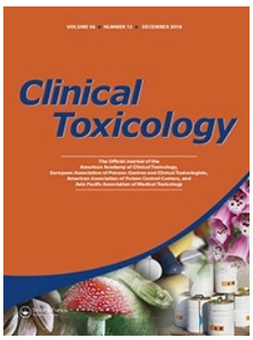

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CLINICAL RESEARCH

Subacute and chronic neuropsychological sequalae of acute organophosphate pesticide self-poisoning: a prospective cohort study from Sri Lanka

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ABSTRACT

Context: Some epidemiological evidence implicates acute organophosphate (OP) pesticide poisoning in long-term neurocognitive deficits. However, no study has prospectively followed up poisoned patients long-term from the time of intoxication. We aimed to determine whether clinically significant acute OP self-poisoning leads to subacute and chronic neurocognitive deficits, in a prospective follow up study.

Methods: Employing Mini Mental State Examination, Digit Span and Cambridge Neuropsychological Test Automated Battery (CANTAB), we compared multiple cognitive functions in 222 patients hospitalized with acute OP pesticide self-poisoning with a control group of 52 patients hospitalized with paracetamol overdose, at three time points: on discharge following clinical recovery, 6 weeks and 6 months post-ingestion. Intergroup comparisons at each time point were done in multiple regression models, adjusting for sex, age, education and psychiatric comorbidities. OP within-group analysis was done to determine a dose–response relationship.

Results: After adjusting for covariates, the OP poisoned group had significantly poorer working memory (Digit Span) and episodic memory (CANTAB Paired Associates Learning); impaired spatial planning (CANTAB Stocking of Cambridge); and slower response speed in the sustained attention task (CANTAB Rapid Visual Information Processing), in the post-discharge assessment. Only working memory and episodic memory measures were impaired in the OP group at 6 weeks, whereas no significant intergroup differences were observed at 6 months. The OP subgroup who had complete red cell acetylcholinesterase inhibition on admission had poorer episodic memory when tested post-discharge than those who had partial inhibition, but no significant subgroup differences were observed at 6 weeks or 6 months.

Discussion: Acute OP pesticide poisoning may cause neuropsychological impairment that outlasts the cholinergic phase on a subacute time scale; but does not cause measurable chronic neuropsychological deficits.

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Organophosphate; pesticide; poisoning; cognition; memory; neuropsychological effects; self-harm

Introduction

Organophosphate (OP) pesticides are extensively used as crop insecticides around the world. Human intoxications occur in the form of chronic low-level occupational or environmental exposure, or as acute accidental or intentional poisoning. In the acute phase of poisoning, acute cholinergic overactivation is caused when OPs inhibit the enzyme acetylcholinesterase that degrades acetylcholine in central and peripheral cholinergic synapses. Apart from the well-defined effects of acute cholinergic crisis, long-term cognitive changes following chronic low-level exposure to OP pesticides have been investigated in many studies ([1–4] for reviews); and acute accidental OP pesticide poisoning have been reported in four large-scale epidemiological studies [5–8].

The four studies on acute OP poisoning have assessed apparently healthy, pesticide or plantation workers using an array of neuropsychological tests, between several months [5,6] to few years [7,8] after acute accidental poisoning. The intoxication was ascertained retrospectively based on medical records that documented that 1) the subjects experienced symptoms and had cholinesterase inhibition [7], 2) the treating physicians detected clinical features [5], or 3) the subjects sought outpatient medical attention [8] or hospitalization [6]. These rather limited, retrospective measures of the severity of intoxication preclude establishing a dose–response relationship between OPs and cognitive impairment, that would make a case for causal inference. Furthermore, lack of repeated neuropsychological assessments since the subacute stage of poisoning has prevented delineating how their cognitive profiles change with time.

Worldwide, occupational exposures are not currently the most common reason for significant acute OP poisoning.

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Supplemental data for this article can be accessed here.

Self-poisoning using pesticides remains a common mode of self-harm in agricultural communities in the developing world, including Sri Lanka, where pesticides can be easily accessed [9,10]. We reported two small-scale clinical studies where patients with OP pesticide self-poisoning were prospectively recruited based on a history of self-ingestion of an OP pesticide and presence of signs of systemic intoxication [11,12]. In these studies, the OP-poisoned group – compared to a matched control group of patients hospitalized with intentional paracetamol overdose – showed impaired cognitive processing of visual and auditory information, even after clinical recovery from the cholinergic phase of intoxication. However, the smaller samples precluded adjustments for potential confounding factors such as psychiatric comorbidities prevalent in that population. Neither could we follow up significant numbers of participants long-term to determine how those subacute deficits changed over time.

The objective of the present study was to determine whether acute intentional poisoning of OP pesticides is associated with subacute and chronic neuropsychological deficits, in a prospective study with periodic follow-up assessments. If acute OP pesticide poisoning causes any subacute or long-term impairment, we hypothesized that any impairment observed in an OP-poisoned patient at a given post-exposure time point should be over and above what is observed at the same post-exposure time point in a patient who attempted self-poisoning of an agent that does not affect the nervous system. To this end, we recruited and followed up a control cohort of patients with intentional paracetamol overdose.

To determine the potential toxic effects of OPs on different areas of the brain, we selected neuropsychological tests that tap into three neurocognitive domains with rather distinct neuroanatomical underpinnings: visual attention and visuomotor skills (occipital lobe and fronto-parietal circuits); visual memory and learning (medial temporal lobe); and planning and working memory (prefrontal cortex).

Methods

Study design and setting

We conducted a prospective cohort study, recruiting patients admitted to the Clinical Toxicology Departments of two hospitals in the Central Province of the Sri Lanka: The Teaching Hospital Peradeniya – a tertiary care toxicology center in the island, and the District General Hospital Nuwara Eliya. We prospectively recruited two cohorts of hospitalized patients: the test cohort of patients acute OP self-poisoning (*OP group*) and a control cohort of patients with intentional paracetamol overdose (*control group*). This control group was selected since they tend to be similar to the test cohort in terms of the degree of impulsivity and psychiatric co-morbidities; but not having central nervous system deficits caused by the agent ingested. Each cohort was followed up with neuropsychological assessments at three time points: within first few days post-discharge (subacute phase), and 6 weeks and 6 months (chronic phase) post-ingestion. Then we compared the performance of the two groups at each time point, adjusting for age, sex, education and psychiatric comorbidities.

The ethics approval for the study was granted by the Human Research Ethics Committee of the Faculty of Medicine, University of Peradeniya. Informed written consent was taken from all participants. For those who were unconscious, intubated or otherwise severely ill, the consent was initially obtained from the next of kin, and the consent from the patient was obtained once they recovered. Those who were younger than 18 years, informed written consent was also taken from the parents or guardians. The eligible study participants were recruited from the study hospitals from February 2013 to October 2017.

Study participants

The ingestion of an OP pesticide was based on a reliable history provided by the patient or a witness, the container provided, and/or pesticide confirmation in laboratory testing. Those who had 1) two or more cholinergic features of intoxication (viz. miosis, excessive sweating/secretions, bradycardia, dyspnoea/lung signs, limb or tongue fasciculations and impaired consciousness) and/or 2) on-admission red blood cell acetylcholinesterase (RBC-AChE) levels below 10 Units/g Hb were considered having significant acute intoxication and were recruited to the OP group. RBC-AChE was measured using Testmate ChE (EQM Research, Inc., Cincinnati, OH) a validated method for bedside assessment [13].

The patients hospitalized with intentional paracetamol overdose were recruited to the control group. None of them developed liver failure or hepatic encephalopathy.

We considered individuals between 16 and 70 years of age for recruitment. We excluded those who were acutely psychotic or aggressive, and those who had preexisting neurological diseases that can affect cognitive functions, a history of head injury with potential neurological damage, motor dysfunction of the upper limbs, gross uncorrected visual or hearing impairment, alcohol dependence or substance abuse from both groups.

Clinical data

All patients were admitted under the care of a specialist physician. All OP-group participants underwent a neurological examination and RBC-AChE measurement on admission. The patients were monitored frequently during the first few hours following the initial assessment, and thereafter at least twice a day by the ward doctors. OP-poisoned patients were managed according to the standard protocols [14], atropine dosing and timing being determined by the need of each patient. Pralidoxime was administered by the treating physicians to some patients with OP poisoning. Patients with paracetamol toxicity, when indicated, were treated with *N*-acetylcysteine. All patients were seen by a psychiatrist. Those who had preexisting or newly diagnosed psychiatric illnesses were managed by the psychiatry teams of the respective hospitals.

In addition to the clinical records maintained by the ward staff, medically qualified clinical research assistants recorded demographic information, circumstantial evidence related to the episode of poisoning, clinical data and laboratory investigation findings in a structured data sheet. On discharge, the participants were given an appointment for the first neuropsychological assessment.

Neuropsychological assessment

Neuropsychological assessments were conducted at the Clinical Neurophysiology and Cognition Laboratory at the Department of Physiology, Faculty of Medicine, University of Peradeniya. We tested the participants of both groups at three time points: within few days after discharge from the hospital (i.e., post-discharge), 6 weeks and 6 months after poisoning. To allow complete elimination of atropine, we ensured the OP patients were first tested at least 2 days (i.e., approximately 12 half-lives) after their last dose. The participants were instructed not to consume alcohol from the evening the day before testing, sleep at least 6 h in the previous night, and not to smoke or consume coffee on the day of testing. Those who were on psychiatric drugs or other long-term medications were allowed to continue their medications as usual. Each participant was compensated with 1500 Sri Lankan Rupees (equivalent to around 10 US Dollars during that time) for the time they spent on each visit.

The participants received the test instructions in their first language which was Sinhala or Tamil. First, we administered the version of the Mini-Mental State Examination (MMSE) adapted to Sri Lanka [15,16]. This was followed by verbal Digit Span and a selection of tests from the Cambridge Neuropsychological Test Automated Battery (CANTAB) – a language-independent computerized test battery [17] which has also been adapted for use in Sri Lanka [18]. Overall, three cognitive domains were assessed:

a. visual attention and visuomotor skills – using CANTAB *Reaction Time* (RTI) and CANTAB *Rapid Visual Information Processing* (RVP) tasks;
b. visual episodic memory and learning – using CANTAB *Paired Associates Learning* (PAL) test; and
c. working memory and spatial planning – using Digit Span (forwards and backwards) and CANTAB *Stockings of Cambridge* (SOC) tasks.

In each session the order of testing was MMSE, Digit Span, PAL, RTI, SOC and RVP.

We administered the CANTAB using a Motion® JB3500 tablet PC, with a 12.1-inch touch screen and an attached response pad with reaction time buttons. The participants first completed a motor screening task to familiarize with the computer and its touch screen. The detailed rationale, implementation and interpretation of the individual CANTAB tests are provided in the CANTAB Test Administration Guide [17] and previous publications [19–23]. The individual CANTAB tests that we used, and the definitions of their outcome measures reported in this paper are briefly described below:

Paired Associates Learning

PAL is a test of visual episodic memory and associative learning [19]. The participants had to remember an increasing number of patterns (2, 3, 4, 5, 6 or 8 depending on the *stage*) housed in several boxes (6 or 8) which opened and closed randomly. Successfully completing stages with more patterns in a minimum number of trials (thus minimum errors) in each stage signifies better performance. We measured the PAL test performance using five indices: 1) *number of patterns succeeded on* within the maximum number of 6 attempts at each stage, 2) number of *stages completed on first trial* (out of 6), 3) *first trial memory score* (i.e., total number of patterns correctly located in first trial), 4) *mean trials to success* per stage, 5) *total errors (adjusted)* and 6) *total trials (adjusted),* the adjustments being made for the latter stages not performed due to failing the task at earlier stages.

Reaction Time

RTI is a test of visual reaction time and motor speed. The test comprises a *simple reaction time* task and a visuospatial *five-choice reaction time* task.

Stockings of Cambridge

SOC is test of spatial planning and spatial working memory [20]; a modified version of the Tower of London task [24], with identical test problems. In each test problem, participants had to move and rearrange a set of colored balls between three pockets to copy a target pattern, in minimum number of moves. The task started with 2-move problems and became increasingly difficult up to 5-move problems. We measured test performance by the *number of problems solved in minimum moves* (out of 12 problems) and *mean moves to solve 4-move problems* and *5-move problems*.

Rapid Visual Information Processing

This is a test of sustained visual attention. The participants observed a pseudo-random sequence of digits and responded to a predefined 3-digit target sequence (3–5–7) while ignoring the distracters. Better performance is indexed by a higher *probability of hit*, shorter *mean response latency* for correct responses; and higher *A′ (A prime)* – a measure of the subject's accuracy in discriminating the target from the distractors [25,26].

Statistical analyses

Intergroup comparisons

Intergroup comparisons of outcome measures were carried out in multiple linear regression models adjusting for age in years, sex, years of education, and diagnosis of depression. For "PAL number of patterns succeeded on" there was a highly skewed distribution with a ceiling effect, with the majority of the subjects succeeding in the most difficult stage (i.e., locating 8 patterns hidden in 8 boxes). Therefore, these data were dichotomized based on whether a participant completed or failed to complete all stages. Intergroup

comparison of the odds of failing to complete all stages of PAL was done in a multiple logistic regression model adjusting for the above confounding factors. We did not directly compare the OP group with a healthy community-living sample. However, the *clinical mode* of the CANTAB SOC task that we used in this study has age, sex and education adjusted Sri Lankan norms [18]. Using those norms, we determined whether each participant of the present study was significantly impaired (i.e., below predicted mean – 2SD) in the SOC task for his/her age, sex and years of education.

Analyses within the OP group

RBC-AChE has the same enzyme structure as AChE in the cholinergic synapses; and RBC-AChE activity has been found to correlate with acute cholinergic toxicity [27] and neuromuscular transmission in OP pesticide poisoning [28]. On this basis, we conducted a series of secondary analyses within the OP group to investigate whether there was a dose–response relationship between RBC-AChE inhibition and subacute/chronic cognitive impairment. RBC-AChE levels were significantly skewed with a floor effect; the majority having complete inhibition. Therefore, we compared the outcome measures between the OP subgroup who had complete RBC-AChE inhibition and the subgroup who had partial RBC-AChE inhibition in regression models, adjusting for age, sex, education and diagnosis of depression.

The logistic regressions and the risk estimate calculations were done using STATA/IC 11.1 (StataCorp LP, College Station, TX). The linear regressions and all other data analyses were conducted using IBM® SPSS® version 20.0.0. All statistical comparisons were two sided and statistical significance was defined as a p value less than 0.05.

Results

Sample characteristics

The participant recruitment and follow-up counts are summarized in Figure 1. There was a significant attrition from the point of post-discharge neuropsychological assessment through the successive stages of follow-up. Two-hundred and twenty-one eligible patients with OP pesticide poisoning (*OP group*), and 52 patients with paracetamol overdose (*control group*) underwent at least one neuropsychological assessment. Their sample characteristics are summarized in Table 1. The OP group was significantly older and had less formal education than the control group. Seventy-one percent of the OP group and 46% of the control group were males, the proportion in the OP group being significantly higher. Similar proportions (around 14%) of the participants in each group had preexisting depression or were diagnosed having depression in post-poisoning admission: all of them were on antidepressants during the 6-month follow up period. Of the participants who were not diagnosed with a psychiatric illness during the in-ward psychiatric assessments, none were subsequently diagnosed during the 6-month follow-up period.

Of the 221 participants in the OP group, 80 (36.2%) ingested profenofos, 24 (10.9%) phenthoate, 21 (9.5%) chlorpyrifos, 11 (5.0%) fenthion, 11 (5.0%) diazinon, 9 (4.1%) quinalphos, and 1 (0.5%) ingested azinophos methyl. In 64 (29.0%) cases the specific OP formulation could not be confirmed. As per the acute cholinergic features, of the OP group 130 (58.8%) had miosis, 93 (42.1%) respiratory changes/lung signs, 71 (32.1%) impaired consciousness, 50 (22.6%) excessive sweating/secretions, 50 (22.6%) fasciculations and 36 (16.3%) had bradycardia. RBC-AChE levels were missing in 2 OP group patients. Of the remaining 219, 141 (64.4%) had complete RBC-AChE inhibition (i.e., 0 U/gHb). While being hospitalized, 15 (6.8%) in the OP group had developed hypotension and 5 (2.3%) had seizures and 72 (32.6%) were intubated due to respiratory failure or impending respiratory failure. It is noteworthy that although 141 OP-group patients had complete RBC-AChE inhibition, only 54 of them required ventilatory support. Two-hundred and seven (93.7%) received atropine, and 29 (13.1%) received pralidoxime during their hospital stay.

Comparison between the OP group and the controls

The OP group underwent the first neuropsychological assessment after a median duration of 12 days (range 3–38), and the control group after a median duration of 10.5 days (range 2 – 22) following exposure. Group differences (linear regression unstandardized B coefficients for group factor with 95% confidence intervals and p values) adjusted for confounding factors are summarized in Figures 2–4. Figure 2 presents general test performance scores; Figure 3 shows data on the trials/moves spent to succeed on PAL and SOC problems solving tasks; and Figure 4 shows the response speeds in attention tasks. In the PAL task, the majority of each group completed all stages (i.e., up to 8-pattern stage) within the maximum of 6 attempts allowed at each stage, in all three testing sessions, except the OP group in the post-discharge assessment (Figure 5). Group comparisons of PAL error and successful completion are summarized in Table 2.

Post-discharge assessment

Of the comparisons of 17 outcome measures of the 6 neuropsychological tests, the OP group performed significantly poorer than the control group in 8 measures, whereas no significant intergroup differences were observed in 9 measures, in the post-discharge assessment. Compared to the control group, the OP group 1) had shorter forward and backward Digit Spans; 2) took more trials to succeed, and more total trials (adjusted) in the PAL memory task; 3) solved less problems with minimum moves, took more trials to solve 4-move and 5-move problems in the SOC planning task; and 4) was slower to respond to the target sequences in the RVP sustained attention task. Although other outcome measures did not show statistically significant intergroup differences, the direction of the adjusted mean differences favored poorer performance in the OP group compared to the control group in 15 out of 17 outcome measures – i.e., all except RTI mean simple and 5-choice reaction times

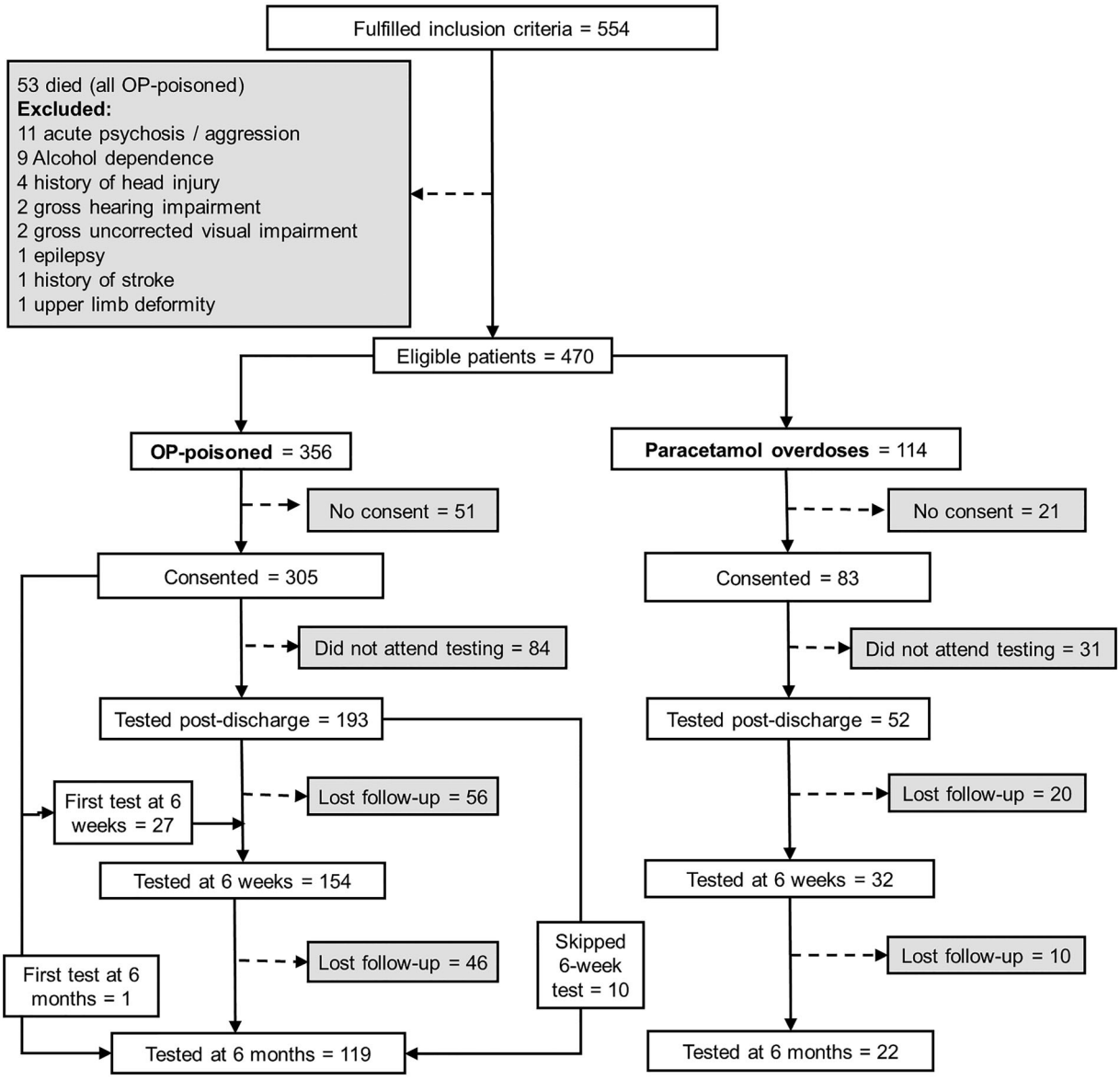

Figure 1. Participant recruitment and follow-up.

Table 1. Sample characteristics.

	OP group ($n = 221$)	Control group ($n = 52$)	p Value
Number (%) of males	158 (71.5%)	24 (46.2%)	0.0004
Mean age (SD) in years	40.0 (13.8)	26.3 (7.7)	<0.0001
Mean years of education (SD)	8.44 (3.58)	12.17 (1.54)	<0.0001
Number (%) diagnosed with depression	33 (14.9%)	7 (13.5%)	0.787

(Figures 2–4). The odds of the OP group failing to complete all patterns of the PAL task was 2.2 times that of a control group, and this was marginally significant (Table 2). When compared with the age-, sex- and education adjusted Sri Lankan norms, SOC performance was significantly impaired in 22.6% ($n = 43$) of the OP group and 7.7% ($n = 4$) of the control group.

Six-week assessment

At 6 weeks, only 3 of the 17 outcome measures, viz. the forward and backward Digit Span and PAL stages completed in first trial, were significantly impaired in the OP group compared to the control group. No significant intergroup differences were observed in the other 14 outcome measures, however – like we observed in the post-discharge comparison – the direction of the adjusted mean differences favored poorer performance in the OP group compared to the control group in 15 out of the total of 17 outcome measures (Figures 2–4). Compared to Sri Lankan norms, SOC number of problems solved in minimum moves was significantly impaired in 14.3% ($n = 22$) of the OP group and 3.1% ($n = 1$) of the control group. MMSE score, RTI mean simple reaction time and SOC mean moves taken to solve 5-move problems

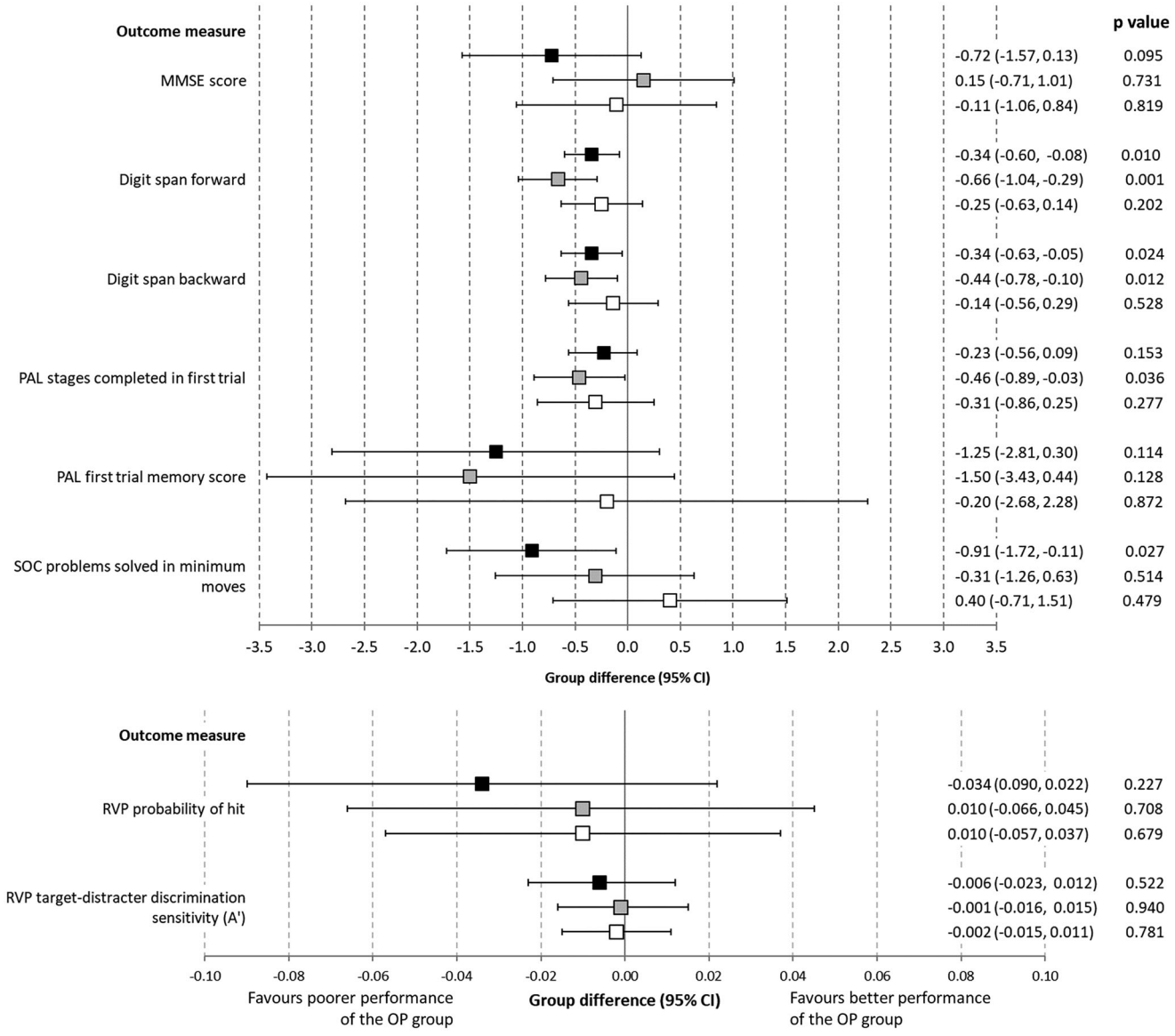

Figure 2. Group differences (unstandardized B coefficients and 95% confidence intervals) of neuropsychological test scores, at post-discharge (■), at 6-weeks (▒) and at 6 months (□) post-exposure. Note: Regression coefficients are adjusted for sex, age in years, years of education and presence/absence of depression. MMSE: Mini Mental State Examination; PAL: Paired Associates Learning; SOC: Stockings of Cambridge; RVP: Rapid Visual Information Processing.

favored better performance in the OP group but the differences were not significant.

Six-month assessment

At 6 months, no significant intergroup differences were observed in any of the outcome measures. Although not statistically significant, the direction of the adjusted mean difference favored poorer performance in the OP group compared to the control group in 11 out of the total of 17 outcome measures and the rest favored poorer performance in the control group (Figures 2–4). Compared to Sri Lankan norms, SOC performance was significantly impaired in 9.3% ($n = 11$) of the OP group and 9.1% ($n = 2$) of the control group.

Presence of depression was not significantly associated with outcome measures either at the post-discharge assessment or at 6 months. In the 6-week assessment, presence of depression (once adjusted for other factors in the regression models) was associated with 1) shorter Digit Span backward (B = −0.37, 95% CI: −0.70, −0.05, $p = 0.024$); 2) more mean trials to success ($B = 0.34$, 95% CI: 0.01, 0.67, $p = 0.045$), total errors (adjusted) ($B = 14.39$, 95%CI: 4.75, 24.03, $p = 0.004$) and total trials (adjusted) ($B = 2.22$, 95% CI: 0.54, 3.91, $p = 0.010$) in the PAL task; and 3) lower probability of hit (B = −0.062, 95% CI: 0.0114, 0.019, $p = 0.019$) and target-distracter discrimination sensitivity (B = −0.016, 95% CI −0.031, −0.001, $p = 0.032$) in the RVP task.

RBC-AChE and cognitive functions in the OP group

Within-group analysis was based on 219 subjects who had on-admission RBC-AChE measured. Of these, 192 were assessed post-discharge, 153 at 6 weeks and 118 at 6 months. The numbers who had complete RBC-AChE inhibition at each of these time periods were 123 (64.1%), 96 (62.7%), and 72 (61.0%), respectively. Differences between the subgroup

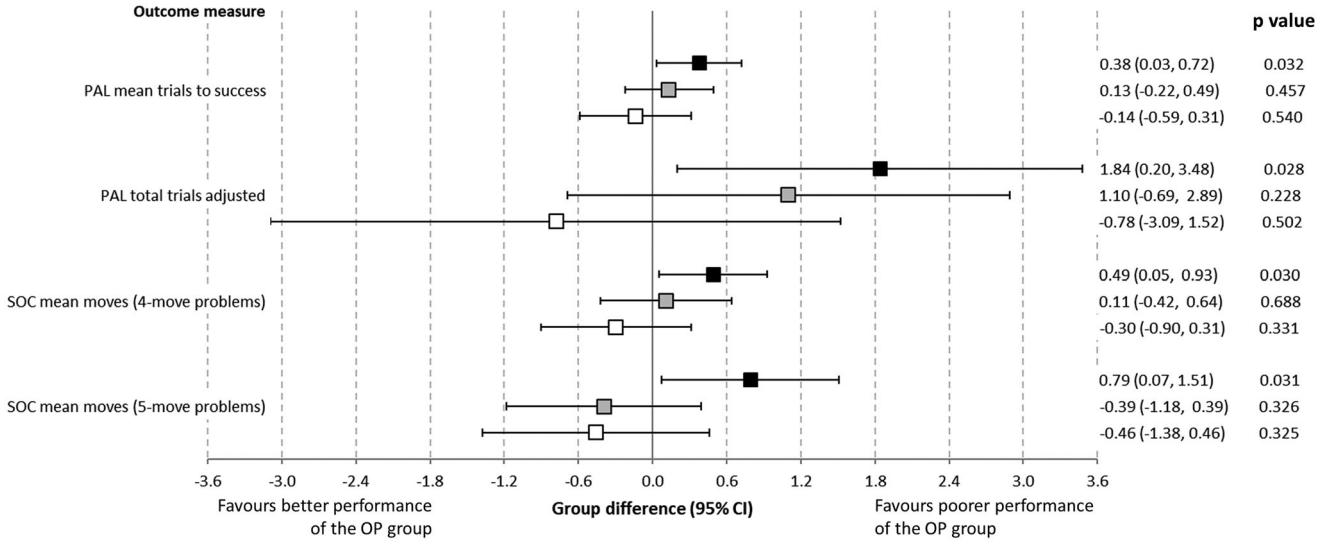

Figure 3. Group differences (unstandardized B coefficients and 95% confidence intervals) of Paired Associates Learning (PAL) trials and Stockings of Cambridge (SOC) mean moves taken to solve 4- and 5-move problems, at post-discharge (■), at 6-weeks (■) and at 6 months (□) post-exposure. Note: Regression coefficients are adjusted for sex, age in years, years of education and presence/absence of depression.

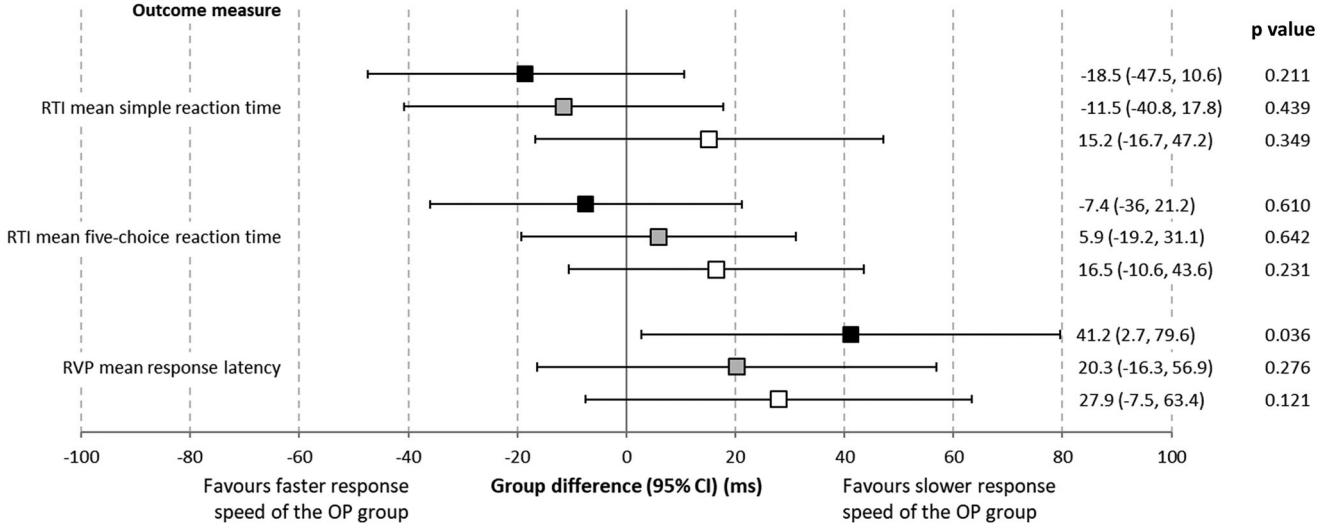

Figure 4. Group differences (unstandardized B coefficients and 95% confidence intervals) of the response speed measures in reaction time and attention tasks, at post-discharge (■), at 6-weeks (■) and at 6 months (□) post-exposure. Note: RTI, Reaction Time; RVP, Rapid Visual Information Processing. Note: Regression coefficients are adjusted for sex, age in years, years of education and presence/absence of depression.

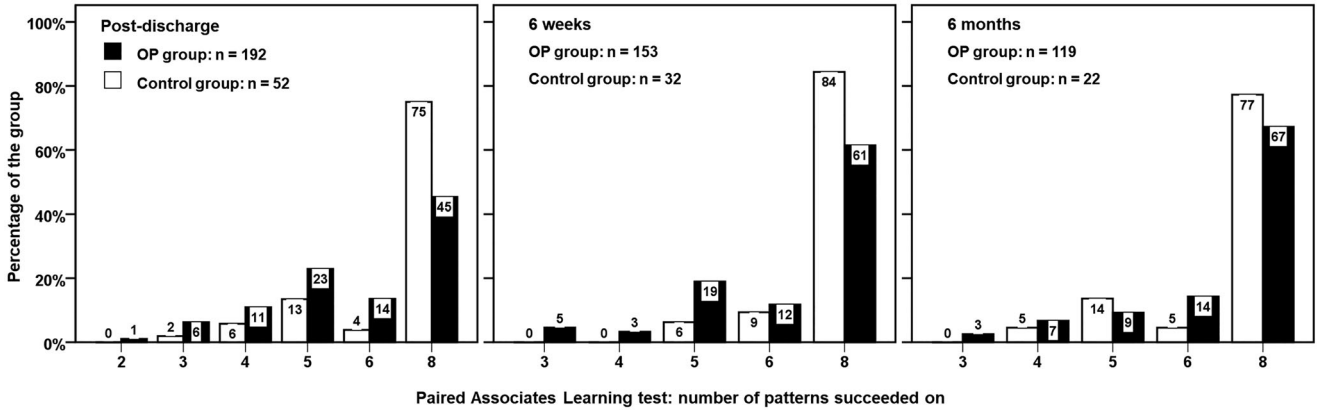

Figure 5. Percentages of subjects who succeeded in completing different numbers of patterns in the Paired Associated Learning task.

Table 2. Group comparisons of Paired Associates Learning (PAL) failure to succeed on all patterns and errors.

	Failure to succeed on all patterns		PAL total errors (adjusted)	
Assessment time-point	Odds ratio[a] (95% CI)	*p* Value	Group difference[b] (95% CI)	*p* Value
Post-discharge	2.20 (0.99, 4.87)	0.051	8.95 (−1.13, 19.03)	0.082
6 weeks	1.25 (0.40, 3.95)	0.700	4.04 (−6.18, 14.25)	0.437
6 months	0.88 (0.25, 3.03)	0.836	−4.94 (−18.27, 8.40)	0.465

[a]Odds of the OP group/odds of the control group.
[b]OP group – control group.
Note: Regression coefficients are adjusted for sex, age in years, years of education and presence/absence of depression.

Table 3. Comparisons of Paired Associates Learning (PAL) failure to succeed on all patterns and errors between OP subgroup with Complete RBC-AChE inhibition and partial inhibition.

	Failure to succeed on all patterns		PAL total errors (adjusted)	
Assessment time	Odds ratio[a] (95% CI)	*p* Value	Group difference[b] (95% CI)	*p* Value
Post-discharge	1.63 (0.84, 3.18)	0.149	9.94 (1.21, 18.66)	0.026
6 weeks	0.98 (0.44, 2.16)	0.959	−0.78 (−9.22, 7.67)	0.856
6 months	0.76 (0.31, 1.88)	0.552	−3.18 (−13.05, 6.69)	0.525

[a]Odds of the complete inhibition group/odds of the partial inhibition group.
[b]Complete inhibition group – partial inhibition group.
Note: Regression coefficients are adjusted for sex, age in years, years of education and presence/absence of depression.

that had complete RBC-AChE inhibition and partial inhibition (linear regression unstandardized B coefficients for group factor with 95% confidence intervals and p values) adjusted for potentially confounding factors are summarized in Supplemental Figures 1–3 and Table 3.

In the post-discharge assessment, the subgroup with complete RBC-AChE inhibition performed significantly poorer than the partial-inhibition subgroup in 3 out of 17 outcome measures – all in the PAL task: first trial memory score (B = −1.56, 95% CI: −2.85, −0.27, $p = 0.018$), total trials (adjusted) ($B = 1.51$, 95% CI: 0.10, 2.91, $p = 0.036$), and total errors (adjusted) ($B = 9.93$, 95% CI: 1.21, 18.66, $p = 0.026$). Even though all other comparisons were not statistically significant, it is noteworthy that the direction of the adjusted mean difference favored poorer performance in the subgroup that had complete RBC-AChE inhibition in 16 out of the total of 17 outcome measures (Supplemental Figures 1–3). No significant subgroup differences were observed in any outcome measure at 6 weeks or at 6 months. The general direction toward poorer performance in the complete RBC-AChE) also disappeared with time, with that group tending to show lower (although not necessarily significant) performance in 12 out 17 measures at 6-weeks, and only 7 out of 17 at 6 months.

The heterogeneity of the OP compounds precluded us analyzing each formulation in isolation.

Discussion

We conducted a large prospective cohort study, to determine the extent of subacute and chronic cognitive sequelae of acute OP pesticide poisoning. After adjusting for confounding factors, there were significant neurocognitive deficits in the OP group when compared with the controls (8 of the 17 outcome measures), in the post-discharge assessment conducted on average 12 days post-exposure. Those impairments were in episodic memory (PAL), working memory (Digit Span), spatial planning (SOC) and sustained attention (RVP) domains. These deficits were short-lived, with only 3 of the 17 outcome measures (in episodic memory and working memory) being impaired in the OP group at 6 weeks, and none at 6 months. Broadly, the results indicated the OP group had poorer neuropsychological test performances in the immediate post-discharge period; but the differences disappeared over the next 6 months. There was some evidence of a dose–response relationship on subacute neurocognitive deficits: the subgroup who had complete RBC-AChE inhibition on admission (compared to those who had only partial AchE-inhibition) performed poorly in 3 out of 17 outcome measures – all in the episodic memory domain. However, the extent of acute RBC-AChE inhibition did not significantly predict any of the test performances 6 weeks or 6 months after poisoning.

Four previous epidemiological studies reported chronic cognitive sequelae of acute OP pesticide poisoning [5–8] and two studies report neuropsychological sequelae of victims of the 1995 Tokyo subway sarin attack [29,30]. Their setting, design, and the neuropsychological test results of specific cognitive domains are summarized in Table 4. Chronic deficits reported in some of those on Digit Span [5,6,30] and sustained attention [6,7] are consistent with the subacute deficits that we observed, but considerable differences in the post-poisoning time (i.e., months to years versus few days to few weeks in our study) preclude further interpretation of those comparisons. The chronic deficits, however, can be more closely examined among the present and the published data. The two earliest studies compared the OP poisoned and the matched control groups in univariate analyses, and showed significantly poorer performance in the OP poisoned group in many outcome measures [5,6]. However, all subsequent studies that adjusted for potential confounders–like we did in the present study–showed no significant impairment in the OP poisoned group in measures of attention, memory or visuoperceptual skills: the only exception was impaired Digit-Symbol test (visuomotor speed) performance reported by Wesseling et al. [8] and Yokoyama et al. [29]. This shortage of objective evidence of chronic cognitive

Table 4. Summary of the findings of the previous studies on acute OP poisoning.

Study	Savage et al. 1988 [5]	Rosenstock et al. 1991 [6]	Steenland et al. 1994 [7] (all poisoned cases)	Steenland et al. 1994 [7] (hospitalized poisoned cases)	Wesseling et al. 2002 [8]	Yokoyama et al. 1998 [29]	Nishiwaki et al. 2001 [30] (high exposure group)	Nishiwaki et al. 2001 [30] (low exposure group)
Country	USA	Nicaragua	USA	USA	Costa Rica	Japan	Japan	Japan
Study population	Pesticide poisonings in Colorado and Texas rosters	Men working with pesticides	Men from California pesticide illness records	Men from California pesticide illness records	Banana plantation workers	Victims of 1995 Tokyo subway sarin attack	Rescue workers and police at the Tokyo sarin attack	Rescue workers and police at the Tokyo sarin attack
Mode of poisoning	Unintentional (mostly occupational)	Occupational exposure	Occupational exposure	Occupational exposure	Occupational exposure	Accidental exposure	Accidental exposure	Accidental exposure
Control Group	Matched for age, sex, education, social class, socioeconomic status, race.	Age- and sex-matched friends or siblings	Friends not working with pesticides	Friends not working with pesticides	Fellow workers not treated for any pesticide poisoning	Unexposed healthy individuals	Age- and occupation-matched controls	Age- and occupation-matched controls
Exposure and / or severity ascertainment[a]	Symptoms documented by physician.	Hospital records.	Sought medical attention. Symptoms and RBC-AChE inhibition.	Hospital admission. Symptoms and RBC-AChE inhibition.	Received medical attention, but no hospitalization.	Cholinergic signs, plasma ChE inhibition.	Immediate hospitalization.	Attending hospital as outpatients.
Time between exposure and testing	>3 months	>9 months	Not specified (years)	Not specified (years)	27 months (average)	6–8 months	3 years (2 years 10 months to 3 years 9 months)	3 years (2 years 10 months to 3 years 9 months)
Statistical Analysis	Comparison of means	Paired *t* test	Regression, adjusted for confounders	Regression, adjusted for confounders	Regression, adjusted for confounders	Analysis of covariance adjusted for age, sex.	Univariate comparison and multiple regression	Univariate comparison and multiple regression
Cognitive domain (test):								
Attention, sustained (digit vigilance)		–†			+			
Attention, sustained (Continuous performance)			–	– *		+		
Attention (trails A)		–†			–			
Executive functions, abstract thinking (Similarities)	– †							
Executive functions, cognitive flexibility (Trails B)	–							
Executive functions, task switching Wisconsin Card Sorting)	– †							
Executive functions, verbal fluency (Thurston Word Fluency)	– †							
Language (vocabulary)	– ‡	–			–			
Language (WAIS verbal IQ)	– ‡							
Language (Comprehension)	– *							
	– †							

(*continued*)

Table 4. Continued.

Study	Savage et al. 1988 [5]	Rosenstock et al. 1991 [6]	Steenland et al. 1994 [7] (all poisoned cases)	Steenland et al. 1994 [7] (hospitalized poisoned cases)	Wesseling et al. 2002 [8]	Yokoyama et al. 1998 [29]	Nishiwaki et al. 2001 [30] (high exposure group)	Nishiwaki et al. 2001 [30] (low exposure group)
Language (Peabody Reading Comprehension)								
Language (Peabody Reading Recognition)	– †							
Memory (Story Memory Test – Learning)	–							
Memory (Story Memory Test – Memory)	–							
Memory, verbal (Rey Auditory Verbal Learning)		–			–			
Memory, visual (Benton Visual Retention)		– †			–		+	–
Memory, visual (Pattern Memory)			+	+				
Memory, verbal (Paired Associates Learning)						+		
Memory, working (Digit Span)	– † (forward)	– † (average)			–	+ (sum)	–	+ (forward), – (backward)
Memory, working (Serial Digit Learning)			+	+				
Visuomotor speed (Digit-Symbol)	– ‡	– †			– *	– *		
Visuomotor speed (Symbol Digit)			–	– *				
Visuomotor speed (Simple reaction time)		–	–	–	–	+	–	+
Visuomotor speed (Choice reaction time)							–	+
Visuoperceptual skills (Block Design)	–	– †			–			
Visuoperceptual skills (Picture Completion)	+					+		
Visuoperceptual skills (WAIS picture arrangement)	+							
Visuoperceptual skills (WAIS Object Assembly)	–							

[a]All studies ascertained exposure and/or severity of acute intoxication retrospectively.

Note: The cells are left blank if a particular test is not applied in a study. WAIS, Wechsler Adult Intelligence Scale; RBC-AChE, red blood cell acetylcholinesterase.

–, favors poorer performance in the OP poisoned group; +, favors better performance in the OP poisoned group.

*$p < 0.05$, †$p < 0.01$, ‡$p < 0.001$.

impairment is also in contrast with the OP-exposed individuals having more self-reported complaints [29,31,32].

A dose–response relationship helps elucidate whether acute OP pesticide poisoning causes any cognitive impairment, and some evidence from the present and previous studies support this assertion. We found complete RBC-AChE inhibition on-admission (compared to partial inhibition) associated with deficits in episodic memory in a subacute, but not a chronic time-course. Steenland et al. [7] and Nishiwaki et al. [30] –in their subgroup analyses–have used hospital admission (against outpatient treatment) as a criterion of the severity of poisoning. Only Steenland et al. [7] found that the hospitalized subgroup (but not the full sample who had medical attention) was significantly impaired than the healthy controls in measures of sustained attention (Continuous Performance test) and visuomotor speed (Symbol-Digit test). Scarcity of clinical data in the acute stage of poisoning seems to have limited those previous investigators from utilizing any clinical measures or biomarkers to examine a dose–response relationship.

Muscarinic cholinergic neurons project to a wide area of the cerebral cortex, thus cholinergic overactivation and synaptic dysfunction could occur in the prefrontal and parietal cortical areas and temporal lobe including hippocampus [33,34]. The subacute deficits that we observed in episodic memory, spatial planning and working memory, and attention are consistent with dysfunction of those cholinergic circuits. The dose–response relationship that we observed in episodic memory encoding (as indexed by PAL performance) warrants further discussion. Neuroimaging studies indicate that performance of PAL and other episodic memory tasks correlates with the degree of activation of the hippocampus [35,36]. Hippocampus has been found to be damaged by OP nerve agents in experimental animals [37–40] and hippocampal gray matter volume has been found to be decreased in humans exposed to acute single dose of OP nerve agent sarin [41]. This damage is purported to be caused by cholinergic overactivation, which in turn overactivate the excitatory glutamatergic circuits of the hippocampus, resulting in excitotoxic neuronal inflammation, ionic imbalances, edema and cell death [42]. However, our neuropsychological tests did not find evidence of permanent damage following acute OP pesticide poisoning. Unlike experimental animals exposed to large doses of extremely toxic OP nerve agents without receiving antidotes, our sample was exposed to less toxic OP pesticides, and all patients who were significantly intoxicated were managed according to the standard protocols [14]. Atropine, by occupying muscarinic receptors of the central nervous system not only antagonize the cholinergic stimulation but also could be protecting the post-synaptic cells of the cortical regions from permanent excitotoxic damage. Interpreting our longitudinal data in the light of previous clinical and animal studies, we believe a transient derangement of synaptic connectivity (that lasts up to few weeks), rather than a permanent neuronal damage, underlies the course of neuropsychological deficits of acute OP pesticide poisoning. In this regard, it should be noted that the OP formulations ingested by the present sample are highly lipophilic [43]. The main effect of higher lipid solubility would be a higher volume of distribution and a longer half-life. Therefore, it is possible for the compounds to remain in the brain tissue beyond the recovery of peripheral signs, thus potentially contributing to the neurocognitive impairment that outlast the cholinergic phase of poisoning. Neither did we find previous research on acute OP poisoning that reported neurocognitive functions to correlate with the lipid solubility of the exposed OP compounds.

Significant attrition in the follow-up is a limitation in the present study (Figure 1). This was further complicated by some subjects attending 6-week and/or 6-month assessments but not the post-discharge assessment. Nevertheless, the response rate is better than those of the previous studies, including that of the series of victims of the Tokyo subway sarin attack followed up at various intervals after the incident [29,30,44]. Many patients may not have revisited for the follow-up owing to practical inconveniences, losing interest in the study, or their (and their families') need to forget the incident of self-harm. None of these would have introduced a systematic bias since such reasons similarly apply to both OP group and the control group. However, it is still possible that those who felt worse either attended (to get a "check-up") or withdrew (losing motivation) introducing a systematic bias, but we do not have information to favor either motive. Given the effect sizes we observed at 6-months in many outcome measures (Figures 2–4) it is unlikely that higher follow up rates would have made significant differences in the comparisons and the overall interpretation of the results. It is noteworthy that the majority of the OP-group patients who had complete RBC-AChE inhibition did not require ventilatory support. Although this phenomenon has also been noted and in patients poisoned with S-alkyl OP pesticides including profenofos, and has been discussed in detail previously [45], we do not have a clear explanation for those observations. Animal studies show that RBC-AChE is more sensitive than brain-AChE to profenofos [46], chlorpyrifos [47], diazinon [48] – the formulations ingested by the majority of the OP-group patients.

Conclusions

In conclusion, our longitudinal data indicate acute OP pesticide poisoning is associated with impaired memory, attention and executive functions that last beyond clinical recovery, with some memory deficits persisting at 6 weeks post-exposure. These deficits might possibly impact the everyday functioning of those patients during the post-discharge period. Slowed responses in sustained attention tasks, but not in simple psychomotor tasks, implicate that increasing task demands could be associated with poorer task-adjustment; potentially increasing the risk in daily activities such as driving and operating machinery. That said, acute OP pesticide poisoning did not seem to cause chronic central nervous system effects detectable at a neurobehavioral level. Given these null results, it is unlikely that an acute less severe exposure (such as might occur in accidental poisoning in pesticide workers) would cause long-term

neuropsychological/neurobehavioural deficits. An avenue for the future research is to examine whether OP pesticides cause functional and structural changes at the brain-level using neurophysiological and neuroimaging techniques. Apart from the choice of assessment techniques and outcome measures, careful measurement of the severity and biomarkers of acute intoxication, and exploring a dose–response relationship is important in establishing or refuting a causative association.

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