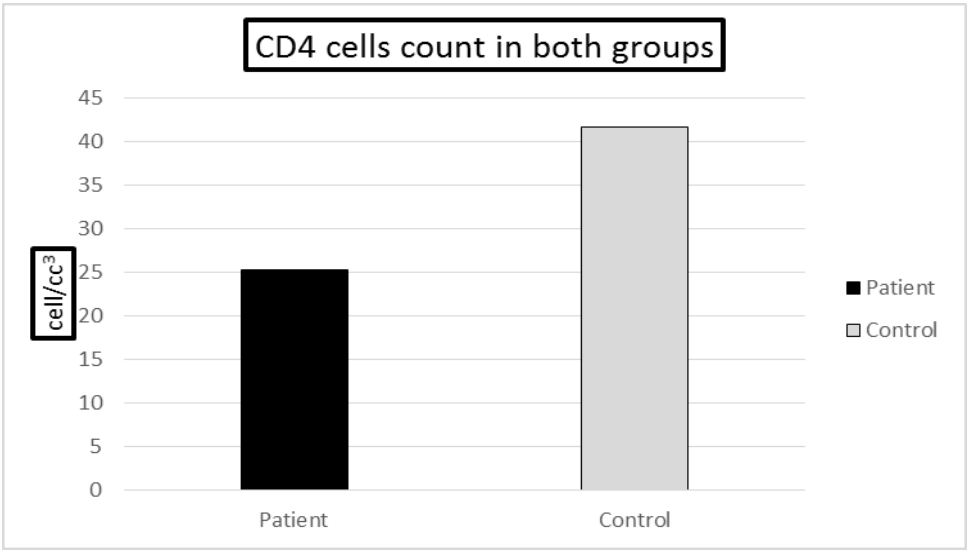

Fig. 1. Showing mean of CD4 cell count of groups B (patients) and group A (control).

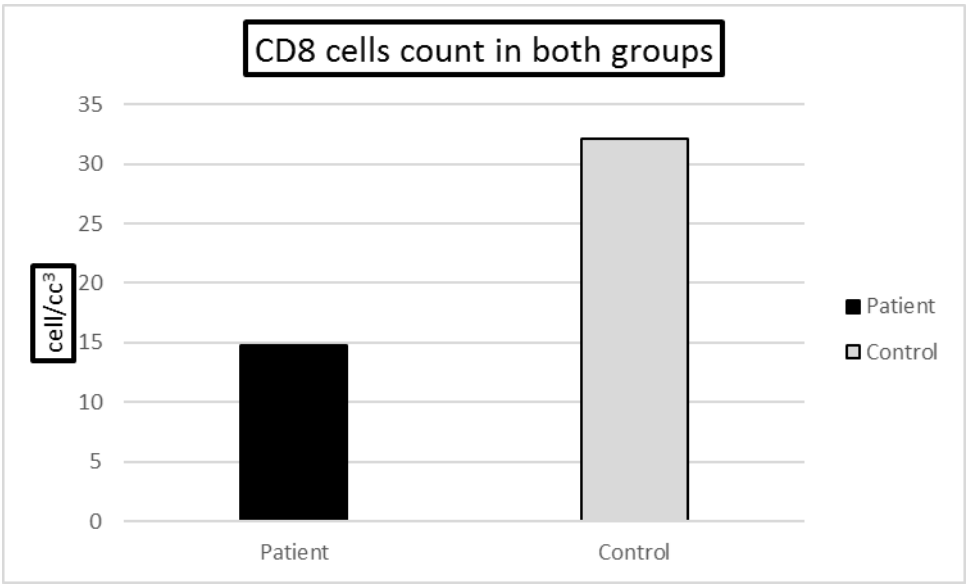

Fig. 2. Showing mean of CD8 cell count of groups B (patients) and group A (control)

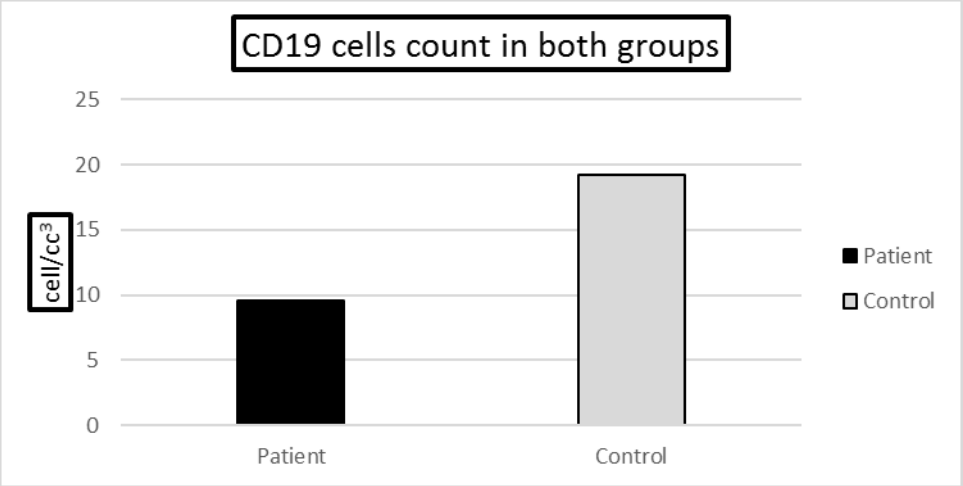

Fig. 3. Showing mean of CD19 cell count of groups B (patients) and group A (control).

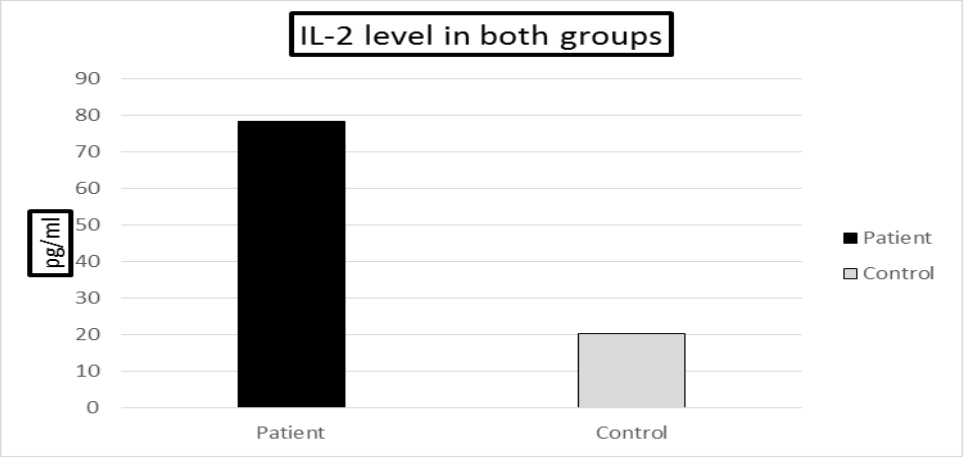

Fig. 4. Showing mean of IL2 level (pg/ml) of groups B (patients) and group A subjects (control).

Discussion

Organophosphorus pesticides (OPs) are widely used throughout the world as insecticides in agriculture and as eradicating agents for termites*(*Corsini et al., 2013).Among them, malathion is widely used and legally allowed by ministry of agriculture in Egypt to control pests which attack several important crops(Amer et al., 2002).

Several pesticides, including organophosphate compounds have the potentiality to produce immunotoxicity. The immune response plays an important role in the pathophysiology of numerous diseases(Corsini et al., 2013).

The current study was carried out todemonstrate some clinical effects and to investigate some biochemical changes associated with acute malathion (one type of organophosphorus insecticide) intoxication on the immune system.

Our clinical study depended on the work ofCorsini et al., (2008)who reported that the first phase of immunotoxicity studies should include the determination of CD3, CD4, CD8 (CD4/ CD8 ratio), CD19 and CD16/56 positive cells.

Regarding demographic data, the current study revealed that malecases (72.7%) were more than female cases(27.3%). This finding is in agreement with study conducted in Chennai by Shivaprasad et al., (2001), in which male patients (74%) outnumbered female (26%). However, in a study conducted in Nepal male: female was1:2 (Rehiman et al., 2008). This may be explained by the fact that the majority of male cases were farmers who usually handle with organophosphus insecticides.

The mean age of cases was (30.1±8.5) years and the majority of affected patients belong to age group (18-27) years (42.4%).Banerjee et al., (2012) reported that majority of the patients belonged to the young age group (Mean±S.D.: 34.47±9.21).

Suicidal intent was the most common mode of toxicity (69.7%) while, accidental one was (30.3%). This result approximately similar to Banerjee et al., (2012) who reported that poisoning with suicidal intent (82.02%) was more common than the accidental one (17.98%).

The majority of affected patients were intoxicated by ingestion route (81.8%). Yuruzem et al., (2008) demonstrate that oral ingestion (86.5%) was

found to be the most common route of poisoning.

The mean interval between intoxication and admission to the hospital was 8.3 hours and the maximum number of patients (54.5%) presented with delay 5-12 hrs. In a study conducted at Chennai, maximum patients (89.69%) presented within 6 hours(Shivakumaret al., 2002). This delay can be attributed to living of farmers in villages where they go to primary health care unit at first before going to P.C.C of Minia University Hospital which is tertiary health care.

Tachycardia (66.7%), normal blood pressure (51.5%), normal temperature (78.8%), normal respiratory rate (63.6%) were the evident vital signs in our study. Banerjee et al., (2012) reported that bradycardia represented (38%), tachycardia (8%), hypothermia (6%) and hypertension (9%) while Rehiman et al., (2008) showed thatbradycardia represented (52%), tachypnea (34%).

Lacrimation, salivation & sweating occurred in (78.8%) of caseswith statistically significant difference. Gannur et al., (2008) reported that salivation developed in (68%) of cases.

Intact sensorium (48.5%), miosis (84.8%), vomiting and diarrhea (87.9%), urinary incontinence (78.8%), chest crepitation and wheezes (81.8%), muscle twitches (72.7%) and muscle weakness (69.7%) were the commonest systemic manifestations and occurred with statistically significant difference. Gannur et al., (2008), reported that miosis (74%), diarrhea (17%), convulsions (4.92%) developed. Chugh et al., (2005) reported that vomiting (80%), convulsions (26.6%), incontinence (40%), miosis (100%), muscle twitches (40%).

The variability in the clinical presentation depends on nature of organophosphorus insecticides compounds, amount consumed, the type of exposure, the quantity absorbed, and time gap between exposure and presentation in the hospital (Eddleston, 2008; Paudyal, 2008).

Pseucholinestrase enzyme levels were significantly reduced with mean value (1257.6 ± 603.7 U/l) and most cases were in the group level 1000-1400 U/l. Significant decrease was observed in acetylcholinesteraseactivity in RBC of malathion poisoning cases in comparison to control subjects (Vandana et al., 2008). The decrease of this enzyme can be explained as malathion like other organophosphoruscompounds inhibit acetylcholinesterase enzyme resulting in accumulation of acetylcholine (Ach) and overstimulation of cholinergic synapses (Eddleston, 2008).

CD4 T cells are mainly cytokine-secreting helper cells (CD4+ lymphocytes) that play an important role in maximizing the capabilities of the acquired immune response.CD4 T helper cells can be divided into two major types;type 1 (Th1) helper T cells secrete interleukin- 2 and interferon (IFN)andtype 2 (Th2) helper T cells secrete interleukin-4, 5, 6, and 10 (Yazawa et al., 2003).

CD8 T cells (Cytotoxic T lymphocyte, CD8+) induce death of cells that are damaged, dysfunctional and infected with viruses or pathogens. CD19 B cells are B-lymphocytes. CD19 is an antigen, found on the surface of B-cells. CD19 cells regulate innate immunity(Yazawa et al., 2003).

Cytokines (e.g. IL-2) are specific chemical mediators which have a central role in the immune response as a protection against infectious agents and reduction of allergic and autoimmune responses (Gately et al., 1998).

The current study revealed statistically significant decrease in the levels of CD4, CD8 and CD19. These results are in agreement with those of El Sharkawy et al., (2003)who reported that the effect of malathion on the percentage of T-lympocytes blastogenesis in response to PHA (phytoheamoagglutinin) mitogen stimulation revealed highly significant decrease in lymphocyte percentage.

The previous findings can be explained as follow: 1)Malathion acts directly or indirectly on lymphoid cell distribution, Ig metabolism, T or B cell functions, macrophages cooperation and macromolecular biosynthesis (Zabrudski et al., 2003).2) Immune suppression after malathion exposure is possible as organophosphates bind to esterase, a vital membrane bound protein that helps immune cells to interact with and destroy foreign organisms (Li et al., 2006). 3)These effects may be free radicals-mediated as several studies suggest that cytokines are at times released during oxidative stress (Krieger et al., 1998).

As regards IL-2 levels, our study revealed a significant increase in IL-2 levels ($P<0.05$) in group B subjects, in comparison with group A, after acute malathion toxicity. Our findings are in accordance with Vandana et al., (2008) who noticed an elevation of IL-2 levels in their research. Our results are not in accordance with (Barnett and Rodgers, 1994; andelbriel et al. 2000) who showed decreased IL-2 production in rats.Vandana et al., (2008)suggested thatthe increase in IL-2 was due to interaction between malathion and early stage of lymphocyte activation, so increased IL-2 level wasa result of proliferation of T cells. In addition, they stated that cytokines are specific cellular mediators of immune system, synthetized by lymphoid cells and non-lymphoid cells and bind to specific receptors on the target cells.Malek, (2008) reported that although previous studies of this cytokine have focused primarily on T-cells, new findings have highlighted that IL-2 is also produced by other cell types (e.g. dendritic cells) and in various different settings. For these reasons, IL-2 can be defined as a pleiotropic cytokine with different functions.

A positive significant correlation between IL-2 and CD4was elucidated.Type 1 (Th1) CD4 T helper cells secrete interleukin-2 so, the increase in CD4 T cells is associated with increase IL-2 level (Yazawa et al., 2003).

Positive insignificant correlation between IL-2 and CD8. Cytotoxic T lymphocyte, (CD8+) induce death of cells(Yazawa et al., 2003). So, CD8 does not involved in IL-2 secretion and this may explain

insignificant correlation.

A negative significant correlation between IL-2 and CD19 were observed. This finding may be due to presence of other cytokine that affect B lymphocyte maturation and activation.Malathion modulates immune response. Th1-like response is enhanced with release of cytokines IFN & IL-2. IL-2induces B cell maturation. Th2-derived IL-4 stimulates B cell activation and Ig secretion, and Th1-derived IFN causes antagonistic response. Th2 response is partially inhibited with decreased IFN production as a result of Ach accumulation. So, the end result is reduction of CD19 count although elevation in IL-2 level (Spergel et al., 1999).

Insignificant statistical correlation betwe en pseudocholine esterase enzyme and CD4, CD8, CD19 and IL-2. This result disagrees with Galloway et al., (2004) who reported that a correlation between PChE suppression and immune responses by organophosphates has been suggested. The decreased activity of AChE after malathion exposure inhibits the E-rosetting of T-lymphocytes Moreover, inhibition of AChE results in an accumulation of the amino acid neurotransmitter acetylcholine (ACh), which stimulates lymphocytes, elevates concentrations of cellular c-GMP and increases the motility and cytotoxicity of lymphocytes leading to reduction in the IFN levels in serum. The difference between 2 studies may be due to many factors as malathion concentration, administered amount, bioavilability of malathion.

In our study, critical cases that were admitted at ICU represented (27.3%) of all cases. Çolaket al., (2014) reported that (15%) patients were admitted to the intensive care unit. Early admission to hospital and receiving first aid and specific measures as emisis, gastric lavage and antidote therapy (atropine & oxime) may explain the decrement in severe and critical cases in Çolaket al., (2014) in comparison to our study result.

The mean value of atropine ampoules was 11.6±4.3 ampoules and using 10-15 ampoules of atropine was the most common used dose in this study (45.5%) with significant statistical difference when compared with other doses. The mean value of toxogonin ampoules was 7.5±3.2 ampoules. Administration of 5-9 toxogonin ampoules represented (54.5%) of toxogonin doses and showed significant statistical difference when compared with other doses. The mean duration of patients' hospital stay was 8.6±4.8 days. Admission for 4-7 days was the most common hospital stay duration (36.4%).

The need for high doses of atropine andtoxogonin and prolonged hospital stay may be a result of delayed presentation of most patients as the mean value of delay in this study reached 8.3 hours giving malathion more time to cause more morbidity in patients.

Out of 33 cases,3patients of malathion toxicity died (9.1%) due to ARDS that occurred as a complication of mechanical ventilation. This finding is comparable to the study done in Turkey where mortality rate was (9.1%) and patients died due to sudden respiratory and cardiac arrest (45%), respiratory failure (25%), CNS depression (5%) and septic shock (25%). (Yurumez et al., 2007). Another study showed that following admission, a total of 56 (5.78%) patients died during their hospital stay andrespiratory failure was the primary cause of death in 21 patients (37.5%) followed by CNS depression (33.92%), cardiac arrest (21.44%), and septicemia (7.14%)(Banerjee et al., 2012). The difference between studies regarding mortality rate can be attributed to delay presentation of patients and difference in malathion concentration, ingested amount & absorbed fraction

Conclusion

The assessment of immunotoxicity in human is a very difficult task. Also, the assessment of the immunotoxicity of pesticides in agricultural settings, where co-exposures to several compounds is common, is needed. Acute malathion intoxication carries a risk of immunotoxicity as it affects T cells proliferation and secretion of cytokines i.e. IL-2. Due to the paucity of data, especially new pesticides, it is clear that there is demand to continue to improve immunotoxicology studies. A concerted effort is required to organize and standardize a study protocol.

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الملخص العربى

بعض التأثيرات الاكلينيكية والبيوكيميائية المصاحبة للتسمم الحاد بالملاثيون على جهاز المناعة بمدينة المنيا ، جمهورية مصر العربية

أحمد حفناوي عباس[١] وهند محمد مؤنس [2] وعمرو رضا زكي [3] ومنال عويس حسن ونجلاء عدلى عبدالعظيم[4]

تسببت المبيدات الحشرية في زيادة انتشار الأمراض المرتبطة بالتعديلات في الاستجابة المناعية.تهدف هذه الدراسةلتوضيح بعض التأثيرات الاكلينيكية وبحث بعض التغيرات البيوكيميائية التي تحدث مع التسمم الحاد بالملاثيون في الإنسان بشأن النظام المناعي. وقد عقدت الدراسةخلال فترة ستة أشهر بدأت من ١ نوفمبر ٢٠١٤ حتى ٣٠ من أبريل ٢٠١٥ ، وقد تم اختيار ٤٣ شخصا من كلا الجنسين للمشاركة في هذه الدراسة وتراوحت أعمارهم من ١٨-٤٥ عاما. وتم تقسيمهم إلى مجموعتين : المجموعة الأولى (الضابطة) : تضمنت١٠أشخاصمن الأصحاء ظاهريا و المجموعة الثانية : اشتملت على ٣٣ مريضا تعرضوا للملاثيون. وقد تم التحقيق من مستوى انزيم سودوكولين استيراز ومستوىانترلوكين ٢ (IL -2) ، وعد خلايا مجموعة من خلايا التمايز (CD4، CD8 و CD19) لجميع الأشخاص . أظهرت نتائج الدراسة زيادة حالات التسمم في الذكور. وكان التناول بالفم هو اكثر الطرق للتسمم. تراوح متوسط أعمار المرضى (٣٠،١±٨،٥) عام. لوحظ وجود تغير احصائي مهم في العلامات الحيوية والاعراض الموضعية وبعض الاعراض الخاصة باجهزة الجسم. وجد انخفاض فيمستوى انزيم سودوكولين استيراز في جميع الحالات.كما وجد انخفاض ذات دلالة احصائية مهمة في عدد خلايا CD4، CD8 و CD19في أشخاص المجموعة الثانية(المرضى) مقارنة بالمجموعة الضابطة, في حين وجدت زيادة كبيرة في مستويات IL -2 في المجموعة الثانية(المرضى) مقارنة بالمجموعة الضابطة.ظهر ارتباط ايجابي مهم احصائيا بين CD4 و IL -2 وظهر ارتباط سلبي مهم احصائيا بين CD19 و IL-2. لم توجد اي ارتباطات ذات دلالة احصائية مهمةبين مستوى انزيم سودوكولين استيراز و(CD4، CD8 وCD19) وبين مستوى IL-2و (CD8 ومستوى انزيم سودوكولين استيراز). مثلت الحالات الجرجة (٢٧،٣%) من الحالات. كان متوسط اقامة الحالات بالمستشفى (٨،٦ ± ٤،٨) يوما. وخلصت الدراسة الى أن التسمم الحاد بالملاثيون يمكن أن يحمل مخاطر على وظائف جهازالمناعة. هناك حاجة ماسة لتحسين الدراسات المتعلقة بتسمم جهاز المناعة والتي تتطلب جهود مركزة لتكوين وتوحيد بروتوكول لهذة الدراسات.

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