Research Article

Biomarkers of Organophosphate Pesticides and Attention-Deficit/ Hyperactivity Disorder in Children: A Case-Control Study

Manal Abd El-Salam1, Amal A Hegazy2*, Marwa Elhady3, Gamil Elsayed Ibrahim4 and Rania Hussein5

1Pediatrics Department, Faculty of Medicine, Al-Azhar University, Cairo, Egypt
2Family and Community Medicine Department, Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia
3Community and Occupational Medicine Department, Faculty of Medicine, Al-Azhar University, Cairo, Egypt
4Flavour and Aroma Chemistry Department, National Research Center, Cairo, Egypt
5Neuropsychiatric Department, Faculty of Medicine (for girls), Al-Azhar University, Cairo, Egypt

Abstract

Children are frequently exposed to significant hazard for organophosphate poisoning (OP) in their environment; since the brain is growing during this period and it is extra liable to neurotoxicants. The aim of this work was to determine the relationship between urinary concentrations of dialkylphosphates (DAP) metabolites, which are biomarkers of OP pesticide exposure, and attention-deficit/hyperactivity disorder (ADHD) in Egyptian children ages seven to twelve years. A case-control study was conducted at Azhrara University hospital. We recruited 40 children diagnosed as ADHD according to “the diagnostic and statistical manual of mental disorder (DSM-5) criteria” by psychiatrist. Control group of 40 healthy children who did not have any psychiatric or neurologic disorders, age and sex matched. Exposure was determined by analyzing six urinary metabolites of DAP. We investigated the association between ADHD subtypes, coincidence of epilepsy and OP exposure. Significant increase in urinary level of all DAP metabolites for children with ADHD in comparison to control group except Diethyl-dithio-phosphate. ADHD children with epilepsy had statistically significant elevated level of urinary Dimethyl-dithio-phosphate, Diethyl- phosphate and Dimethyl-phosphate metabolites in comparison to those without epilepsy. By logistic regression analysis, Children with high level of urinary Dimethyl-dithio-phosphate (OR=2.29), Diethyl-phosphate (OR=2.40), Dimethyl-phosphate (OR =2.02), and Dimethyl-thio-phosphate (OR=1.82) have two fold increased risk of developing ADHD than those having lower concentration of these metabolites. Our findings back up the theory that existing environmental concentration of organophosphate pesticide exposure may lead to the development of ADHD and increase the risk of epilepsy in ADHD children.

Keywords: Organophosphate; Pesticides; ADHD; Dialkylphosphates; DAP; Epilepsy

Introduction

Farming and occupant use of organophosphate (OP) pesticides has increased than before in recent decades after forbidding some persistent pesticides. Even though there is confirmation of the results of OP on neurodevelopment and behaviour in adults, however, the data about their outcome in children is restricted [1]. Both Farming and inhabitant settings used a lot of million pounds of OP in 2001. The Environmental Protection Agency (EPA) anticipates nutrition, drinking water, and inhabitant pesticide apply as vital sources of exposure [2]. Inhabitant pesticide use is common, but the major origin of exposure to pesticides for infants and children would be the meal, according to the National Academy of Sciences [3].

Taken into consideration children are frequently exposed to significant hazard for organophosphate poisoning; since the brain is growing during this period and it is extra liable to neurotoxicants [4]. Regarding the body weight of children and the amount of pesticides tend to be high. Young children whose age range from six to eleven years having greatest urinary levels of dialkyl-phosphate metabolites (biomarkers of organophosphate exposure), contrast to another group of different age [5]. Body of young children containing minor levels of metabolizing enzymes (paraoxonase or chlorpyrifosoxonase) that disrupt OP pesticides than adults [6,7], indicating that children may be higher susceptible to risk of exposure.

Dialkyl-phosphates (DAP) are metabolites resulting from the degradation of various OP pesticides by the act of esterases. The degradation of most organophosphate pesticides creates only six alkyl-phosphate metabolites which point to that a single investigative technique is adequate to detect exposure to different substances [8]. The six DAP metabolites were three "dimethyl-alkyl-phosphate (DMAP), including dimethyl-phosphate (DMP), dimethyl-thio-phosphate (DMTP), and dimethyl-dithio-phosphate (DMDTP), and three diethyl-alkyl-phosphate (DEAP) molecules, including diethyl-phosphate (DEP), diethyl-thio-phosphate (DETDP), and diethyl-dithio-phosphate (DEDTP) [9].

Numerous biological techniques might cause connection between ADHD and OP pesticides. The main action of these toxic substances, mostly due to acute toxicity, is suppression of acetylcholin esterase [10], and disturbance in cholinergic signals [11]. When the dose lower than that causing acetylcholineesterase suppression, specific OP influence various neurochemical targets, numerous neurotransmitter and second messenger systems, including growth factors [12,13]. OP pesticides exposure during growing might have constant effects on multiple neural systems that lead to ADHD behaviors, such as inattention and cognitive deficits, which is similar to the symptoms due to nicotine exposure during development [14,15]. Globally ADHD is considered one of the main prevalent neurobehavioral disorders in children, affecting three to ten percent of children [16-18].

*Corresponding author: Amal A Hegazy, Family and Community Medicine Department, Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia, Tel: +201063635064; E-mail: renalahmed@gmail.com

Received April 19, 2017; Accepted April 24, 2017; Published April 28, 2017


Copyright: © 2017 El-Salam MA, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
ADHD manifestations mostly continue into youth and adult, and are linked to considerable social and medical costs [19,20]. A lot of studies pointed to that neurodevelopmental troubles, encompassing ADHD, outcome from combined connections of hereditary and ecological causes. There is increasing evidence regarding the role of different toxicants in neurodevelopmental disorders, including ADHD. The growing brain is completely susceptible to substances which are toxic to neurons at low level that does not cause serious effects on adulthood. Even though many successful hard work from public health activities and regulatory agenda restricting the level of exposure to industrial chemicals, although alarms are present as result of their effect, yet at little concentration, on the health of children and development of neurons [21]. Our objective is to determine the association between concentrations of dialkyl-phosphate metabolites in urine, which are biomarkers of organophosphate pesticides exposure in Egyptian children ages seven to twelve years and attention-deficit/hyperactivity disorder.

Materials and Methods

This case control study was conducted at Alzhraa hospital, AL-Azhar University, Cairo, Egypt, between July 2015 and May 2016. It comprised 40 children diagnosed as ADHD according to the diagnostic and statistical manual of mental disorder (DSM-5) criteria [18] by psychiatrist. They were recruited from psychiatric outpatient clinic at Alzahraa University hospital. Another group of 40 healthy children was selected randomly at the same period from outpatient pediatric clinic at Alzahraa University hospital and did not have any psychiatric or neurologic disorders, age and sex matched children served as a control group. Their age ranged between 7 to 12 years old. Informed written parental consent was obtained prior to enrollment in the study in adherence with the guidelines of the ethical committee of Alzhraa hospital, AL-Azhar University, Cairo, Egypt.

Children with any systemic medical illness (e.g., cardiac, respiratory, hepatic, renal diseases, neuromuscular disabilities) or any psychological disorders other than ADHD and parental psychosocial factors were excluded from the study.

Clinical history and examination

All the studied children were subjected to clinical examination and interview with their parents to fill the questionnaire. It included demographic data, presenting symptoms, any medical disorders, presence of seizures, current medications and history of exposure to passive smoking, chemical pollutant, household or environmental pesticides.

Diagnosis of ADHD

Children were diagnosed as ADHD according to the DSM-5 criteria [18] by psychiatrist and children were categorized into (subtypes): hyperactive, inattentive or mixed ADHD. Additionally, Conner test was done to all the included children. This test is a diagnostic tool used to screen for ADHD in children and adolescents. The test is used to assess the behaviour, emotion and academic problem and it includes the DSM-5 criteria for diagnosis of ADHD. In this study we used the parent form of Conner test. It is a set of rating scales consist of 43 items with score 0-3 for each. It offers straightforward administration and scoring with excellent validity and reliability. Scores above 60 are usually a sign for having ADHD. Scores between 61 and 70 are usually a sign that the child may have a moderately severe problem. Score more than 70 indicates a more severe problem. Completion of the test takes from 60-90 minutes.

Electroencephalogram (EEG)

Digital interictal EEG was performed using a Nihon Kohden 1200 digital EEG instrument. Intermittent photic stimulation activation procedure was done for all cases. Chloral hydrate sedation was infrequently used in unhelpful children (30 mg/kg maximum 1 gm/dose) that were given 30 minutes before recording with complete monitoring of the vital data during the recording. Regarding history of seizures and EEG results we divided ADHD group into two groups, the first one is ADHD with epilepsy and the other is ADHD without epilepsy.

Laboratory investigation

Urine collection and urinalysis: Samples of urine were collected, transported on ice to the laboratory without delay, and kept back frozen at (-20°C) at the time of examination. Before analyzing, the urine samples were liquefied and homogenized using a vortex mixer. Six DAP as biomarkers of organophosphates exposure were analyzed by gas chromatography (GC): DMP, DEP, DMTP, DETP, DMDTP and DEDTP.

Samples of urine were prepared for GC examination according to a modified method of Moate et al. [22]. “Aliquots of the samples underwent azotropic distillation with methanol and evaporation under a nitrogen stream. Sample extracts were then derivatized with 2,3,4,5,6-pentafluorobenzylbromide to convert phosphate acids to esters. Extracted samples were analyzed on a gas chromatograph (Agilent 7890A GC system equipped with an OF 5380 pulsed flame photometric detector (PPFD)”. Creatinine concentrations were measured using the Jaffe reaction [23].

Each metabolite was converted from its untransformed concentration microgram/liter (µg/L) to the corresponding molar concentration (nmol/L) by dividing by its molecular weight. The concentrations of urinary DEP metabolites were expressed as µg per gram (µg/gm) creatinine.

Statistical analysis: Statistical analysis was performed using the Statistical Package for Social Sciences (version 20; SPSS Inc., Chicago, IL, USA). Quantitative data were expressed as mean ± SD. Differences between two groups were analyzed with independent T student test. Differences between more than two groups were analyzed with ANOVA test. Logistic regression analysis was done to determine the predictors of ADHD in the studied children. Receiver operating characteristic curves (ROC) were used to identify the optimal cut-off points of urinary metabolites levels for prediction of ADHD. P-value <0.05 was considered to be significant.

Results

This study carried out at forty children with ADHD (60% male and 40% female); twenty (50%) of them were hyperactive, ten (25%) were inattentive and ten (25%) were mixed type ADHD. The age of onset ranged between two to five years with a mean duration of illness of 6.25 ± 3.55 years. Twenty seven of them (67.5%) received medication. Twenty two of them (55%) had EEG abnormalities but only 18(45%) of them had confirmed diagnosis of epilepsy. Another 40 healthy children were selected as controls (62.5% male and 37.5% female). There was no significant difference between cases and control regarding age, gender, socioeconomic status, passive smoking, weight and height (p=0.632, p=0.053, p=0.622, p=0.820, p=0.528, and p=0.871, respectively). As demonstrated in Table 1, there was statistically significant increase in urinary level of all DAP metabolites for children with ADHD than control group (p<0.000 except DEDTP (p=0.948).

We observed significant association between ADHD subtypes
(hyperactive, inattention or mixed) regarding urinary level of DEDTP, DMTP and DMP (p=0.002, p=0.001 and p=0.001, respectively); however there was no significant difference in the rest of dialkylphosphates metabolites [DMDTP (p=0.469), DETP (p=0.183) and DEP (p=0.389). In addition, by Post hoc: LSD test we found significant increase in urinary metabolite of DMP and DME in children with hyperactive versus inattention and mixed type while there was no significant difference between inattention versus mixed type (Table2).

Furthermore ADHD children with epilepsy had statistically significant elevated level of urinary DMDTP (p=0.030), DEP (p=0.007) and DMP (p=0.002) metabolites in comparison to those without epilepsy as shown in Table 3. Logistic regression analysis for ADHD predictors of DAP metabolites demonstrated in Table 4. The common predictors of urinary DAP metabolites associated with ADHD were DMDTP (OR=2.29), DEP (OR=2.40), DMP (OR=2.02) and DMTP (OR=1.82). Children with high level of urinary DMDTP, DEP, DMP, and DMTP were have two fold increased risk of developing ADHD than those having lower concentration of these metabolites.

The cut off points for dialkylphosphates metabolites for ADHD cases were revealed in Table 5; it revealed that DETP is 100% sensitive and 65% specific, DMTP is 95% sensitive and 55% specific and DMDTP is 80% sensitive and 90% specific in prediction of ADHD. However in ROC curve (Figure 1) shows that the most sensitive predictor tests of ADHD are DEP followed by DMDTP and DETP.

**Discussion**

OP pesticides exposure was widespread in our children, with hundred percent of them having DAP metabolites detected in their urine. Our results suggest a connection between childhood urinary dialkyl-phosphate metabolite concentrations, which are biological biomarker of organophosphate pesticides exposure, and ADHD particularly hyperactive-impulsive type. These findings are consistent with previous studies that reported increased ADHD behavioral problems in school-age children with higher organophosphate metabolite concentrations in urine. Lizardi et al. [24] reported adverse link of child urinary DAP and attention related performance errors in children at seven years of age. In this research the children with high level of urinary DMDTP, DEP, DMP, and DMTP were have two fold increased risk of developing ADHD than those having lower concentration of these metabolites. Yu et al. [9] found that higher concentration of DMP in children urine may have a double to triple increased risk of being diagnosed with ADHD. Furthermore, Bouchard et al. [2] reported that Children with elevated levels of dialkyl-phosphate in urine, particularly dimethyl-alkyl-phosphate (DMAP) were more likely to be diagnosed as having ADHD.

<table>
<thead>
<tr>
<th></th>
<th>Control (n=40)</th>
<th>ADHD (n=40)</th>
<th>test</th>
<th>t-test</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DEDTP (nmol/L)</td>
<td>Mean ± SD Range</td>
<td>38.60 ± 8.35 17-51</td>
<td>38.47 ± 8.99 24-53</td>
<td>0.065</td>
<td>0.948</td>
</tr>
<tr>
<td>DMDTP (nmol/L)</td>
<td>Mean ± SD Range</td>
<td>43.55 ± 9.06 26-68</td>
<td>75.95 ± 14.31 45-98</td>
<td>12.095</td>
<td>0.000*</td>
</tr>
<tr>
<td>DETP (nmol/L)</td>
<td>Mean ± SD Range</td>
<td>90.82 ± 11.31 76-114</td>
<td>117.80 ± 38.03 75-184</td>
<td>4.300</td>
<td>0.968</td>
</tr>
<tr>
<td>DMTP (nmol/L)</td>
<td>Mean ± SD Range</td>
<td>115.58 ± 24.02 94-183</td>
<td>191.88 ± 52.07 128-312</td>
<td>8.418</td>
<td>0.000*</td>
</tr>
<tr>
<td>DEP (μg/gmcreatinine)</td>
<td>Mean ± SD Range</td>
<td>60.50 ± 14.21 46-77</td>
<td>88.52 ± 15.42 67-125</td>
<td>3.990</td>
<td>0.000*</td>
</tr>
<tr>
<td>DMP (nmol/L)</td>
<td>Mean ± SD Range</td>
<td>195.82 ± 25.46 178-247</td>
<td>292.22 ± 68.64 178-451</td>
<td>3.877</td>
<td>0.002*</td>
</tr>
</tbody>
</table>

**Table 1**: Comparison between cases and control group regarding level of dialkylphosphates metabolites.

| DEDTP (nmol/L) | Mean ± SD Range | 42.85 ± 8.79 28-53 | 32.00 ± 6.43 24-39 | 7.096 | 0.002* |
| DMDTP (nmol/L) | Mean ± SD Range | 74.95 ± 17.55 38-94 | 72.40 ± 10.93 58-89 | 0.773 | 0.469 |
| DETP (nmol/L) | Mean ± SD Range | 85.25 ± 12.57 73-108 | 92.40 ± 12.55 75-108 | 1.781 | 0.183 |
| DMTP (nmol/L) | Mean ± SD Range | 211.20 ± 52.20 137-312 | 141.80 ± 17.96 128-173 | 8.712 | 0.001* |
| DEP (μg/gmcreatinine) | Mean ± SD Range | 86.50 ± 17.47 67-113 | 94.20 ± 17.97 78-125 | 0.968 | 0.389 |
| DMP (nmol/L) | Mean ± SD Range | 328.65 ± 65.51 226-451 | 266.80 ± 49.54 229-357 | 9.309 | 0.001* |

**Post hoc: LSD test**

| DME (nmol/L) | Hyperactive vs. Inattention | 0.001* | 0.033* | 0.234 |
| DMDTP (nmol/L) | Hyperactive vs. Mixed | 0.673 | 0.222 | 0.483 |
| DETP (nmol/L) | 0.153 | 0.524 | 0.077 |
| DMTP (nmol/L) | 0.000* | 0.646 | 0.003* |
| DEP (μg/gmcreatinine) | Inattention vs. Mixed | 0.206 | 0.947 | 0.248 |
| DMP (nmol/L) | 0.011* | 0.000* | 0.236 |

*Significant
A study was done at United State observed that more than ninety percent of children have noticeable urinary residues of DAP [25]. The half-life of DAP metabolites range from twenty four to twenty seven hours and three to six days for complete removal from the body, the recognition of high rate of DAP point to that children are constantly exposed to OP pesticides [26]. Children may get bigger exposure than adults since they eat, drink, and respire more per unit of body weight. In addition, children are further critical to the effects of toxic substances as their bodies have lower detoxification abilities [27].

The associations between organophosphate pesticides and ADHD in children may be explained by several biological mechanisms. A main mechanism of organophosphates, mainly with regard to acute poisoning, is suppression of acetylcholinesterase [28] and disturbances in cholinergic signaling are considered to take place in ADHD [29]. At dosages minor than those required to suppress acetylcholinesterase, certain organophosphates affect different neurochemical targets, including disruption of the nuclear transcription factors, interference with neural cell development, alteration of synaptic formation [30], reduced muscarinic and nicotinic acetylcholine receptor expression, DNA synthesis inhibition, generation of reactive oxygen species [31], dysregulation of neuronal signal pathways and has neuronal excitatory effects [32]. These effects occur at lower organophosphate concentrations that did not inhibit acetylcholinesterase so cholinergic transmission remains unaffected [33].

Our results revealed that elevated urinary organophosphate metabolite levels are associated with increase coincidence of epilepsy in ADHD children. Verhulst et al. [33] reported that thirty percent of children intoxicated with OP developed tonic-clonic-
Conclusions and Recommendation

Our children continuously exposed to organophosphate pesticides in their environment. The present study support the building up information that connecting elevated concentrations of pesticide exposure to unfavorable neuro-developmental results. Results of this study back up the theory that existing environmental concentration of organophosphate pesticide exposure may lead to the development of ADHD and increase the risk of epilepsy in ADHD children. In view of these findings, we recommend the implementation of measures in the form of policies and regulations to control the use of pesticides in the farm and homes. Additionally, increase the public awareness and health education of the general population about the hazard of pesticides and how to avoid the exposure especially in children.

References