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Pesticides Usage in Agriculture among Rural Women in Egypt: Association Between Serum Organo-Chlorine Pesticide Residues and Occurrence of Diabetes

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Abstract: Organochlorine pesticides were widely used in Egypt; large quantities had been released into the environment, where they became persistent substances both in the environment and in the food chain. The non organized use of pesticides in Egypt has caused immense damage to the environment and human health. Recent studies have reported an association between exposure to organo-chlorines pesticides (OCPs) and impaired blood-glucose regulation. The study aimed to investigate the relation between exposure to specific agricultural pesticides and diabetes incidence among some Egyptian women. The population study consisted of 51 rural women, who help their families in agriculture. For all participants in the study, questionnaires were filled up and, blood samples were obtained for determination of blood glucose levels and of OC pesticides residues. The dieldrin and DDD residues were found almost in all samples, followed by the heptachlor, heptachlor-epoxide and the aldrin respectively and finally the DDE. There was only a significant positive correlation between the heptachlor residue and the blood glucose level of the participants. Evaluation of the attitudes and behavior of the studied population showed a significant association between the heptachlor and DDE residues in the sera of women who didn't use or have pesticides in their home. This can confirm that the presence of OC pesticides in several environmental compartment like food and soil, may pose another risk factor for the human health. These findings may have relevance to the general population, who suffer greatly from environmentally persistent compounds and the increasing prevalence of diabetes and a further extended study is needed.

Key words: Diabetes · Environmental exposure · Farmer attitudes · Organochlorine pesticides

INTRODUCTION

Persistent organic pollutants (POPs) including organo-chlorine pesticides, are organic compounds that, to a varying degree, resist photolytic, biological and chemical degradation. POPs are often halogenated (chlorinated) and characterized by low water solubility and high lipid solubility, leading to their bioaccumulation in fatty tissues. They are also semi-volatile, enabling them to move long distances in the atmosphere before deposition occurs [1]. Organochlorine pesticides (OCPs) were widely used in Egypt, by then; large quantities had been released into the environment, where they became persistent substances both in the environment and in the food chain [2-4]. In the human body, they persist for long periods, accumulating in adipose tissue and in the lipid component of serum, causing serious health problems. Sharaf *et al.* [5] found that endrin; DDT and its derivatives and dieldrin were the main contaminants of breast milk, the maternal serum, umbilical serum and abdominal adipose tissue of some Egyptian women giving birth by cesarean section. Amr [6] analyzed the health profiles for 300 pesticide formulators and 300 applicators, where peripheral neuritis, psychiatric manifestations, electroencephalographic (EEG) changes and hepatorenal dysfunction and finally cytogenetic changes among formulators and applicators, were proved to be more than those of controls. Diabetes is one of the most prevalent

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chronic diseases in developing countries, conferring a significant burden in terms of medical complications and health-care costs. In 2030 there will be approximately 6.7million cases of diabetes in Egypt [7].

The African region is expected to have the largest proportional increase in adult diabetes numbers by 2030 [8, 9]. Number of reports have been emerged and showed a possible relation between exposure to persistent organic pollutants (POPs) and the incidence of diabetes, prevalence of diabetes, levels of serum insulin and glucose [10-13]. Cranmer *et al.* [14] studied a population of individuals exposed to dioxin from a pesticide manufacturing site having inadequate production controls and waste disposal, he reported that his studied group showed normal glucose levels, while their plasma insulin concentrations were significantly higher in individuals with higher dioxin serum levels and this exposure resulted in hyper-insulinemia and insulin resistance. Other studies have reported an association between exposure to organo-chlorines pesticides (OCPs) and impaired blood-glucose regulation. Lee *et al.* [15] found a strong dose–response relationship between serum levels of six persistent organic pollutants (POP's): PCB 153, two dioxin congeners and three pesticides residues (oxychlordane, dichlorodiphenyldichloroethylene (DDE) and trans-nonachlor) and diabetes. Everett *et al.* [16] and others reported an association between serum levels of both PCB-126 and p,p'-DDT (dichlorodiphenyltrichloroethane), DDE, the major metabolite of DDT, with diabetes [16-18] and hexa-chlorobenzene (HCB) was also linked to diabetes in cross-sectional studies [17]. Lee *et al.* [19] suggested that OC pesticides and five non-dioxin-like congeners were positively associated with type 2 diabetes by increasing insulin resistance. The results of these studies propose that the background levels of POPs including OC pesticides contribute to the burden of diabetes. Our study aimed to investigate the relation between exposure to specific agricultural pesticides and occurrence of diabetes among some Egyptian rural women.

MATERIALS AND METHODS

Sampling has been done, in Kafrelsheikh governorate, Egypt. It consisted of 51 rural women, who help their families in agriculture. Inclusion criteria for the participators of the study, was that, not complaining from chronic medical problems. For all participants in the study, the following steps were taken. They were interviewed through a questionnaire which includes: Socio-demographic data (age, education and income), history of medical problems and history of pesticide exposure. Blood samples were collected for each participant by venipuncture, in 2 ml collection tubes for analysis of glucose (Random Blood Sugar) and in other separate tubes, for analysis of OC pesticides residues. The blood samples were allowed to clot at room temperature for an hour then centrifuged and the serum was obtained, stored at-80°C on-site until being transported on dry ice to the laboratories for analysis. Serum glucose was measured by using the hexokinase and glucose-6-phosphate dehydrogenase coupled method for glucose [20].

Determination of Pesticides Residues:

Extraction and Instrumentation: Extraction of pesticide residues from samples collection were applied according to the method of Liu and Pleil [21]. Each serum sample (2 ml) was mixed with 6ml de-ionized water (Milli Q water purification, USA). Sodium chloride (2g) was added to each sample to saturate the sera solutions. These solutions were extracted by 12 ml hexane (for each sample) through vortex (Heidolph REAX, Germany) for 1min. The extracted mixture was centrifuged at 1600 g for 30 min (Sigma 2K15, Germany). The organic phase was separated from the aqueous phase and re-extracted twice using 6ml of hexane in each time. The extracts were combined and dried over sodium sulfate anhydrous and then the solutions were evaporated under nitrogen flow. The extracted samples were analyzed by Hewlett Packard GC Model 5890 equipped with Ni^{63} Electron Capture Detector (ECD) and Flame Ionization Detector (FID) and fitted with HP-101 capillary column (Cross linked methyl silicon Gum), 30 m length, 0.25 mm diameter and 0.25 µm film thicknesses. The oven temperature was programmed to start at 160°C and raised to 220°C with rate of 5°C/min and was held for 30 min. Injection and detector temperatures were 220°C and 300°C, respectively. The flow rate of carrier gas (nitrogen) was obtained by adjusting it at the pressure of 10 psi (pound/in^2).

Concentrations of pesticide residues in different analyzed samples were calculated as nano gram/ml serum. To determine method's accuracy, a recovery study was performed on blood samples that were fortified by the investigated pesticides. The recovery values were calculated from calibration curves constructed from the concentrations and peak areas of the obtained chromatograms with standards of OC pesticides. Blank analysis was performed in order to check interference from the sample. Mean recoveries ranged from 90 to 94%

with S.D < 6 indicating excellent repeatability, with relative standard variation (RSD) is usually more than 10 % for methods involving a simple preparation procedure, the RSD is in the order of 5-10 % [22]. Values below the method detection limit (MDL) were set to zero or ND.

Pesticide Standards: Standards of organochlorine (OC) pesticides include: hexachlorobenzene (HCB); aldrin; heptachlor; heptachlor-epoxide; endrin; DDT: 1,1,1-trichloro-2,2,bis(p-chlorophenyl) ethane; DDE (1,1-dichloro-2,2-bis(p.chlorophenyl) ethylene; DDD: 1-chloro-2,2-bis(p-chlorophenyl) ethane.

- All standards over 99 % purity and purchased from Chem. Service, Inc. (West Chester, PA).
- Standard solution mixtures were prepared in acetone from stock individual standards and stored at-18°C. Working solutions were prepared by dilution with hexane and stored at 4°C.

Chemicals: All solvents used (hexane and acetone) were reagent grades and purchased from Merck (Merck, Darmastadt, Germany).

- Sodium chloride and sodium sulfate anhydrous of analytical grade were used after purification by heating at 300°C overnight.

RESULTS

Table 1 summarizes the socio-demographic characteristics of the studied group. More than 88 % were married and 53 % were illiterate. About 92 % with family income less than 500 LE with average family size 5 members, very few women were reported as smokers (3.9%). The studied population has an average age of 34.5 ± 8.5 (years old). Table 2 shows the mean and standard deviation of: random blood sugar levels, height, weight and body mass index (BMI) among the studied group. The random blood sugar levels have a mean of 98 ± 14 mg/dl, the mean BMI was 33.84 ± 11.31 kg/m^2. Table 3 shows that approximately (43.1%) of the participants had pesticides in their home (68%) have pesticides in a safe place, (43.1%) they worked in the fields, helping their husband in application and or formulation of pesticides especially during the rice agriculture seasons and they didn't keep or use empty

Table 1: Socio-demographic characteristics of the studied population.

Characteristics	Number	%
Marital Status		
Married	45	88.2
Widow	6	11.8
Smoking Status		
Smoker	2	3.9
Nonsmoker	25	49.0
Passive Smoker	24	47.1
Farmer's education		
Illiterate	27	52.9
Read and write	5	9.7
Educated	19	37.4
Family income		
800 or more	1	2.0
800-500	3	5.9
Less than 500	47	92.1
Total	51	100

Table 2: Levels of random blood glucose, height, weight, BMI among the studied population.

Item	Mean± SD	Minimum	Maximum
Random blood glucose (mg /dl)	98.14±40.11	74.00	314.00
Height (cm)	154.75±9.27	150.00	169.00
Weight (Kg)	79.27±17.89	53.00	111.00
BMI (kg/m^2)	33.84±11.31	20.96	48.24

Table 3: Activity related to handling of pesticides among the studied population.

Activity		Number	%
Have pesticides in their homes	Yes	22	43.1
	No	29	56.9
Pesticides in safe place	Yes	15	68.2
	No	7	31.8
Preparing pesticides for farming with family member	Yes	22	43.1
	No	29	56.9
Empty pesticides package	Use after washing	0	0
	Not used	51	100.0
Total		51	100.0

Table 4: Mean ± SD, Median, Minimum, Maximum concentrations and Percent detectable of some organo-chlorine pesticides (OCPs) measured in serum of the studied group.

OCPs (ng/g lipid)	Mean ± SD	Medium	Minimum	Maximum	Percent detectable
Alpha-BHC	10.86 ±5.38	10.54	5.80	22.00	13.7
Gamma-BHC	141.44 ±75.94	175.40	5.60	175.40	9.8
Delta-BHC	4.80	4.80	4.80	4.80	1.96
Heptachlor	61.43±141.90	32.10	7.80	926.60	82.3
Aldrin	81.25±103.30	31.60	4.12	282.40	68.62
Heptachlor -epoxide	118.92±265.93	21.40	0.74	876.00	92.1
Gama-chloredane	-	-	-	-	0
Endosulfan	55.47±22.57	52.60	21.34	92.80	60.78
Dieldrin	116.73±73.17	98.80	30.40	384.00	100
DDE	57.86±46.94	54.00	4.56	230.00	25
Endrin	-	-	-	-	0
DDD	596.23±443.30	358.00	193.40	1,580.00	100
DDT	-	-	-	--	0
Methoxychlor	181.07±89.39	136.20	123.00	284.00	5.88

(-) under the limit of detection

Table 5: Correlation of organochlorine pesticides (OCPs) with the random blood glucose levels of the studied population

	Random Blood Glucose	
Pesticides residues	R	P-value
Heptachlor	0.400	0.009*
Aldrin	0.046	0.794
Heptachlor epoxide	0.192	0.196
Endosulfan	- 0.106	0.572
Dieldrin	-0.095	0.508
DDE	-0.144	0.491
DDD	-0.238	0.093

*Highly significant p-value<0.01

Table 6: Association of the detectable organo-chlorine residues in serum among rural women and the presence of the used agriculture pesticides in their homes.

		Have pesticides				
		Yes		No		
OCPs		No	%	No	%	P-value
Alpha-BHC	Free	19	86.4	25	86.2	0.65
	Detectable	3	13.6	4	13.8	
Gamma-BHC	Free	19	86.4	27	93.1	0.36
	Detectable	3	13.6	2	6.9	
Heptachlor	Free	8	36.4	1	3.4	0.004*
	Detectable	14	63.6	28	96.6	
Aldrin	Free	9	40.9	7	24.1	0.16
	Detectable	13	59.1	22	75.9	
Heptachlo- epoxide	Free	1	4.5	3	10.3	0.41
	Detectable	21	95.5	26	89.7	
Endosulfan	Free	10	45.5	10	34.5	0.42
	Detectable	12	54.5	19	65.5	
DDE	Free	15	68.2	11	37.9	0.03*
	Detectable	7	31.8	18	62.1	
Methoxychlor	Free	22	100	26	89.7	0.12
	Detectable	0	0	3	10.3	

*Statistically significant p-value<0.05

pesticides packages in their homes. Studying the frequency distribution of the health profiles among the studied population, it revealed the absence of acute pesticides poisoning symptoms and the only case of poisoning present was that of a food poisoning.

For each organo-chlorine residue, Table 4 shows the number of detectable cases, with description of the mean ± standard deviation, the minimum and the maximum of 14 organochlorine pesticides residues measured in the serum of the studied group. From this table we notice that all most the studied group was free from the gamma-chloredane, endrin and DDT. The dieldrin and DDD residues were found almost in all samples, followed by the heptachlor-epoxide, heptachlor, aldrin and endosulfan respectively and finally the DDE. Table [5] shows the correlation between the blood glucose levels in the sera of the studied population and the pesticide residues detected in most cases. There was only a highly significant positive correlation between the heptachlor residue and the blood glucose levels. Table [6] represents the association of the detectable organochlorine residues in sera of rural women and the presence of the used agriculture pesticides packages in their homes. There was a significant association between the following OC residues heptachlor and DDE residues in the sera of women who didn't use or have pesticides in their home.

DISCUSSION

The non organized use of pesticides in Egypt has caused immense damage to the environment and human health [2, 3, 6, 23,]. Mansour [2, 3] showed that the use of about 1 million metric tons of pesticides in the agricultural sector over a period of 50 years represented a major hazard in Egypt, where specific health and environmental problems have been occurred. Exposure to persistent chlorinated pesticides now-days has been declined, but there are indications that the presence of OC pesticides in food may be an additional risk factor for human health [4]. As well as several other reports reveal that OC pesticide residues are still detectable in several environmental compartment [24, 25]. In addition the farmers' attitudes, toward pesticides and their behavior in using them, garnered new insights as to how pesticides should be better controlled and regulated in Egypt [26]. Ddiabetes has not usually, been considered to be an environmentally induced disease [27], but there was a significant association, between serum pesticide levels and diabetes ranged from a 20 percent to 200 percent increase in risk among pesticides applicators in the study of Montgomery *et al.* [28]. On the other hand, Everett *et al.* [16] and Lee *et al.* [19, 29] and demonstrated dose-response relationships between serum concentrations of different organochlorine compounds and the prevalence of diabetes.

In our study, DDD and Dieldrin were the dominant OC pesticides in all analyzed samples, followed by the heptachlor epoxide and finally the heptachlor. DDT was not detected in all the analyzed samples (Table 4) and this is matched with the discontinuation of using DDT, either in Egypt or internationally [30]. Although the study of Masoud *et al.* [25] in Egypt, showed that DDT concentrations were higher than maximum residue level (MRL) at mixing waters between irrigation and drain water in summer in the area of our study. In Egypt, the largest agricultural use of DDT has been on cotton, before its ban. So this compound especially its metabolites still contaminates the Egyptian environment mostly because of their persistence and long range of transport. The DDT and its metabolites have been detected in food from all over the world and this route is likely the greatest source of exposure for the general population. DDE and DDD (DDT metabolites) was the second most frequently found residue in a survey of domestic animal fats and eggs and in fish samples from Egypt, however, these residues have declined steadily over the past 20 years [31]. These residues, where also detected among twenty seven pesticides, analyzed by Masoud *et al.* [25] from different zones and different sites of irrigation canals in Kafrelsheikh governorate.

Dieldrin binds strongly to soil particles and hence is very resistant to leaching into groundwater. Dieldrin residues have been detected in air, water, soil, fish, birds and mammals, including humans and human breast milk. As aldrin is readily and rapidly converted to dieldrin in the environment and in organisms, the levels of dieldrin detected likely reflect the total concentrations of both compounds. In Egypt, the estimated dietary intake of dieldrin by breast fed infants of 1.22 µg/kg body weight/day. Diet is the main source of exposure to the general public. Dieldrin was the second most common pesticide detected in a survey of US pasteurized milk [31]. Heptachlor has been used primarily against soil insects and termites; it has also been used against cotton insects. It binds readily to aquatic sediments and bio-concentrates in the fat of living organisms. Heptachlor is metabolized in animals to heptachlor epoxide. So, heptachlor and heptachlor-epoxide were among the most frequently detected OC residues [31]. In epidemiologic studies, serum concentrations of organo-chlorine are commonly used in exposure assessment, but little is known about the reasons behind the findings of large inter-individual variation in concentrations. Serum concentrations of organo-chlorine among women are associated with personal attributes such as food habits, age, body mass index (BMI), breast feeding [32] and place of residence [17].

We examined the association between the serum levels of the mostly detected organo-chlorine pesticides residues and the serum glucose levels of the studied group (Table 5). Of the seven residues, one residue (heptachlor) was strongly associated with the serum glucose levels in the studied population (p-value < 0.01). This could be explained by the fact that heptachlor is a frequent component of chloredane and is structurally very similar. Chloredane was detected, as one of the highly persistent chlorinated hydrocarbon in the Egyptian soil, sediments [24] and food-chain organisms [4]. In addition to the study of Montgomery *et al.* [28] has considered the diabetogenic actions of seven specific pesticides including the heptachlor (aldrin, chlordane, heptachlor, dichlorvos, trichlorfon, alachlor and cyanazine) for which the odds of diabetes incidence increased with days of use of pesticides applicators [28]. Some evidence proved also that heptachlor it-self affects lipid metabolism [33]. Organochlorine pesticides are well known to be lipophilic; people with higher BMI may be more likely to store higher levels of these pollutants than people with lower BMI, with equivalent exposure. This association was not strong with BMI (19) and (18) and we confirm this finding also. The study of Cox *et al.* [34] showed that the direction of the association of BMI with serum levels of organo-chlorines is not clear; both positive and negative associations have been reported in their literature [34].

When we evaluate the attitudes and behavior of the studied population towards pesticides, we found a significant association between heptachlor and DDE residues in the sera of women who didn't use or have pesticides in their home (Table 6) and this confirm that the presence of OC pesticides in other environmental compartment like food and soil may pose another risk factor for the human health. OC pesticide exposure is a complex and challenging process due to the multiplicity of their pathways of exposure, in addition and they shouldn't be treated as a homogeneous group of compounds. Although, these findings based in an occupationally exposed study, the findings may have relevance to the general population, who suffer greatly from environmentally persistent compounds and the increasing prevalence of diabetes, so there is a need for further extended study.

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