

Pesticides Residues in Egyptian Diabetic Children: A Preliminary Study

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Abstract

Pesticides exposure has been linked with many childhood diseases including endocrine and immune disorders. The aim of the present study is to monitor the levels of pesticides residues in a group of Type 1 Diabetic Children (T1D) in our locality and to explore if there is correlation between presence of pesticides and risk of occurrence of T1D. One hundred and ten Egyptian children; their ages ranged from 1.2 to 10 years were studied. The control group comprised 35 completely healthy children, while the study group included 75 children (newly diagnosed as T1D). Children were chosen from those attending Mansoura University Children Hospital. Blood samples were collected from both groups for detection of pesticides residues. The results reveal that lindane is the most common organochlorine pesticide detected followed by o,p-DDD and p,p-DDE as DDT metabolites; while the most prevalent organophosphate compound is malathion. It could be concluded that Egyptian children have measurable levels of several pesticides residues and there is increased risk of developing T1D in children exposed to some types of pesticides. Additionally, biomonitoring of these toxicants provide clinical toxicologists and physicians with reference values to be compared with other populations and could be correlated in the future studies with diseases claimed to be due to pesticide exposure especially in children.

Keywords: Pesticides; Organochlorine; Organophosphorus; Diabetic children

Introduction

Pesticides comprised several chemical compounds, which are used to increase agricultural products by preventing losses due to pests. Among the major groups of pesticides; Organochlorine (OC) pesticides are one of the most toxic and more potent due to their persistence and stability. They were prohibited from use throughout the world for more than 20 years ago [1]. Currently, OC are replaced by the less toxic organophosphorus compounds despite of being also restricted for use in many developed countries due to their toxic health effects [2].

However, many of the banned pesticides are still sold or manufactured for export to developing countries [2,3]. Furthermore, OC pesticides are subject to transport over long distances and can be detected even in areas where they have never been used. They can bioaccumulate and biomagnify in food chains. They are lipophilic and persistent with long half-lives [4]. In Egypt many studies have been documented the presence of OC in water; milk and its products; vegetables and fruits [5,6].

Exposure of children to pesticides may occur through placenta during fetal life, lactation and diet, or contact with contaminated house dust, carpets, chemically treated gardens or pets treated with insecticides [1,7]. Children are more vulnerable than adults to toxic effects of environmental pollutants because of their unique behavior and dietary pattern. Pesticide exposure has been linked with many childhood diseases particularly congenital malformations, growth disorders, cancer, malabsorption, immunological dysfunction, neurobehavioural and endocrine diseases [3,8,9].

Although pesticide exposure information is readily available for many areas in the world, toxicological data regarding pesticides exposure in human especially among Egyptian children remains largely unstudied [10]. The goal of the present study is to monitor the levels of pesticides residues in a group of type 1 diabetic (T1D) children in our locality and to explore if there is correlation between presence of pesticides and risk of occurrence of T1D.

Subjects and Methods

Subjects

This study was conducted on 110 children who were presented with their mothers to Mansoura University Children Hospital, Endocrinology and Diabetes Unit. The study began at September 2008 and ended by April 2010 when the targeted cases were collected. They were divided into two groups:

Study group: 75 children aged more than one year and ≤ 10 years who were newly diagnosed as type 1 diabetes (within the first month) and fulfilled the inclusion criteria.

Control group: 35 children who were completely healthy selected from the outpatient endocrinology clinic when they came with their siblings for follow up.

Exclusion criteria:

1. Any child with associated disease, endocrine disorders, birth defect, physical or mental retardation, or congenital anomalies e.g. cardiovascular or musculoskeletal.
2. Children with family history of diabetes.
3. Allergy, atopy, asthma or any associated autoimmune disease.

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Each child was subjected to

1. History taking from the mother to get sociodemographic criteria regarding age, sex and residence.
2. Complete medical examination.

Sampling

1. A written informed consent was taken from mothers of the studied children to participate in the research.
2. 5 ml of blood sample was collected from each child in polyethylene tubes without anticoagulant or serum separator. Blood was precipitated for 30 min and centrifuged at 3600 round per minute (rpm) for 15 min.
3. All serum samples were stored in Eppendorf tubes at -70°C, and then they were put in an ice tank to be transported to the National Research Center for pesticides residues analysis.
4. All instruments and vials used during sample preparation were cleaned with hexane and acetone and stored until usage.

Measurement of pesticides levels

Pesticide standards: Standards of Organochlorine (OC) pesticides include: Hexachlorobenzene (HCB); lindane; aldrin; heptachlor; endrin; p,p'-DDT: 1,1,1-trichloro-2,2-bis(p-chlorophenyl) ethane; o,p'-DDT: 1-(o-chlorophenyl) -1-(p-chlorophenyl)-2,2,2-trichloroethane; p,p'-DDE: (1,1-dichloro-2,2-bis(p.chlorophenyl) ethylene; o,p'-DDE: 1-(ochlorophenyl)-1-(p-chlorophenyl)-2,2-di-chloroethane); p,p'-DDD: 1-chloro-2,2-bis(p-chlorophenyl) ethane, p.p. DDA: 2,2-bis-4-chlorophenyl acetic acid and o,p'-DDD: 1- (o-chlorophenyl)-1-(p-chlorophenyl) -2,2-dichloroethane.

Standards for Organophosphorus (OP) pesticides include diazinon, chlorpyrifos-methyl, malathion, chlorpyrifos and profenofos. All standards were 97-99% pure 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 used solvents (hexane and acetone) were reagent grades and purchased from Merck (Merck, Darmstadt, Germany).

Extraction and instrumentation: Extraction of pesticides residues from different collected samples were applied according to the method of Liu and Pleil [11]. Extraction was done by vortexing with hexane (12 ml) for 1 min; then the mixture was centrifuged at 1600 rpm for 30 min. The organic phase (top) was then separated from the aqueous phase. Afterwards, the aqueous phase was extracted again following the same procedure using 6 ml of the solvent. The extracted samples were analyzed by Hewlett Packard Gas Chromatography (GC) Model 5890 equipped with Ni63 Electron Capture Detector (ECD), and fitted with HP-101capillary 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/in²). Concentrations of pesticide residues in different analyzed samples were calculated as nanogram/ml serum (ng/ml).

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

Demographic data	Studied Groups	
	Control (n=35)	Patients (n=75)
1) Age (years) Mean ± SD	6.1 ± 1.7	6.01 ± 1.9
t: 0.356-, p value: 0.722		
2) Gender	19 (54.3%)	39 (52%)
Female:		
Male:	16 (45.7%)	36 (48%)
χ ² : 0.050 , p value: 0.823		
3) Residence		
Rural	19 (54.3%)	35 (46.7%)
Urban	16 (45.7%)	40 (53.3%)
χ ² : 0.554, p value: 0.457		
P is significant if <0.05		

Table 1: Sociodemographic data of the control group (n=35) and the diabetic children (n=75).

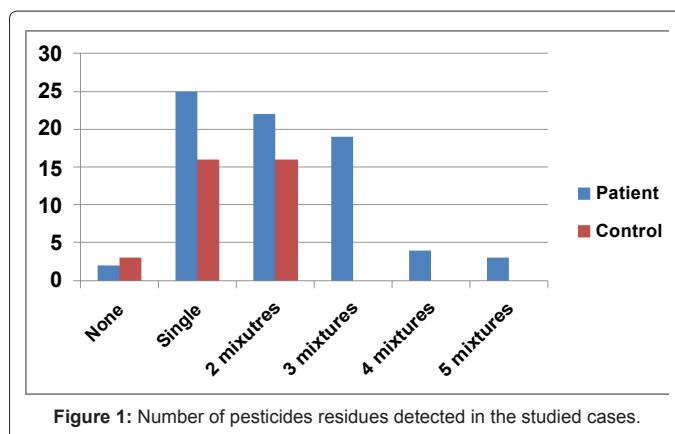


Figure 1: Number of pesticides residues detected in the studied cases.

(RSD) is usually more than 10% for methods involving a simple preparation procedure, the RSD is in the order of 5-10% [12]. The Limit of Detection (LOD) must be around 1 µg/l blood, higher than this limit may be adequate for monitoring occupationally exposed workers or for acute poisoning cases. In the present investigation, LOD was 2 µg/l for OC pesticides and 5 µg/l for OP pesticides.

Statistical analysis

The statistical analysis of data was done by using excel program for figures and SPSS (SPSS, Inc, Chicago, IL) program statistical package for social science version 16. Kolmogrov-Smirnov Z test was used for analysis of data and it was significant. Quantitative data were presented as mean, median; minimum; maximum and frequency. Chi square test (χ²) was used for qualitative data. Mann-Whitney test was used to test significance between groups. Significance was set at p<0.05. Odds ratios and corresponding 95% Confidence Interval (CI) were calculated to estimate the magnitude of association between independent variables.

Results

The sociodemographic data of the control and the diabetic children are presented in Table 1. No significant difference is found between the studied groups as regards age, sex and residence.

Pesticides residues are detected either as a single compound or mixtures as shown in Figure 1.

Pesticides residues detected	Control (n=35)	Patients (n=75)	Statistical data		
	N (%)	N (%)	Odds ratios	95% Confidence Interval	p
Organochlorines (OC)					
Lindane	19 (54.3%)	53 (70.7%)	2.02	(0.88-4.65)	0.09
Endrin	0	8 (10.7%)	1.52	(1.32-1.75)	0.04*
o,p-DDD	0	16 (21.3%)	1.59	(1.36-1.86)	0.00*
p,p-DDE	0	16 (21.3%)	1.59	(1.36-1.86)	0.00*
o,p-DDT	9 (25.7%)	6 (8%)	0.25	(0.08-0.77)	0.01*
p,p-DDA	0	4 (5.3%)	1.49	(1.30-1.70)	0.16
Organophosphates (OP)					
Malathion	11 (31.4%)	49 (65.3%)	4.11	(1.74-9.69)	0.00*
Profenofos	6 (17.1%)	2 (2.7%)	0.13	(0.02-0.69)	0.00*
Chlorpyrifos-Methyl	12 (34.3%)	6 (8%)	0.16	(0.05-0.49)	0.00*

Lindane: (hexachlorocyclohexane isomer: γ -HCH); o,p'-DDT: 1-(o-chlorophenyl)-1-(p-chlorophenyl)-2,2,2-trichloroethane; p,p'-DDE: (1,1-dichloro-2,2-bis(p-chlorophenyl) ethylene); o,p'-DDD: 1-(o-chlorophenyl)-1-(p-chlorophenyl)-2,2-dichloroethane; p,p-DDA: "2,2-bis-4-chlorophenyl acetic acid". *p is significant if <0.05.

Table 2: The frequency and odds ratio (OR) of pesticides residues in the studied groups.

Pesticides residues detected	Control (n=35)		Patients (n=75)		p
	Mean	Median (Min-Max)	Mean	Median (Min-Max)	
Organochlorines (OC)					
Lindane	<LOD	<LOD	0.48	0.54 (0.00-0.87)	0.00*
Endrin	<LOD	<LOD	0.91	0.90 (0.88-0.96)	0.00*
o,p-DDD	<LOD	<LOD	1.04	0.75 (0.16-4.3)	0.00*
p,p-DDE	<LOD	<LOD	0.37	0.28 (0.1-0.97)	0.00*
o,p-DDT	<LOD	<LOD	0.20	0.00 (0.01-0.61)	0.00*
p,p-DDA	<LOD	<LOD	1.07	1.07 (0.51-1.64)	0.00*
Organo-phosphates (OP)					
Malathion	0.03	0.03 (0.02-0.05)	0.54	0.61 (0.11-1.01)	0.00*
Profenofos	0.03	0.03 (0.03-0.04)	0.46	0.47 (0.47-0.47)	0.034*
Chlorpyrifos-Methyl	0.02	0.03 (0.01-0.04)	0.24	0.00 (0.00-0.72)	0.254

LOD=limit of detection; Min=minimum; Max=maximum; *p is significant if ≤ 0.05

Table 3: Concentrations of pesticides residues (ng/ml) in the serum of the studied groups.

The frequency and odds ratio of pesticides residues in the studied groups are shown in Table 2. Lindane is the most common organochlorine pesticide detected in the diabetic group (70.7%); followed by o,p-DDD and p,p-DDE (21.3% each); while the most prevalent organophosphorus compound is malathion. Children exposed to malathion, lindane, p,p-DDE, o,p-DDD, endrin and p,p-DDA as seen from the highest odds ratio (4.11, 2.02, 1.59, 1.59, 1.52 and 1.49 respectively) have the highest risk to develop T1D than the control healthy group.

The concentrations of pesticides residues in the serum of the studied groups are illustrated in Table 3. In diabetic children, the organochlorine pesticides; p,p-DDA; o,p-DDD; endrin and the organophosphorus malathion have the highest concentrations. On the

other hand, organochlorines are not detected in the control group while malathion has the highest concentration.

However, pesticides residues which are not detected in any of the serum of the test group include: hexachlorobenzene (HCB); aldrin, heptachlor, p,p'-DDT; o,p'-DDE; p,p'-DDD; diazinon and chlorpyrifos as they are below the Limit Of Detection (LOD).

Discussion

Several childhood diseases such as allergic disorders, type 1 diabetes and cancer have been linked to environmental exposures [8]. Type 1 diabetes mellitus (T1DM) is the most common chronic metabolic condition seen in children. Its global incidence is rising by around 3.4% per year but the reasons for this increase remain unclear. Researches that highlight environmental "triggers" of T1DM are on the rise [13,14].

Pesticides are relevant environmental pollutants. Studies regarding the levels of these toxic chemicals in humans especially in children are scarce [10,15]. To our knowledge, this work is the first one aiming to monitor the levels of pesticides residues in a group of type 1 diabetic children (T1D) in our locality and to explore if there is correlation between presence of pesticides and risk of occurrence of T1D.

In the present study, many types of OC and organophosphorus pesticides or their metabolites (i.e. lindane, endrin, o,p-DDD, p,p-DDE, o,p-DDT, p,p-DDA) were detected in serum of diabetic children (either single residue or mixed residues). Only the organophosphorus compounds were found in the control healthy children.

Regarding organochlorine pesticides, the current work stated that lindane was the most common in 53 cases (70.7%) followed by o,p-DDD and p,p-DDE in 16 cases each and endrin in 8 cases. On the other hand, malathion is the commonest organophosphorus compound detected (65.3%). Those compounds show the highest odds ratio indicating an increased risk of occurrence of type 1 diabetes in the exposed children.

To our knowledge, this is the first study concerned with the relation between pesticides and T1D. The present results were consistent with Lopez-Espinosa et al. [16] who detected p,p-DDT, lindane and aldrin in adipose tissue of 12% of studied children. Other OC residues are found in different percentages i.e. p,p-DDE (79%), o,p-DDT (17%); o,p-DDD (15%) and dieltrin (8%). More or less similar, Luzardo et al. [17] mentioned that endrin was present in 22% of the children studied from Canary Islands (Spain).

Porta et al. [18] detected p,p-DDT, o,p-DDT, o,p-DDE, p,p-DDE, p,p-DDD, o,p-DDD and HCH in more than 85% of the studied population in Spain.

The present findings indicated that although an Egyptian Ministerial Decree prohibited the import and use of OC in 1996, some of these toxic pesticides are still illegally applied making exposure to these compounds unavoidable [19,20]. This can be also attributed to the persistence of these compounds for decades in the environment and food chains [21].

Similarly, in spite of the ban of OC pesticides by the United States and Canada more than two decades ago, DDE was detected in a majority of blood samples collected in the US (1999-2004), and in Canada (2007-2009), while DDT was detected in 5-10% of samples due to a longer half-life of DDE and the direct exposure to DDE in foods [22,23]. DDD is generally not detected in serum, due to its greater water solubility and lower persistence [21].

In the present study, p,p-DDA; o,p-DDD and endrin had the highest

mean concentrations among diabetic children (1.07; 1.04 and 0.91 ng/ml respectively). Regarding organophosphorus compounds; malathion had the highest concentration (0.54 ng/ml).

In contrast, other researchers had reported that lindane and endrin levels were below the limit of detection in most persons [16,22,24]. This may be attributed to rapid metabolism of endrin; so it does not accumulate in the body. Another possible explanation is good OC banning measures in the studied areas or due to different analytical methods used. In the present work, endrin is detected in the serum of diabetic children which could be attributed to the high dose of endrin or very recent exposure [22].

Unfortunately, there are no limits or standards that regulate the pesticide levels in biological samples. This is primarily because biomonitoring of these compounds is relatively uncommon and very little is known about how levels correlate with harmful health effects [22].

In Egypt, many studies were carried out to assess OC concentrations in different matrices including soil, air, water and food, with only very few reports in human restricted to milk samples, hence, it is difficult to compare the values in the present work with other researches in our country [19]. On the other hand, making a comparison on the basis of historical reference groups in other societies has several limitations due to many variables such as geography, time, type of samples and dissimilar demographic criteria, diversity of analytical methods and results expression. These factors can bias the level of exposure to the chemicals of interest.

In addition to the aforementioned results, three types of Organophosphorus (OP) residues were detected in the serum samples of the studied children in the following order of frequency: malathion, chlorpyrifos-methyl and profenofos with mean serum concentrations (0.54, 0.46 and 0.24 ng/ml respectively).

It is well known that OPs had short half-lives range from hours (12-24 hours for malathion) to weeks [25]. The detection of parent compounds reflects very recent exposure over the previous few days [26]. So, the fact that we could measure these chemicals in the studied samples is suggestive of a higher magnitude of exposure than was expected.

In the US, a mixture of OP residues were detected in the blood and/or urine of nearly all persons sampled [27]. Several birth cohort studies have detected chlorpyrifos and diazinon and other various OP in cord blood [28,29]. Of course, the type of the detected pesticides varied due to different pesticides used in each society and the time of the study.

In accordance with the present findings, many researchers reported OPs exposures in young children. However, most of these studies did not involve measurement of the parent pesticides. Instead, they used urinary metabolites as markers of exposure [30-33].

Virtually, metabolites cannot be attributed to a specific organophosphate pesticide and they might be previously formed in or on the consumed food. It is also difficult to assign health effects to a certain OP compound. [34]. In the present work, measurement of the parent OPs could be considered an advantage and more reliable indicator of recent direct exposure to these compounds.

From the present findings, there is an observed strong association between some types of pesticides (malathion, lindane, p.p.DDE, o.p.DDD, endrin and p.p.DDA) and the risk of occurrence of childhood diabetes in relation to the control non-diabetic group. To the best of

our knowledge, this the first study investigating the association between type 1 diabetes in children and exposure to pesticides.

Very scarce studies concerning the relationship of POPs with diabetes were found. It was reported that low-dose exposure to OC pesticides tended to show the strongest association with type 2 diabetes in adults [34-36]. In addition, Son et al. [37] observed that the risk of diabetes increased in relation to using heptachlor epoxide among pesticide applicators. Moreover, Montgomery et al. [38] stated that heptachlor epoxide, oxychlorane and β -HCH were also strongly associated with metabolic syndrome or insulin resistance in non-diabetic.

Conclusions

From the current work, it could be concluded that Egyptian diabetic children have measurable levels of several pesticides residues and there is increasing risk in children exposed to pesticides to develop T1D. Additionally, biomonitoring of these toxicants provides clinical toxicologists and physicians with reference values to be compared with other populations and could be correlated in the future studies with diseases claimed to be due to pesticide exposure especially in children.

Recommendations

It is recommended to establish regular surveys to set reference values in our population and to identify highly exposed groups. That, in turn, may assist in studying the toxic effects of pesticides and mechanisms possibly related to the etiology of many diseases linked to environmental contaminants. Governments must put strict legislation to reduce exposure to various toxic pesticides especially in the highly susceptible groups i.e. children.

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