Effects of acute organophosphorus or paraquat exposure on neuromuscular function and efficacy of antioxidant therapy in acute paraquat poisoning

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ABSTRACT

Organophosphates (OP) are the most frequently involved pesticides in acute poisoning. Paraquat (PQ) poisoning has the highest case fatality. As the mechanism of PQ toxicity includes free radical generation, antioxidants have been tried as a treatment. Neurotoxic effects of acute OP/PQ poisoning (OP/PQ-P) have been hitherto under-explored.

The aims of the study were to assess the effects of acute OP/PQ poisoning on somatic, autonomic nerves, neuromuscular junction (NMJ), brain stem, cognitive function and psychological status. Further, aims were to evaluate adherence to existing guidelines on the management of OP/PQ poisoning and to find out the efficacy of antioxidant therapy in acute PQ poisoning.

In a cohort study we evaluated the function of peripheral nerves (somatic and autonomic nerves) with the Neuropack MEB-9400A/K EMG/EP (Nihon Koden). Motor and sensory nerve function was tested with nerve conduction studies. Electromyography (EMG) studies were performed on the deltoid and the first dorsal interosseous muscle on the dominant side. Cardiovascular reflexes based autonomic function tests and sympathetic skin response (SSR) was used to evaluate autonomic function. NMJ function was assessed with slow repetitive supramaximal stimulation of the median nerve of the dominant upper limb. Brain stem function, cognitive function and psychological status were assessed with Brain Stem Evoked Response Audiometry (BERA), Mini Mental State Examination (MMSE) and General Health Questionnaire (GHQ) respectively. The data of the patients were compared with age, gender and occupation matched controls. A cross sectional survey was conducted to evaluate adherence to existing guidelines on the management of OP/PQ poisoning. The details of administration of atropine, pralidoxime and Fuller's earth were collected. A randomized double blind placebo controlled clinical trial was conducted to determine the efficacy of antioxidant therapy in acute PQ poisoning. Both arms received intravenous vitamin C (IV vit C) 100 mg, 500 mg, 1000 mg, 3000 mg/day and 3000 mg/8 h for 5 consecutive days. One arm received N-acetylcysteine (NAC) 20 mg/kg in 200 mL of 5% dextrose over 15 minutes followed by 50 mg/kg in 500 mL over 12 hours ($\approx$ 4 mg/h/kg) twice per day for 3 days whereas, the other arm received 200 mL of 5% dextrose over 15 minutes followed by 500 mL over 12 hours twice per day for 3 days as placebo. These were compared to 24 historical and 80 parallel controls who received standard supportive treatment. The survival of the test individuals was compared to the historical and parallel controls by Log Rank (Mantel-Cox) and Tarone-Ware test.

There were 70 OP patients (70 controls) and 28 PQ patients (56 controls). In OP patients, motor nerve conduction velocity (MCV), amplitude and area of compound muscle action potential on distal stimulation (CMAP-D), sensory nerve conduction velocity (SCV) and F-wave occurrence were significantly reduced. At one week the significant impairment in autonomic function were change of diastolic blood pressure 3 min after standing, heart rate variation during deep breathing (HR-DB), SSR-amplitude, post-void urine volume and size of pupil. All except HR-DB were reversed at six weeks. No significant impairment of NMJ function, BERA, MMSE were noted. The prevalence of psychological

distress was significantly higher among the patients. In PQ patients, amplitude of ulnar nerve CMAP-D, median nerve area of CMAP-D and F-wave occurrence of median, ulnar and tibial nerves, blood pressure variation after standing and SSR-amplitude were significantly reduced at the first assessment. All but F-wave occurrence remained impaired at six weeks. A significant decrement response in RNS was observed following exercise in both assessments. No significant impairment of BERA, MMSE were noted. GHQ showed high prevalence of psychological distress among the patients. None of OP/PQ patients showed spontaneous activity, fibrillation potentials, high amplitude of the motor units, polyphasia or reduced interference pattern in EMG. Atropine was commenced in 44% of patients without cholinergic features. 73% of patients developed atropine toxicity. None of the patients received the maintenance therapy of pralidoxime for the recommended duration. Ninety percent of PQ poisoned patients received Fuller's earth, but 15% of them did not receive it in adequate amounts. There were 40 test, 24 historical-controls and 80 parallel-controls in the clinical trial. The median survival time was longer in the patients given antioxidants than in the historical controls [8 days (95% CI 1.8-14.2) vs 1 day (95% CI 0.53- 1.47)]. This difference was significant by both the Log Rank (Mantel-Cox) ($p=0.034$) and Tarone-Ware tests ($p=0.012$).

The absolute risk reduction is 18.33% (95% CI: -2.66% to 39.33%, Number Needed to Treat was 6). The median survival time of parallel controls were 7 days. There was no statistically significant survival difference between the tests vs parallel controls and the individuals who received IV vit C + placebo vs IV vit C+NAC. The Proportional Hazard assumption appeared to hold. Stratified Cox Proportional Hazard model was done as variable sex violated the Proportional Hazard assumption (Chi-square value = 66.6, $p<0.01$). There were no statistically significant changes of antioxidant levels within the groups over five consecutive days.

Some of the effects of OP/PQ on peripheral nervous system persist at least for six weeks. NMJ function is not affected in OP patients. However impairment of NMJ function was observed following exercise in patients with PQ poisoning even at 6 weeks of the exposure. Brain stem and cognitive function were not affected in both groups of patients. High prevalence of psychological distress may indicate need for psychological support, diagnosis of depression and treatment. Adherence to guidelines was not optimal regarding the administration of antidotes in OP/PQ poisoning. Antioxidant therapy did not show promising effects on acute PQ poisoning.

This study was performed in University of Ruhuna, Sri Lanka and the results were included in a thesis with ten research papers in peer reviewed index journals. Further, 21 abstracts were presented in national and international forums including 10 platform presentations. An oration was done at inauguration of Annual Academic Sessions of the Galle Medical association in 2015, based on the findings of organophosphate neurotoxic study. The study received an Academic award by Asian and Oceaninan congress of clinical neurophysiology, an International Travel award by the American academy of clinical toxicology, the best poster presentation at the annual academic sessions of the Galle Medical association and President's award for highly rated scientific publications. The thesis was defended on 10th of May 2012.

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Introduction

Acute pesticide poisoning is a major health problem especially in developing countries. It was estimated that one million serious, unintentional poisonings occurred and an additional two million people were hospitalized for attempted suicide with pesticides annually (1). Organophosphate (OP) and paraquat (PQ) pesticide ingestion is a serious health problem especially in developing countries, since OP compounds were involved in 76% of pesticide poisoning (1,2) and PQ self-poisoning has the highest case fatality (65%) for any poison in Sri Lanka (3).

OP poisoning leads to four well defined neurological syndromes, namely acute cholinergic crisis, intermediate syndrome, organophosphate induced delayed polyneuropathy (OPIDN) and chronic organophosphate induced neuropsychiatric disorders (COPIND) (4). Studies have shown abnormalities in motor and sensory nerve conduction studies in farm workers exposed to OP (5,6). Effects of chronic mixed pesticide exposure on

autonomic nerve function were also demonstrated by Ruijten *et al.* in 1994 (7). There have been no studies which have looked into somatic and autonomic function following acute single exposure to OP.

OP bind to the esteratic site on the acetylcholine esterase (AChE) molecule, phosphorylates the enzyme, and lead to inhibition of its action (4). Accumulated acetylcholine at nerve endings initially stimulates and eventually leads to exhaustion of cholinergic synapses, resulting in neuromuscular junction (NMJ) dysfunction (4).

Oxidative stress is a well recognized mechanism for many neurotoxic agents (8). Paraquat is often used as a neurotoxin in in-vitro experimental models. However, surprisingly, there are no published studies exploring neurotoxic effects of paraquat in humans.

Chronic damage to the central nervous system resulting in cognitive impairment has been shown with repeated low doses of OP exposure over months or years (6,9,10). Delay in cognitive processing with acute OP ingestion has been reported even after recovery from the acute cholinergic phase of intoxication, and this did not improve even six months after the exposure (11).

The two important pathologies behind PQ toxicity are generation of reactive oxygen species (ROS; superoxide anion, hydrogen peroxide, hydroxyl radical) and depletion of NADPH which is necessary for normal function (12). Dandapani M *et al.* (2003) reported oxidative damage following OP poisoning (8). Widespread and robust effects of chlorpyrifos on the genes involve in antioxidant activity has been shown by Theodore A *et al.* (13). ROS are associated with drugs (cisplatin, aminoglycosides) (14,15) and noise induced (16,17) cochlear pathology. Therefore it would be interesting to look in to brain stem auditory evoked potentials (BAEP) following ingestion of OP/PQ.

Psychiatric illness and suicidal attempts are correlated. However literature did not explore the correlation of self-poisoning and psychological distress. The psychological status even after attempting suicide is not always addressed (5) and some individuals have multiple attempted suicides. Some will continue until they succeed. Therefore identification and management of psychological distress following suicidal attempts are important to minimize future suicide risk.

The major mechanism of toxicity in PQ poisoning is production of free radicals. However there is no specific antidote for PQ poisoning. On the other hand it would be important to look into whether there is any space for optimization of management according to guidelines in order to improve the survival.

Therefore, I was interested in exploring the neurological outcome, adherence to the guidelines on the management of OP/PQ poisoning and talried some antioxidant strategies to combat oxidant-induced cellular damage in PQ poisoning.

Materials and methods

Ethics Statement

The study was approved by the Ethical Review Committee, Faculty of Medicine, University of Ruhuna, Sri Lanka. Informed written consent was obtained from the patients and the controls. All clinical investigations were conducted according to the principles expressed in the Declaration of Helsinki.

Neurotoxic study

A cohort studies with matched controls was conducted over two years in a secondary and a tertiary care hospital in the Southern Province of Sri Lanka with the approval of the Ethical Review Committee, Faculty of Medicine, University of Ruhuna. Consecutive patients admitted to the hospitals with OP/PQ poisoning were recruited to the study after obtaining informed written consent.

Somatic, autonomic nerve and NMJ functions, brain stem auditory evoked potentials (BAEP), cognitive function of the survivors were assessed at one and six weeks after the exposure.

Controls were recruited from persons accompanying patients to the hospitals. The controls were matched to ± 3 years of age, gender and occupation (according to International Standard Classification of Occupation; ISCO-88 by International Labor Organization) of the patients.

People who had diabetes mellitus, known neurological disease, history of head injury or who were on long term medication were excluded from the study. Further, controls did not have a history of

acute exposure to any poisons.

Sensory and motor nerve conduction studies, F-wave studies, and electromyography were performed to assess function of somatic nerves. Autonomic nerve function was assessed with cardiovascular reflex based autonomic nerve function tests (heart rate response to Valsalva manoeuvre, heart rate response to deep breathing, heart rate response to standing, and blood pressure response to sustained handgrip), sympathetic skin response (SSR), measurement of the size of the pupil, and residual urine volume. Exercise modified slow repetitive supra-maximal stimulation was used to assess the function of the neuromuscular junction. Cognitive function and psychological status assessed with Mini Mental State Examination (MMSE), digit span test, test of long term memory function and General Health Questionnaire (GHQ) respectively.

The Neuropack MEB-9400A/K EMG/EP (Nihon Koden) measuring system was used for electrophysiological assessment.

Double blind placebo controlled clinical trial (Antioxidant trial)

Patients who were admitted to the secondary care hospital following acute PQ ingestion were recruited for the study. Individuals were randomized into two groups. Both arms received intravenous vitamin C (IV vit C) 100 mg, 500 mg, 1000 mg, 3000 mg/day and 3000 mg/ 8 h for five consecutive days. One arm received N-acetylcysteine (NAC) 20 mg/kg in 200 mL of 5% dextrose over 15 minutes followed by 50 mg/kg in 500 mL over 12 hours ($\approx$ 4 mg/h/kg) twice per day for three days. Whereas the other arm received 200 mL of 5% dextrose over 15 minutes followed by 500 mL over 12 hours twice per day for three days as placebo. These were compared to 24 historical and 80 parallel controls who received standard supportive treatment.

Statistical analysis

Statistical analysis was done by using GraphPad Prism 4 and Statistical Package for the Social Sciences. Normal distribution of the data was tested with Kolmogorov-Smirnov test. If the data were not distributed normally non-parametric tests were used.

The paired T-test was used to compare the results of the first and the second assessment and the unpaired T-test was used to compare the results of the patients and the controls. Multiple linear regression models were used to adjust for potential confounders. ANOVA and Post Hoc comparison were used to analyse inter peak latencies in BAEP. Discrete variables were analysed with the Chi-square test.

The survival status (primary outcome measure) of the test individual vs historical controls/parallel controls and the two arms in the test group in the antioxidant trial were analysed by Kaplan Meier analysis with Log Rank (Mantel-Cox) and Tarone-Ware test. Antioxidant levels between groups for five consecutive days were analysed with Mann-Whitney U test and within groups were analysed with Linear Mixed Model procedure, which is the test for Repeated Measure ANOVA with missing values.

Results

OP neurotoxic study

There were 70 patients (50 males) and 70 controls. Fifty-three patients attended for the second assessment. Plasma ChE activity at four and/or twelve hours after the exposure was available in 33 patients. The median (inter quartile range) of plasma ChE activity at four and twelve hours was 790 (146-2598) mmol/l/min and 431 (136-3068) mmol/l/min respectively.

In the first assessment MNCV of all the motor nerves examined 95% CI median 0.1 to 2.7, ulnar 0.7 to 3.8 and common peroneal 1.1 to 4.5), CMAP amplitude (95% CI 0.2 to 1.8) and SNCV of ulnar nerve (95% CI 2.3 to 6.5), median ($p=0.005$) and ulnar ($p=0.001$) F-wave occurrence in the patients were significantly reduced compared to the controls.

In the second assessment significant reduction was found in SNCV of both sensory nerves examined (95% CI median 0.6 to 5.8, ulnar 1.5 to 5.9), MNCV of ulnar nerve (95% CI 0.1 to 3.5), CMAP amplitude of common peroneal nerve (95% CI 0.3 to 2.6), F-wave occurrence of median and ulnar nerves ($p=0.002$).

No abnormalities were detected in the patients when compared to the standard cut-off values of nerve conduction studies except F-wave occurrence.

In few occasions the decrement response was statistically significant. They are; the decrement response at rest (at fourth amplitude 95% CI -0.2 to -2.7) and two minutes after the exercise (at fourth; 95% CI -0.8 to -5 and fifth amplitude; 95% CI -1 to -5) in the second assessment compared to the controls, decrement response at rest (at fourth and fifth amplitude) and two minutes after the exercise (at fourth amplitude) in the second assessment compared to the first assessment. Patients in the first assessment and the controls showed more than 8% decrement response either to the second, fourth or fifth stimuli in seven and five occasions respectively.

The number of patients who showed abnormal autonomic function compared to standard cut-off values did not show statistically significantly difference from that of controls by Chi-Square test. When compared to the controls at one week the only significant differences consistent with autonomic dysfunction were change of diastolic BP 3 min after standing (95% CI 2.4 to 6.7), HR-DB (95% CI -1.6 to -8.8), SSR-Amplitude (95% CI -1.01 to -0.5), SSR-Latency (95% CI 28 to 163), post-void urine volume (95% CI 9 to 25) and size of the pupil (95% CI 1.3 to 0.7). At 6 weeks significant recovery of autonomic function was observed and only HR-DB was decreased to a minor degree, -5 beats/min [95% CI 2-8].

The inter peak latency of BAEP was not statistically differ in the controls vs the first assessment, controls vs the second assessment and the first vs the second assessment. Significant impairment of cognitive function was seen in total score of MMSE (95% CI -2.5 to -0.3), orientation (95% CI -1 to -0.2) and language (95% CI -0.9 to -0.1) domains of MMSE, digit span test (95% CI 0.1 to 1.7) and test of long term memory function (95% CI 0.3 to 2.3) in the first assessment compared to the controls. When the results of the second assessment compared with the controls no significant difference were seen.

PQ neurotoxic study

There were 28 (21 males) paraquat survivors and 56 controls in the study. The mean (SD) age of the patients and the controls were 29 (12) and 31 (11) years.

Abnormal results of peripheral nerve conduction studies were found at the first assessment. These were significant for amplitude of ulnar nerve CMAP on distal stimulation (95% CI -0.06 to -2.2), median nerve area of CMAP on distal stimulation (95% CI -1.4 to -10.4), and F-wave occurrence in median ($p=0.002$), ulnar ($p=0.004$) and tibial nerves ($p=0.002$) (vs controls). EMG did not show features of denervation or myositis.

Statistically significant autonomic dysfunction was found in change of systolic blood pressure (SBP) three minutes after standing ($p=0.006$), SSR amplitude ($p<0.001$), and residual urine volume ($p=0.02$). The decrement response after exercise augmentation was significantly greater in the patients at one week after the exposure. Parameters of somatic, autonomic nerve functions and RNS reverted to normal at six weeks after the exposure. The inter peak latency was not statistically differ in the controls vs the first assessment, controls vs the second assessment and the first vs the second assessment.

Double blind placebo controlled clinical trial (Antioxidant trial)

There were 26/40, 18/24 and 65/80 males in the test, historical and parallel controls respectively. The mean (SD) ages of the test, historical and parallel controls were 33 (17), 32 (18) and 34 (15) years.

The median survival time was longer in the patients given antioxidants than in the historical controls [8 days (95% CI 1.8 - 14.2) vs 1 day (95% CI 0.5 - 1.5)]. This difference was significant by both the Log Rank (Mantel-Cox) ($p=0.03$) and Tarone-Ware tests ($p=0.01$). The absolute risk reduction is 18.3% (95% CI -2.7% to 39.3%, Number Needed to Treat was 6). There was no statistically significant survival difference between the individuals who received IV vit C + 5% dextrose (placebo) vs IV vit C+NAC and tests vs parallel controls.

The Proportional Hazard assumption appeared to hold. Stratified Cox Proportional Hazard model was done as variable sex violated the Proportional Hazard assumption (Chi-square value = 66.6, $p<0.01$).

No statistically significant changes of antioxidant levels within the groups over five consecutive days.

The median survival time of parallel controls were 10 days (95% CI 3.0-16.9). There was no significant difference compared to the patients who received IV vit C.

Adherence to national guidelines on the management of OP/PQ

A total of 149 OP patients were recruited. Eighty (54%) patients were directly admitted to the collaborating hospitals. Among them, 35 (44%) did not have cholinergic features at the time of presentation to the hospital and 32 patients were treated with atropine before the appearance of cholinergic features. Seventeen (12%) patients neither show features of atropine toxicity nor inadequacy. The number of patients who developed features of atropine toxicity and inadequacy in the two hospitals were 109 (73%) and 11 (7%) respectively. Seventy-eight (52%) and 39 (26%) patients did not receive the loading dose and the maintenance dose of pralidoxime respectively. None of the patients received pralidoxime for the recommended duration.

One hundred and forty-five PQ patients (111 males) were admitted with paraquat poisoning. Fuller's earth was given to 130 (89.6%) patients. One patient refused and one died before the treatment. Twelve (8.3%) patients did not receive Fuller's earth. Among them five died with the median of 3 (range 1-13) days after the hospital admission. Nineteen (14.6%) patients said that Fuller's earth had not passed with their stool. Forty patients either did not observed whether Fuller's earth was passed with their stools or the data was not available. 58%, 10% and 39% of patients received cyclophosphamide, dexamethaone and methylprednisolone respectively.

Discussion

We observed widespread but small magnitude adverse differences of somatic, autonomic nerve and cognitive function in OP poisoned patients compared to the controls. There were no abnormality detected in BAEP and NMJ function.

OP binds to the esteratic site on the AChE molecule, phosphorylate the enzyme, and lead to inhibition of its action (4). The binding between the esteratic site on the enzyme and the phosphorus atom is stable and takes hours or weeks to break off, depending on the compound involved. Studies have shown that a phenomenon of enzyme aging occurs which involves cleavage of a radical from the inhibited enzyme, making it resistant to rephosphorylation. The net result is the accumulation of excess acetylcholine (ACh) at the cholinergic nerve endings all over the body resulting in the characteristic clinical manifestations. Following inhibition, recovery of this enzyme occurs at a rate of about one percent per day (4). Restoration of AChE levels occurs by spontaneous rephosphorylation of the enzyme and by new enzyme synthesis (4).

Dimethoate active metabolite (omethoate) inhibits AChE slowly and BChE activity can be near normal in symptomatic patients (18). Overall ChE activity in dimethoate poisoned patients may be high. In contrast the active metabolite of chlorpyrifos (chlorpyrifos-oxon) is more potent and inhibits BChE more than AChE (18). In chlorpyrifos poisoning, all patients with sufficient AChE inhibition to provide clinical symptoms will have markedly decreased BChE activity (18). Among 26 patients identified as chlorpyrifos ingestion, six patients showed high levels of ChE. All six patients had cholinergic features on admission and were treated with atropine and pralidoxime. High levels of ChE activity in them may be due to incorrect identification of the poison or mixed ingestion. Overall high levels of ChE in our patients were probably due to the patients with dimethoate poisoning.

Diabetes is well known to cause neuropathy. Therefore we excluded patients with diabetes from the study. Occupation matched controls were recruited since there is evidence that occupational exposure to pesticides can cause neuropathies. Very few studies were found in the literature which focused on SNCS / MNCS following acute ingestion of OP. There have been no studies which have looked at the effects of acute OP exposure on peripheral nerve function compared to matched controls. Most large studies have examined nerve function in farm workers who had chronic, probably low level exposure to pesticides.

Reduction of SNCV and MNCV of the patients in our study indicates that there may be demyelination following acute OP exposure.

Reduced amplitude and/or area of CMAP on distal stimulation were observed in several comparisons. These indicate that there may be an axonal damage since amplitude and area under negative curve of CMAP are directly proportional to the number of functioning axons (19). If the whole nerve is affected, F-wave latency should be prolonged. Reduced nerve conduction velocity only in the distal segment may be evidence for distal demyelination with sparing of proximal segment. Since we did not perform segmental nerve conduction studies focal damage cannot be excluded.

F-wave studies are important for recognizing proximal segment involvement since it is produced by antidromic activation of motor neurons. It is also important to identify marginal changes of standard MNCS since an impulse travels a long pathway to produce an F-wave, and additive effects are more obvious in F-wave latency measurement. In our study there was a statistically significant reduction of F-wave occurrence observed in the median and the ulnar nerves in the first assessment compared to the controls. Although there was prolongation of F-wave latency, it was not statistically significant.

The gold standard electrophysiological tool to explore the neuromuscular junction function is single-fiber electromyography (SF-EMG) (19,20). We explored whether repetitive nerve stimulation with exercise might be a useful alternative method.

A few patients had repetitive nerve stimulation abnormalities in the first assessment and this was no different from the controls. Results of RNS six weeks after the exposure showed significant decrement response. However repetitive nerve stimulation does not appear to be a useful method to explore medium to long term NMJ function.

Decrement response is more obvious in fast repetitive stimulation than the slow repetitive stimulation (21). The current study concentrated only on slow repetitive stimulation and this may not be sensitive enough to detect subtle abnormalities.

The study showed impairment of muscle power in both proximal and distal muscles at one week after the exposure in a few patients. Proximal muscle weakness has been observed in previous studies, for example a case of impairment of muscle power and exaggerated tendon reflexes with absent knee jerk was reported in acute methamidophos poisoning (22). Weakness of proximal limb muscles (shoulder abduction and hip flexion) with normal strength in the distal muscles was reported in patients who developed intermediate syndrome following acute OP exposure (23).

The study done by Senanayaka N (1987) showed reduction of tendon reflexes in 9/10 who developed intermediate syndrome. The remaining patient had exaggerated tendon reflexes (23). In the current study one patient showed absent knee jerk, but some patients showed exaggerated tendon reflexes which may be due to persistent cholinergic effects.

Although autonomic function in farm workers has been looked in to, there was no study that looked at autonomic function following acute OP exposure.

Variation of heart rate during rest, deep breathing and isometric muscle contraction were tested in flower bulb farmers who had chronic mixed pesticide exposure by Ruijten M.W.M.M *et al.* (1994). Significant autonomic dysfunction was shown in variation of heart rate during rest and deep breathing (7). We did not assess variation of heart rate during rest but the results in both studies with regard to heart rate variation during deep breathing were similar.

In the current study, from the tests reflecting parasympathetic damage (heart rate response to Valsalva manoeuver, heart rate variation during deep breathing and immediate heart rate response to standing) a positive result was shown only with heart rate response to deep breathing. It is well known that the response of the heart rate can be abolished by atropine (24). The first assessment of the patients was conducted with the mean of three (IQR 1 - 4) days after the cessation of atropine therapy. Even at the sixth week from the exposure, the effects of atropine might not have disappeared completely. On the other hand, only 48/66 patients were able to do the Valsalva manoeuver. The sample size may not be adequate to draw a conclusion.

Even though there was significantly low SBP and DBP recorded in the patients, postural drop was not significant. Blood pressure response to standing reflects sympathetic function. But it begins to give

abnormal results with more severe sympathetic nerve damage (24).

Reduction of amplitude and prolongation of latency in SSR are in favor of sympathetic damage. SSR represents the change in voltage measured at the skin surface following a single electrical stimulus. It depends on the electrical activity arising from sweat glands (19). Latency of SSR is inversely proportional to the number of sweat glands innervated (19). Hence sympathetic innervation to sweat glands was less in the patients compared to the controls. In the second assessment the latency of SSR remained significantly prolonged whereas amplitude improved. We could not eliminate the effects of atropine on sweat glands and changes may have been due to effects of atropine.

It is well known that high residual urine volume is associated with increased risk of urinary tract infections. Since our results show that residual urine volumes of patients were significantly higher than in the controls, it is very important to follow up such patients further.

There was no change in size of the pupil in the second assessment of the patients compared to the controls. Large pupil size in the first assessment of the patients was probably due to the effects of atropine.

The number of patients who could complete the test of blood pressure response to sustained hand grip was significantly lower than the number of controls who could complete the test. In the follow up assessment the patients performed the test better than in the first assessment. The difference may be due to muscle weakness following poisoning.

PQ neurotoxic

This is the first study which measured effects on peripheral nerve function with paraquat ingestion.

The current study showed that nerve conduction velocity was not affected by paraquat ingestion. However the area and the amplitude of CMAP on distal stimulation of some nerves were lower in paraquat survivors.

Nerve conduction velocity and F-wave latency in peripheral nerves were not apparently affected by paraquat reflecting that there was no evidence of demyelination.

In the current study occurrence of F-waves was uniformly reduced in paraquat survivors in the first assessment. The occurrence of F-wave reflects the excitability of motor nerves that determines the probability of a recruitment response in individual axons (25).

An abrupt increase of plasma creatine kinase level in paraquat poisoned patients has been shown revealing damage to the skeletal muscles (26). EMG of the current study did not show any evidence of chemically induced myositis (spontaneous activity) or denervation (fibrillation potentials, high amplitude of the motor units, polyphasia and reduced interference). However significant reduction of area and the amplitude of CMAP in some nerves in the first assessment may give some evidence of muscle damage.

In our study NMJ dysfunction was demonstrated by slow repetitive stimulation after exercise modification (isometric exercise) in patients with self poisoning with paraquat compared to the controls. No abnormalities were detected in muscle power and tendon reflexes.

A much higher number of patients were unable to complete the test of blood pressure response to sustained hand grip in the first assessment than the number of patients who were unable to complete it in the second assessment. This may be due to transient muscle weakness following paraquat poisoning. The current study showed damage to the sympathetic system sparing the parasympathetic system in the first assessment at the first week.

SSR represents the changes in voltage measured at the skin surface reflecting sympathetic innervations to sweat glands (20). Although the SSR amplitude was significantly low in the first assessment, it recovered at the second assessment suggesting the possibility of re innervations.

In the current study, the residual urine volume was significantly higher in the paraquat survivors but clinical sign is unclear. It would have been useful to have followed them up further. Measurement of residual urine volume of poisoned patients is not included in routine practice. Our results suggest that this measurement may be useful as incomplete emptying can lead to other complications like urinary tract infection.

Cognitive function of OP / PQ poisoned patients

Although there was a slight transient cognitive impairment detected with the screening tests following acute OP/PQ exposure, no long term cognitive defects was detected.

Cognitive function was tested with the simple and quick assessment tool of MMSE. The MMSE provides a measure of cognition which covers a broad set of cognitive domains: orientation, registration, short term memory, attention, calculation, visuo-spatial skills and apraxia (27). The sensitivity and specificity of MMSE were 100% and 69% to the cut off level of less than 24 evaluated against the performances at CAMCOG (Cambridge Cognitive Score) (27).

Stephens R *et al.* (1995) reported impaired cognitive function in sheep farmers who were exposed to OP through sheep dipping, which involves immersing each animal in a pesticide solution to control parasitic infections. Exposure to OP was appeared to be splashing on to the skin where protective cloths were seldom worn. The speed of performance of simple reaction time, symbol-digit substitution and syntactic reasoning were significantly slower in the farmers than the controls. No impairment was found in short term memory (by digit span test and visual spatial memory) and long term memory (by serial word learning, category search classification, category search recognitions) in the cases compared to the controls (9,28).

Psychological status of OP / PQ poisoned patients

A high score of GHQ-12 and prevalence of psychological distress were found in the patients compared to the controls.

The performance of GHQ-12 was shown to be equal to longer versions of GHQs (29,30). The GHQ-12 is a brief self report of measure and has excellent psychometric properties as a screening instrument for psychiatric disorders.

Joe S *et al.* (2008) showed that the risk of attempted suicide was significantly associated with being female, being in the age group 18-34 years and having low/medium educational levels, alcohol consumption, use of illicit drugs, symptoms indicative of conduct disorders and depressive symptoms (31,33). The strongest predictor of suicide is a prior experience of non fatal suicide behavior (34). The above factors, co morbid disease, family history of suicide attempts and smoking habits were identified as confounders for GHQ-12 and analysed with multiple linear regression. The model showed significant association with educational level and co morbid disease.

The high GHQ-12 score and prevalence of psychological distress in the test group indicates that the patients require special attention since suicidal behavior may be a salient sign of a high risk of suicide. Suicidal ideation is the least common symptom of major depressive disorder (35).

Mental health services are very important for individuals with suicidal ideation and suicide attempts (36,37). It may not be possible to assess the psychological status of each and every patient with suicidal attempts by a psychiatrist. Expansion of psychological services with counseling and identification of patients who need further psychological support and management is important. The prevalence of self perceived need for mental health care among those with suicidal behaviors is unclear, but those who do not perceive a need are unlikely to use the services (36,37).

Antioxidant trial

The median survival time was longer in the patients who received antioxidants (vitamin C ± NAC) than in the historical controls but there were no difference compared with the parallel controls. Females showed better outcome and may respond well to treatment. The median survival time was not longer in the vitamin C + NAC group over Vitamin C alone.

Survival status against plasma PQ levels of the trial patients showed promising effects with IV vitamin C compared to the parallel controls which was not statistically significant. IV vitamin C may not be effective in severe poisoning however it may be effective in moderately severe poisoning.

Small sample size is the main weakness of the study

The study done by Hong S Y *et al.* (2002) administering the same dose of vitamin C to 10 paraquat patients showed recovery of all patients

within mean (SD) of 21.2 (5.4) admission days (38). The current study showed a 65% (26/40) mortality rate.

High doses of vitamin C (One gram of vitamin C in 500mL of 5% dextrose saline as a loading dose and then 4g of vitamin C daily for 14 days) were given in the retrospective study described by Moon M J and Chun B J (39). The patients in their study also received dexamethasone (10mg every eight hour for 14 days), cyclophosphamide (one gram for two days) and methylprednisolone (one gram for three days). The mortality in the group was 70.1%. Less than half of the patients in the historical controls received cyclophosphamide and methylprednisolone, where as 95% of the patients in the test group received cyclophosphamide and methylprednisolone. Immunosuppressant therapy may also positively affect the higher survival function of the test patients.

Although females showed better survival the markers of oxidative stress in the females were not higher than that in the males. Therefore the better survival function of the females may not be associated with the antioxidant levels.

The adherence to existing guidelines on the management of OP/PQ poisoning

Atropine was commenced in some patients without cholinergic features. Majority of the patients received toxic doses of atropine. None of the patients received the maintenance therapy of pralidoxime for the recommended duration.

It was not recommended to commence atropine in OP poisoning in the absence of cholinergic features. Some patients presented to hospital soon after the ingestion before the appearance of cholinergic features. Pro-poison OP is converted to the active form as a fat soluble OP (eg. fenthione) and released slowly from fat stores into the blood. In such situations careful observation is required to identify the development of the cholinergic features. But in busy medical wards it would be difficult to observe this constantly. Hence it may be considered rational to start atropine before the appearance of cholinergic features, if the ingestion can be confirmed, in order to prevent the occurrence of dangerous cholinergic effects.

In general medical wards the maintenance dose of atropine is administered to the patients via intravenous drip sets. Neither burette sets nor infusion pumps are used. Although the drip rate is adjusted, it is not protected. The position of the wheel in the intravenous drip set used for adjusting the rate may change position with patient movement and rapid flow through is possible, causing features of atropine toxicity. Frequent monitoring should be done and the drip rate adjusted according to the cholinergic features.

Oximes directly bind to and inactivate OP molecules. They accentuate the pharmacological activity of atropine and may be atropine sparing (40). The clinical usefulness of oxime therapy in OP poisoning is currently not clear. Patients poisoned with some OPs, those with dimethyl or S-alkyl chemical groups attached to the phosphate ion may not get a benefit from oximes (41,42). But patients poisoned with diethyl OPs are more likely to be benefitted (42). A clinical trial conducted in Sri Lanka with 45 patients did not show any benefit from pralidoxime plus atropine over atropine alone in the management of OP poisoning (40).

Management of poisoned patients according to the guidelines, titrating of the dose according to the clinical features and availability of antidotes at all times may not be possible in busy general medical wards. Establishment of separate units to manage poisoning patients may improve the quality of management and outcome of the patients. It may be useful to launch Continuous Medical Education programs for the heath care staff in hospitals with regard to the management of poisoning.

Ninety percent of paraquat poisoned patients received Fuller's earth. At least 15% of patients did not receive adequate Fuller's earth to appear in the stools. Majority of patients received immuno-suppression therapy.

Fuller's earth is the only specific management currently available for paraquat poisoning. Proper administration of Fuller's earth may minimize the mortality and morbidity of patients with paraquat poisoning. The reason for 10% of patients not receiving Fuller's earth was not clear in the current study. Except two patients (who did not have evidence of ingestion and who ingested 750ml of paraquat) all other patients should receive Fuller's earth. According to the expert committee on essential medicines, both Fuller's earth and activated charcoal were included in the National List

of Essential Medicines in Sri Lanka. Therefore Fuller's earth and activated charcoal should be available all the time in the hospitals. Hence 100% of patients with paraquat poisoning should receive Fuller's earth. Activated charcoal can be given if Fuller's earth is not available. Fuller's earth is available as powder for oral suspension. Once it is prepared the supernatant does not contain particles of Fuller's earth. Therefore the suspension has to be well mixed before and while it is administered. People who give Fuller's earth to patients may not be aware of this fact, so supernatant may enter the patient while Fuller's earth remained at the bottom of the container. This may be the reason why some patients did not observe Fuller's earth in the stools after administration. Proper administration of Fuller's earth has to be emphasized to the patients and care givers.

Chen *et al.* (2002), Lin J L *et al.* (1999 and 2006) showed promising effects on PQ poisoning with a combination of cyclophosphamide, methyl--prednisolone and dexamethasone (43-45). They used 1g of methylprednisolone (IV) daily for three days, 15mg/kg of cyclophosphamide (IV) daily for two days and preceding 5mg dexamethasone (IV) three/four times per day or 10mg dexamethasone (IV) thrice daily for 14 days. The dose of methylprednisolone in the regimen given at the General Hospital, Matara was higher than that in the previous studies. The dose of cyclophosphamide in the regimen practiced at the two collaborating hospitals were more or less equal to the studies done previously, except the administration of cyclophosphamide via the oral route from the second dose onward at the General Hospital, Matara. The collaborating hospitals in the current study used slightly higher doses of oral dexamethasone compared to the previous studies, but the previous studies administered dexamethasone intravenously.

The reason why a smaller percentage of patients received cyclophosphamide and methylpredni--solone at the Teaching Hospital, Galle is that they had been recruited for the double blind placebo controlled immunosuppression trial (Gawarammana et al. unpublished data).

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