Evaluation of MR perfusion abnormalities in organophosphorus poisoning and its correlation with SPECT

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Abstract

Aim: Acute organophosphate (OP) pesticide poisoning causes substantial morbidity and mortality worldwide. Many imaging modalities, such as computerized tomography (CT), magnetic resonance imaging (MRI), and single photon emission computed tomography (SPECT) of the brain, have been used for quantitative assessment of the acute brain insult caused by acute OP poisoning. Perfusion defects on SPECT in acutely poisoned patients with OPs have been described, however, MR perfusion abnormalities have not been described in the literature. MR perfusion Imaging has the advantage of having higher spatial resolution, no radiation, and better availability.

Materials and Methods: In this prospective study, 20 patients who ingested OP compounds were included. All the patients underwent brain SPECT on a dual head SPECT gamma camera and MRI brain on a 1.5T MR system. Neurocognitive tests were performed for all patients.

Results: SPECT showed perfusion defects in 7 patients and total number of perfusion defects were 29. On MR perfusion, based on the cut-off values of normalized cerebral blood volume (nCBV) ratios and normalized cerebral blood flow (nCBF) ratios, the total number of patients showing perfusion defects were 6 and 8; and the total number of perfusion defects were 29 and 45, respectively. There was significant difference of the nCBV ratios and nCBF ratios between the control group (n = 20) and positive patients group (n = 6 and n = 8, respectively) (P > 0.05). All the defects seen on SPECT were well appreciated on nCBF maps (MRI perfusion) suggestive of 100% correlation.

Conclusion: MR perfusion imaging can be used as an effective modality for evaluation in acute OP poisoning.

Key words: Magnetic resonance imaging; organophosphorus poisoning; single photon emission computed tomography

Introduction

Poisoning due to organophosphate (OP) pesticides is a common cause of significant mortality and morbidity all over the world.[1] In Asia, OP poisoning is the most common form of fatal self harm. It accounts for more than 60% of all deaths, and is thus considered to be more important than hanging or other forms of suicide.[2,3]

Acute OP poisoning usually occurs after suicidal ingestion and may occasionally follow accidental exposure during agricultural spraying.[4] OPs are anticholinesterases that are specifically designed as pesticides and weedicides. Patients with OP poisoning may present with a variety of gastrointestinal, neurological, and cardiac symptoms such as nausea, vomiting, seizures, fasciculation’s,

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neuromuscular weakness, conduction disturbances, and arrhythmias. Multiple case reports are available detailing conventional magnetic resonance imaging (MRI) findings in OP poisoning; however, there are no detailed studies evaluating the role of MR perfusion in OP poisoning. The present study investigates the relationship between perfusion defects seen in the brain after acute OP poisoning on MRI T2* perfusion and single photon emission computed tomography (SPECT) with neurocognitive dysfunction.

Materials and Methods

Participants
This was a prospective study in which 20 patients, with acute OP poisoning presenting within 1 week of poisoning and clinical features consistent with cholinergic crises over a period of 2 years were included. The presence of low serum AChE activity and stability to undergo MRI, SPECT, and neurocognitive tests were also prerequisites for inclusion in the study. An informed written consent was obtained from every patient or his/her relative. Approval for the study was given by the institute ethics committee. All patients were initially subjected to neurocognitive tests followed by SPECT and MRI examination. The mean day of scan for brain SPECT was 7.5 (5 – 10 days) from the date of poisoning and for brain MRI was 8 (6 – 10 days) from the date of poisoning. MR perfusion results were interpreted by two neuroradiologists (with 10 and 5 years of experience). SPECT results were also evaluated by two specialists in Nuclear Medicine (with 10 and 5 years of experience). Twenty age and sex-matched adults who were being scanned for other complaints and had normal structural MRI were used as controls for MR perfusion imaging and neurocognitive tests.

Magnetic resonance imaging
Routine MRI sequences including T1, T2, fluid-attenuated inversion recovery (FLAIR), and diffusion weighted imaging (DWI) were performed on patients using a 1.5T MR system (Aera; Siemens Medical Systems, Germany) using a quadrature birdcage head coil. Dynamic susceptibility contrast (DSC) enhanced imaging was performed after an intravenous bolus of gadopentetatedimeglumine (Dotarem; Guerbet Laboratories, Aulnay-sous-Bois, France) by using a three-dimensional multisolid shifted-echo EPI sequence. (TR: 2010 ms, TE: 30 s, FOV: 230 × 100, matrix: 128 × 128, flip angle: 70°, imaging plane: transverse, total no. of cycles: 60, no. of slices per cycle: 20). The raw perfusion data was transferred to the Syngo MR work place for post processing. The arterial input function (AIF) was obtained by positioning a region-of-interest (ROI) box measuring 20 × 20 pixels on the area representing the M2 segment of the right middle cerebral artery. Relative cerebral blood flow (rCBF) and relative cerebral blood volume (rCBV) maps were generated.

Image analysis for magnetic resonance imaging
The area under the receiver operating curve (ROC) and the corresponding cut-off values of normalized relative cerebral blood volume (nrCBV) and normalized relative cerebral blood flow (nrCBF) ratios in control patients were derived for analyzing perfusion anomalies in the patients. For this, in control patients, ROIs were drawn on the rCBV and rCBF maps in five regions (i.e., frontal cortex, parietal cortex, occipital cortex, temporal cortex, and basal ganglia) on both sides at a fixed level in all control patients. nrCBV and nrCBF were calculated for controls by measuring the ratio of the measurement at the ROI to the ipsilateral cerebellum. Similarly, in patients, nrCBF and nrCBV values were derived for the corresponding five cerebral regions. Cut-off values for abnormal perfusions were deduced from the ROC analysis of the values from control patients.

Single photon emission computed tomography
SPECT was performed using a dual head gamma camera. 15–20 mCi of 99mTc-ECD (ethylene cysteine dimer) was injected via the intravenous route. Data acquisition was performed after 25 minutes. The gamma camera was rotated through 360° in a circular orbit around the head. A total of 128 projections (20 s/projection) were obtained (matrix size: 128 × 128; zoom factor: 1.5). Reconstruction of the data was then done using an iterative reconstruction algorithm and a smoothing filter (Butterworth). Subsequently, the perfusion abnormalities were visualized in all three planes (i.e., axial, sagittal, and coronal).

Image analysis for single photon emission computed tomography
The SPECT findings were evaluated by qualitative as well as semi-quantitative analysis. For qualitative analysis, the frontal, temporal, parietal, occipital cortex, basal ganglia, cerebellum, and brainstem were analyzed. An area of hypoperfusion had to be visible on all three planes to be considered significant. Visual interpretation of the perfusion state was made using a rating scale of 0 to −3, in which 0 was baseline perfusion, −1 mild, and −2 moderate reduction in perfusion. A score of −3 was given to a region of severe deficit which was defined as a clear disconnection in brain ECD uptake in more than 3 continuous slices. For semi-quantitative analysis, ROIs were drawn on the axial slices at the areas showing low perfusion visually and at six regions on both the sides at a fixed level in all the patients (frontal, temporal, parietal, occipital cortex, basal ganglia, cerebellum, and brainstem). Counting ratios were obtained from these ROIs. To calculate lobe to cerebellum ratio (nL:C), the value in each cortical area was divided by the mean value in the ipsilateral cerebellar lobe.

Neurocognitive tests
Trail-making test, PGI memory scale, verbal fluency test, and Bender Visual Motor Gestalt test (BVMG) were used...
to assess the memory, concentration, attention, perceptual motor skills, and executive functions.

Statistical analysis
Students t-test was applied to determine the statistical difference of nRCBV, nRCBF, and nL:C ratios between normal and low perfused areas. If the P value was ≤ 0.05, the difference was considered statistically significant. The significance of the relationship between nRCBV, nRCBF, nL:C ratios, and perfusion of lobes was assessed using ROC curve analysis. Sensitivity and specificity were established to use the potential cut-off values permitting discrimination between normal and low perfused areas. For categorical data, we applied Chi-square test. Mann–Whitney test was used for comparison of perfusion defects. The Statistical Package for the Social Sciences (SPSS) version 15.0 (IBM) was used for analysis.

Results
Seven males and 13 females with acute OP poisoning (accidental = 5, suicidal = 15) with a mean age was 25.4 years (range: 14–62 years) were included in the study. The most common presenting features were salivation (95%) and lacrimation (70%). Few patients had severe neurological manifestations such as fasciculation (10%) and neuromuscular weakness (5%).

Conventional MRI sequences revealed signal abnormalities in the form of T2/FLAIR hyperintensity in the caudate and putamen with mild diffusion restriction in one case only. Eight patients showed abnormal MR perfusion abnormalities. Abnormal low perfusion on SPECT was seen in 7 patients.

Magnetic resonance imaging
On the basis of equal misclassification rates, various cut-off values for all the areas were obtained at different sensitivity and specificity [Tables 1 and 2]. nRCBV ratios of normally perfused areas in OP poisoned patients ranged between 0.78 and 1.32, and were significantly different from those of low perfusion areas, which ranged between 0.10 and 0.36 (P < 0.002). nRCBF ratios of normally perfused areas in OP poisoned patients ranged between 0.67 and 1.35, and were significantly different from those of low perfusion areas, which ranged between 0.11 and 0.53 (P < 0.004). Based on the cut-off values of nRCBV ratios and nRCBF ratios, the total numbers of perfusion defects were 29 and 45, respectively. Based on the cut-off values of nRCBV ratios, the maximum number of defects were observed in the occipital lobe (Right = 5 and Left = 5) followed by the temporal lobe (Right = 3 and left = 3). Based on the cut-off values of nRCBF ratios, the maximum number of defects were observed in the occipital lobe (Right = 8 and left = 7) followed by temporal lobe (Right = 6 and left = 6).

Single photon emission computed tomography
The perfusion defects were initially evaluated based on the qualitative analysis (visual appearance) of brain SPECT, which depicted the abnormal areas that were later evaluated via semi-quantitative methods [Table 3]. Twenty-nine perfusion defects were detected. nL:C ratios of normal perfused areas in OP poisoning patients ranged between 0.99 and 1.47, and were different from those of low perfusion areas, which ranged between 0.34 and 1.06. The maximum number of defects were observed in the occipital lobes (Right = 6 and Left = 6) followed by the right parietal lobe. Perfusion defects were also seen in bilateral frontal, temporal, and basal ganglia regions (Figure 1A, 2A and 3A).

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Table 1: MR perfusion analysis: The value of area under ROC and corresponding cut off values of nRCBV ratios in control patients

<table>
<thead>
<tr>
<th>Areas</th>
<th>Area under ROC curve</th>
<th>Cut-off values</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
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<td>RT FRONTAL</td>
<td>0.678</td>
<td>0.87</td>
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<td>0.821</td>
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<tr>
<td>RT TEMPORAL</td>
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<td>75</td>
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<tr>
<td>LT TEMPORAL</td>
<td>0.675</td>
<td>0.98</td>
<td>80</td>
<td>65</td>
</tr>
<tr>
<td>RT BG</td>
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<td>60</td>
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</tr>
<tr>
<td>LT BG</td>
<td>0.653</td>
<td>0.95</td>
<td>90</td>
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</table>

Table 2: MR perfusion analysis: The value of area under ROC and corresponding cut off values of nRCBF ratios in control patients

<table>
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<tr>
<th>Areas</th>
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<th>Specificity (%)</th>
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Table 3: SPECT analysis: Positive patients (visual appearance)

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<th>Severity</th>
<th>Perfusion defects LT</th>
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<td>F</td>
<td>1</td>
<td>−2</td>
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<td>−2</td>
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<td>−1</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Single photon emission computed tomography and magnetic resonance perfusion

All the defects seen on SPECT were well appreciated on nCBF maps (MRI perfusion) suggestive of 100% correlation (Figure 1B, 2B and 3B). In addition, based on the ROC curve analysis, 16 defects were seen on nrCBF maps, which were not seen on SPECT. These additional defects were seen in bilateral frontal, parietal, temporal, and occipital regions. The nrCBF maps also showed a defect in one patient in whom SPECT was normal. Almost perfect agreement was seen between nrCBF and SPECT (Kappa coefficient = 0.894).

The nrCBV ratio maps showed perfusion defects in 6 patients, as against 7 patients on SPECT. On nCBV maps, 2 defects were seen in the right parietal region versus 3 defects seen on SPECT and 5 defects each in the right and left occipital lobe versus 6 defects on SPECT. There were 3 defects each in the right temporal lobe and left temporal lobe versus 2 defects each on SPECT. Using kappa coefficient, the substantial agreement was seen between nrCBV and SPECT (0.659).

Neurocognitive function tests

On admission prior to imaging, detailed tests of neurocognitive functions were carried out in all 20 patients who had consumed OP compounds. Five patients could not perform Trail A and B tests as these require the capability to read and write. BVMG and tests for visual retention could not be performed in 2 and 4 patients, respectively, as they also require writing skills. Verbal fluency and Trail B tests were impaired in 15 patients. The PGI memory scale revealed impairment in visual retention (16/16), visual recognition (20/20), and verbal retention for dissimilar pairs (20/20). BVMG was abnormal in 18/18 patients. The correlation between the perfusion defects detected on brain SPECT and MRI Perfusion with performance on neurocognitive testing was not significant (P > 0.05).

Discussion

Acute OP poisoning leads to acute, intermediate, and delayed neurological syndromes. Initially, there is an acute cholinergic crisis, which often requires management in the intensive care unit and may be life threatening. This is followed by an intermediate syndrome which is characterized by cranial nerve palsies, respiratory muscle weakness, and proximal muscle weakness. The patients often require respiratory support at this stage. Delayed effects are characterized by polyneuropathy. Following OP poisoning, morphologic changes in the brain are seen within a few hours. OP poisoning leads to inactivation of cholinesterase by phosphorylation of the serine hydroxyl group that is present at the active site of acetylcholinesterase. Cholinesterase accumulates in the central nervous system leading to overstimulation of the nicotinic and muscarinic receptors. A type II paralysis occurs due to accumulation of acetylcholine at the neuromuscular junction. The cholinergic neurons also react with other neurotransmitter systems leading to gamma aminobutyric acid inhibition and N-methyl-D-aspartate activation. This may be a cause of the seizures and respiratory depression. OP poisoning also leads to an inflammatory response via activation of microglia, increase in glial fibrillary acidic protein expression, cytokine levels, prostaglandin/isoprostanoid levels, macrophage activity/mast cell degranulation, and altered chemokine levels.

Our study sample was composed of total 20 patients of OP poisoning. The most common clinical presenting feature in our study group was vomiting (55%), salivation (95%), and lacrimation (70%), which is in concordance with most of the previous studies. The mean time of acquisition of SPECT and MRI was day 7 and 8, respectively. The MR imaging findings have been documented in few reports in the literature. Wang et al. have reported restriction...
of diffusion in the splenium of the corpus callosum in a patient suffering from delayed OP-induced neuropathy.\(^\text{[12]}\)

In another case report, Panda et al. described T2/FLAIR hyperintensity in bilateral basal ganglia in a young patient who had ingested Ops.\(^\text{[13]}\) Teke et al. also reported atypical hyperintense lesions on T2-weighted imaging in the mesencephalon and cerebellum.\(^\text{[14]}\) In our study, only 1 patient had T2/FLAIR hyperintensity involving the corpus striatum on conventional MR imaging; however, no perfusion defect was seen in this patient on either SPECT or MR perfusion. This relative absence of signal abnormalities on conventional MRI sequences may be related to the time of image acquisition, which was earlier in our study in the 1\(^{\text{st}}\) week as compared to other studies. We also obtained apparent diffusion coefficient (ADC) values for all controls patients. The difference between ADC values of control and poisoning patients was not significant. Testylier et al. have documented reduction in ADC in mice poisoned with Soman, suggesting cytotoxic brain edema as a possible pathophysiology.\(^\text{[15]}\)

**Magnetic resonance perfusion**

To our knowledge, there is no study addressing MR perfusion in acute OP poisoning till date. T2* (DSC) perfusion imaging was performed in all our patients. Normalized rCBV and rCBF values were obtained with respect to the ipsilateral cerebellum in our study. Global hypoxia may potentially be seen in any area of gray or white matter; however, cerebellar involvement has not been seen in previous SPECT studies. For this reason, the ipsilateral cerebellum has been used as an internal standard. There was significant difference of the nCBV ratios and nCBF ratios seen between the control group (n = 20) and positive patients group (n = 6 and n = 7, respectively) with (P > 0.05). This is likely due to difference in contrast uptake dynamics between low and high perfusion areas caused by altered capillary permeability and blood–brain barrier seen in
hypoxic areas.\textsuperscript{[16]} Mean nCBV ratios of normal perfused areas in patients with OP poisoning ranged between 0.78 and 1.32, and are significantly different from those of low perfusion areas, which ranged between 0.10 and 0.36. Mean nCBF ratios of normal perfused areas in OP poisoned patients ranged between 0.67 and 1.35, and are significantly different from those of low perfusion areas, which ranged between 0.11 and 0.53. Mean values of nCBV ratios and nCBF ratios showed significant differences between normal and low perfusion areas ($P < 0.002$ and $P < 0.004$, respectively). Based on the cut-off values of nCBV ratios and nCBF ratios, the total perfusion defects were 29 and 45, respectively. Based on the cut-off values of nCBV and nCBF ratios, the maximum number of defects were observed in the occipital lobe followed by the temporal lobe. The frequent occurrence of the defects in the occipital lobes and also slightly more on the right side is in concordance with a previous study on SPECT.\textsuperscript{[17]}

Single photon emission computed tomography
The role of SPECT in evaluation of OP poisoning patients has been well-documented in the literature. A total of 29 perfusion defects were detected. nL:C ratios values showed significant differences between normal and low perfusion areas ($P < 0.001$). The maximum number of defects were observed in the occipital lobe (Right = 6 and Left = 6) followed by the right parietal lobe. Mittal et al.\textsuperscript{[17]} have described regional defects in CBF on 99mTc SPECT in acute OP poisoning. All their patients, except one, had one or more perfusion defects in the brain. It was seen that the occipital lobes were most affected. Moreover, more perfusion defects were also found in the right cerebral hemisphere of the brain. The results of the present study are similar and showed hypoperfused areas in the parietal and occipital regions. However, in our study, right lateralization was not seen on SPECT. In another case report by Günes et al.\textsuperscript{[18]} perfusion defects were seen in the parietal and frontal regions. Yilmazlar et al.\textsuperscript{[19]} studied 16 patients of OP poisoning with SPECT and found multiple perfusion defects, mainly in the parietal regions. On comparison with SPECT, additional perfusion defects were seen on nCBF maps. Moreover, there was some difference between the areas showing reduced perfusion on SPECT and MRI. The reason for these differences is not clear and may be secondary to the difference in technique and the pharmacokinetic properties of the radiotracer.

Neurocognitive tests
The neurocognitive tests used in the study assessed various domains of memory. No significant difference was noted for Trail A test between the controls and patients. However, the patient group took longer to complete the Trail B test as compared to controls, suggesting impairment in attention, mental flexibility, sequencing, motor function, and visual search. Patients were able to speak lesser number of words as compared to controls on verbal fluency tests. Abnormalities in verbal fluency tests represent impairment in the speed and flexibility of verbal thought processes. This suggests involvement of both the frontal and temporal lobes. Patients also performed significantly worse than controls on memory tests, thus indicating a generalized impairment in visual, verbal memory, and new learning. Performance of patients on BVMG at the time of admission was also poorer as compared to that of the controls, suggesting impairment in perceptual motor development and skills.

A retrospective study by Rosenstock et al.\textsuperscript{[20]} found that the following OP poisoning patients performed poorly on tests involving problem solving, visuomotor sequencing, visual memory, and auditory attention. Steenland et al.\textsuperscript{[21]} also found impairment in the performance of OP poisoning patients during tests for assessing motor speed, hand–eye coordination, simple reaction time, sustained visual attention, and learning/memory. Ozyurt et al.\textsuperscript{[22]} compared (99m) Tc-hexamethylpropylene amine oxime SPECT findings and neuropsychological sequelae in carbon monoxide and OP poisoning in 14 patients. They found that neuropsychological sequelae correlated with the sites of perfusion defect. However, no significant relationship was seen between the pattern and number of defects on SPECT and MRI perfusion with neurocognitive dysfunction. This is in agreement with a recent study by Mittal et al.\textsuperscript{[17]} This discordance between neurocognitive tests and imaging may be due to the timing of our imaging. It is possible that the neuropsychological sequelae may manifest at a later date once the acute stage of the illness has passed.

Acute phase brain SPECT findings have already demonstrated the predictive significance for long-term neuropsychological sequelae. Functional neuroimaging techniques such as MR perfusion/SPECT, by showing the distribution of differentially perfused (hypoperfused) cerebral areas, helps in demonstrating the regional brain involvement and evaluating neurotoxic effects. They also help in monitoring clinical prognosis of OP poisoning. Mittal et al. have also stated that detection of perfusion defects may help in predicting neurocognitive deficits.\textsuperscript{[17]} Thus, perfusion imaging using SPECT or perfusion MRI may be a good diagnostic tool when clubbed with the clinical picture in determining the prognosis of the patients.\textsuperscript{[19]} Management of the acute phase of OP poisoning is performed based on the clinical symptoms of the patient. Atropine and oximes are the mainstay of pharmacological intervention during the treatment of OP poisoning. However, there is no evidence-based data to establish the efficacy of these therapies.\textsuperscript{[23]}

Limitation
Our sample size was small, therefore, we were unable to demonstrate a correlation between MR perfusion and neurocognitive testing. Although signals from large arteries
at peripheral parts were not included within the ROI, these factors may have influenced the rCBF values calculated from average signal intensity in an ROI. Moreover, to validate the rCBF values measured from MR perfusion images against the criterion standard, further studies are required.

**Conclusion**

There was significant correlation between SPECT and MR perfusion defects in our patients. Quantitative measurement of MR perfusion such as rCBF represents a promising incremental step forward in the discrimination of normal and hypoperfused areas. Based on our study, we suggest that MR perfusion can be utilized as an adjunct to SPECT perfusion imaging for delineating the extent of brain injury and earlier prognostication. With further validation, it may also replace SPECT in the evaluation of OP poisoning because there is no radiation exposure and all the required information can be acquired in a single sitting at a higher spatial resolution.

**Financial support and sponsorship**

Nil.

**Conflicts of interest**

There are no conflicts of interest.

**References**