Association of acute toxic encephalopathy with litchi consumption in an outbreak in Muzaffarpur, India, 2014: a case-control study.

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Association of acute toxic encephalopathy with litchi consumption in an outbreak in Muzaffarpur, India, 2014: a case-control study


Summary

Background Outbreaks of unexplained illness frequently remain under-investigated. In India, outbreaks of an acute neurological illness with high mortality among children occur annually in Muzaffarpur, the country’s largest litchi cultivation region. In 2014, we aimed to investigate the cause and risk factors for this illness.

Methods In this hospital-based surveillance and nested age-matched case-control study, we did laboratory investigations to assess potential infectious and non-infectious causes of this acute neurological illness. Cases were children aged 15 years or younger who were admitted to two hospitals in Muzaffarpur with new-onset seizures or altered sensorium. Age-matched controls were residents of Muzaffarpur who were admitted to the same two hospitals for a non-neurological illness within seven days of the date of admission of the case. Clinical specimens (blood, cerebrospinal fluid, and urine) and environmental specimens (litchis) were tested for evidence of infectious pathogens, pesticides, toxic metals, and other non-infectious causes, including presence of hypoglycin A or methylenecyclopropylglycine (MCPG), naturally-occurring fruit-based toxins that cause hypoglycaemia and metabolic derangement. Matched and unmatched (controlling for age) bivariate analyses were done and risk factors for illness were expressed as matched odds ratios and odds ratios (unmatched analyses).

Findings Between May 26, and July 17, 2014, 390 patients meeting the case definition were admitted to the two referral hospitals in Muzaffarpur, of whom 122 (31%) died. On admission, 204 (62%) of 327 had blood glucose concentration of 70 mg/dL or less. 104 cases were compared with 104 age-matched hospital controls. Litchi consumption (matched odds ratio [mOR] 9·6 [95% CI 3·6 – 24]) and absence of an evening meal (mOR 7·8 [95% CI 3·3 – 18·8]) in the 24 h preceding illness onset were associated with illness. The absence of an evening meal significantly modified the effect of eating litchis on illness (odds ratio [OR] 7·8 [95% CI 3·3 – 18·8], without evening meal; OR 3·6 [1·1 – 11·1] with an evening meal).

Tests for infectious agents and pesticides were negative. Metabolites of hypoglycin A, MCPG, or both were detected in 48 (66%) of 73 urine specimens from case-patients and none from 15 controls; 72 (90%) of 80 case-patient specimens had abnormal plasma acylcarnitine profiles, consistent with severe disruption of fatty acid metabolism. In 36 litchi arils tested from Muzaffarpur, hypoglycin A concentrations ranged from 12·4 μg/g to 152·0 μg/g and MCPG ranged from 44·9 μg/g to 220·0 μg/g.

Interpretation Our investigation suggests an outbreak of acute encephalopathy in Muzaffarpur associated with both hypoglycin A and MCPG toxicity. To prevent illness and reduce mortality in the region, we recommended minimising litchi consumption, ensuring receipt of an evening meal and implementing rapid glucose correction for suspected illness. A comprehensive investigative approach in Muzaffarpur led to timely public health recommendations, underscoring the importance of using systematic methods in other unexplained illness outbreaks.

Funding US Centers for Disease Control and Prevention.

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Introduction In India, seasonal outbreaks of an acute unexplained neurological illness have been reported since 1995 from Muzaffarpur, Bihar, the largest litchi (lychee) fruit cultivation region in the country. These recurring outbreaks begin in mid-May and peak in June, coinciding with the month-long litchi harvesting season. Children from poor socioeconomic backgrounds in rural Muzaffarpur comprise most of those affected. Illness is characterised by acute seizures and changed mental
status, frequently with onset in the early morning, and is associated with high mortality. A wide spectrum of causes has been proposed for this illness, including infectious encephalitis, exposure to pesticides, and a potential association with litchi fruit consumption.\(^1\)\(^2\) Despite numerous investigations, neither a cause nor risk factors for illness have been confirmed among affected individuals.

In 2013, the National Centre for Disease Control, India (NCDC) and the US Centers for Disease Control and Prevention (US CDC) initiated an investigation, focusing on characterising the clinical and epidemiological features of illness, and assessing potential infectious causes. The laboratory investigation found no evidence of a known infectious cause, and clinical data indicated that the illness was consistent with a non-inflammatory encephalopathy. These results led to consideration of various non-infectious causes, including pesticides or herbicides used to spray orchards and agricultural fields, insecticides used in vector-borne disease control efforts, heavy metals, or exposure to unusual medications. Notably, a common laboratory finding was low blood glucose (<70 mg/dL) on admission, which was also associated with increased mortality. These findings focused our attention on the possibility that children in Muzaffarpur were exposed to an environmental toxin, which resulted in low blood glucose and, subsequently, seizures and encephalopathy.

Published reports of a toxic hypoglycaemic syndrome in the West Indies\(^6\) that was due to the effects of hypoglycin A, a toxin found in the ackee, which is a fruit in the same botanical family as litchi, raised the prospect of a litchi-associated toxin. Of specific interest was the potential role of methylenecyclopropylglycine (MCPG), a homologue of hypoglycin A, and a substance naturally found in the litchi seed and fruit known to cause hypoglycaemia in animal studies by inhibiting \(\beta\)-oxidation of fatty acids and gluconeogenesis.\(^3\)\(^4\)\(^6\) We aimed to investigate the cause and risk factors for this illness.

**Methods**

**Study design**

In 2014, NCDC and US CDC investigated this syndrome, using hospital-based clinical surveillance, an epidemiological case-control study, and comprehensive and novel
laboratory testing methods on human biological and environmental specimens to determine risk factors associated with this illness, assess the aetiologic role of naturally occurring toxins such as MCPP and hypoglycin, and exclude the role of novel infectious pathogens, selected pesticides, and toxic elements.

Hospital-based clinical surveillance
Surveillance was done at the Shri Krishna Medical College Hospital (SKMCH) and the Krishnadevi Deviprasad Kejriwal Maternity Hospital (KDKMH), the chief referral medical centers in Muzaffarpur district, India.

A case was defined as new-onset seizures or altered sensorium in the previous seven days in a child aged 15 years or younger admitted to either SKMCH or KDKMH. Patients admitted for febrile seizures, defined as a seizure in a child 6 months to 6 years whose only finding is fever, and a single generalised convulsion of less than 15 min duration who recovers consciousness within 60 min of the seizure were excluded. Ill children who met the case definition and were admitted at either of the two referral hospitals in Muzaffarpur were prospectively enrolled. Demographic and clinical data were collected with standardised questionnaires.

According to district level clinical guidelines, a patient’s blood glucose was assessed at presentation, ideally before administration of any treatment; treating clinicians provided intravenous dextrose therapy to all patients suspected to have the outbreak illness. Lumbar puncture was done according to the clinician’s decision; cytological (white blood cell [WBC] count) and biochemical (protein and glucose) examination were done on collected CSF specimens. Blood and urine specimens were collected on all enrolled patients at the time of admission. Detailed neurological examination was done within 12 h of admission on a subset of case-patients. Brain MRI (including fluid attenuation inversion recovery [FLAIR] sequence) and EEG diagnostic testing, not normally available at the treating hospitals, were done when possible.

Case-control study
Every alternate surveillance case-patient who survived at least 6 h beyond the time of admission was prospectively enrolled in an age-matched case control study if he or she was a resident of Muzaffarpur district. We calculated a sample size of 100 cases and 200 controls, assuming 80% power, 50% exposure of the key risk factors among controls, and ratio of controls to cases of 2:1. Due to a rapid increase in cases and restricted human resources, enrolment was modified on June 16, 2014, to every fourth eligible case-patient to attain the calculated sample size. Blood and urine specimens were collected from each enrolled control. Each case-patient and control household was visited to collect data for observed exposures.

Environmental specimen collection
Between May 19, and June 13, 2014, litchi fruit samples were collected from orchards in the five blocks of Muzaffarpur district with the highest reported number of cases in 2013 and 2014. In each block, six or more fruits were collected in each of the following categories: unripe, ripe plucked from tree, and ripe fallen on the ground. Each fruit was stored at –20°C within 3 h of collection and subsequently transferred to –70°C until analysis.

Laboratory testing
CSF and serum specimens from case-patients were tested at NCDC using PCR for viruses, including Japanese encephalitis virus, West Nile virus, and enteroviruses. A
subset of case-patient CSF and serum specimens collected in both the 2013 and 2014 investigations was submitted for assessment of additional infectious agents, including potential novel pathogens, to the US CDC Pathogen Discovery Laboratory (Atlanta, GA, USA). Blood and urines specimens of cases from 2014 were examined at the US CDC for metabolites of pesticides and toxic elements using established mass spectrometry methods. At the National Institute of Occupational Health, India (NIOH), red blood cell acetylcholinesterase and plasma butyryl cholinesterase activity were measured (appendix p 5), and litchi fruit samples were analysed for pesticide residues using the Quick Easy Cheap Effective Rugged and Safe method (appendix p 8). A novel assay was developed at US CDC to analyse case and control specimens from 2013 and 2014 for metabolites of hypoglycin A and MCPG using liquid chromatography-tandem mass spectrometry. Plasma acylcarnitine and quantitative and qualitative urine organic acid profiles were assessed at the Emory Genetics Laboratory (Atlanta, GA, USA) using established mass spectrometry methods to identify evidence of derangement in fatty acid metabolism, which was postulated to occur in the case of MCPG or hypoglycin A toxicity as a result of impaired β-oxidation (appendix p 9). Laboratory scientists were blinded to case or control designation of the specimens under assessment. In a collaboration between the US Department of Agriculture (USDA) and the US CDC, a quantitative assay was designed to assess MCPG and hypoglycin A content in soapberry arils (appendix p 10).

Ethical approval
Ethical approval for this investigation and case-control study was obtained from the institutional review boards of NCDC and the US CDC. Written informed consent was obtained in the local language (Hindi) from the parent or guardian of each child enrolled. While laboratory testing on collected case-patient CSF specimens was done as part of the investigation, the decision of whether or not to collect CSF was solely made by the treating physician based on his or her clinical judgment. Participants and their parents or guardians were informed that some laboratory test results would only be available months later, and, although not of specific immediate benefit to the participating child, could help health officials to understand the cause of the outbreak, and thus benefit the community. In 2015, when final laboratory results were available from NCDC and US CDC, these results were communicated to district health officials and treating clinicians who conveyed them to participating families.

Statistical analysis
Data were entered in Epi-Info version 7.0 (CDC, Atlanta, GA, USA) and analysed with Stata version 13.0 (Stata, College Station, Texas, USA) and SAS/STAT software version 9.3 (SAS Institute Inc, Cary, NC, USA). Matched bivariate analyses as well as unmatched bivariate analyses controlling for age were done; risk factors for illness expressed as matched odds ratios (mOR; matched analysis) and odds ratios (OR; unmatched analysis) with 95% CI. Potential interactions between exposures were examined in stratified analyses, controlled for age. A p value less than 0.05 was considered significant.

Role of the funding source
The funder had no role in study design; in the collection, analysis, or interpretation of data; in the writing of the report; or in the decision to submit the paper for publication. The corresponding author had full access to all the data in the study and all authors had final responsibility for the decision to submit for publication.

Results
Between May 26, and July 17, 2014, 390 patients meeting the case definition were admitted to the two referral hospitals in Muzaffarpur. Among these, 213 (55%) were boys, median age was 4 years (range 6 months–14 years), and 280 (72%) were aged 1–5 years (table 1). Among case-patients with recorded measurements, 11 (16%) of 68 were classified as wasted and 46 (65%) of 71 were classified as stunted. Most patients (273; 70%) were from Muzaffarpur district; cases were reported from all 16 blocks of Muzaffarpur district. Clustering of cases was not observed; each affected child seemed to be an isolated case in a village (approximate population per village 2500). The outbreak peaked in mid-June, with 147 cases reported during June 8–14, 2014, and declined substantially after June 21, 2014 (figure).

Caregivers reported that affected children were previously well and 366 (94%) had sudden onset of
symptoms less than 24 h before admission. Further, 224 (66%) of 342 patients with recorded data reported illness onset between 0300 h and 0800 h. Of patients with recorded data, 326 (94%) of 348 reported one or more seizures and 345 (95%) of 362 reported altered mental status before admission; 301 (87%) of 347 patients were unconscious on presentation. Seizure semiology was characterised by intermittent generalised tonic or tonic-clonic seizures; duration and frequency of the seizures varied (appendix p 10). Several patients had convulsive or non-convulsive status epilepticus. Vomiting was reported in 59 (18%) of 337 patients with recorded data. Of 357 patients with recorded admission measurements, the median temperature was 37.2°C (99°F; range 35.6–40.6), and 219 (61%) were afebrile (≤37.5°C ≤99.5°F). Among 386 patients with recorded data, 122 died (case fatality rate 32%).

On detailed clinical assessment of 52 patients, 48 (92%) showed no focal neurological deficits. Brain MRI of 16 patients showed no focal lesions, signal abnormalities, or changes suggestive of inflammation; eight patients (50%) showed mild to moderate cerebral oedema. Clinical severity did not noticeably differ between participants with and without cerebral oedema. EEG in 30 cases showed findings consistent with generalised encephalopathy in 22 (73%); seven showed epileptiform discharges. Of 62 patients with CSF collected for analysis, 52 (84%) had normal WBC counts (<0.5 x 10⁶ cells per L), 58 (94%) had normal protein (<450 mg/L), and 49 (79%) had normal glucose (<2.50 mmol/L) concentrations. Of 327 patients with blood glucose measurement on admission, the median blood glucose level was 2.66 mmol/L (range 0.44–23.98), and 100 (31%) patients had glucose concentration of 1.67 mmol/L or less, 171 (52%) patients had glucose concentration of 2.78 mmol/L or less, and 204 (62%) patients had glucose concentrations of 3.89 mmol/L or less. Of 349 patients with available information, 239 (69%) had a record of receiving dextrose therapy during hospital stay; of these, 173 (73%) survived.

Of 331 patients with recorded data, 149 (45%) were referred from another health-care facility, such as a primary health centre or private clinic; the remainder presented directly to the referral hospitals. In a multivariable model controlling for hypoglycaemia, presence of fever on admission, and receipt of dextrose therapy during hospital stay, patients referred to the hospital from another health facility were twice as likely to die as those who came directly to the referral hospital (OR 2.3 [95% CI 1.2–4.1]).

Between June 1, and July 10, 2014, 104 cases and 104 controls were enrolled. Exposures that were significantly associated with illness on matched bivariate analysis included smoking (matched odds ratio [mOR] 9.6 [95% CI 3.8–24.1]), visiting a fruit orchard (6.0 [2.7–13.4]), and absence of an evening meal (defined as eating the last

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**Table 2:** Exposures associated with illness in matched bivariate analysis of case control study in Muzaffarpur, June–July, 2014

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Cases (N=104)</th>
<th>Controls (N=104)</th>
<th>mOR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ate litchi*</td>
<td>67/103 (65%)</td>
<td>23/102 (23%)</td>
<td>9.6 (3.8–24.1)</td>
</tr>
<tr>
<td>Visited fruit orchard*</td>
<td>52/100 (52%)</td>
<td>18/98 (32%)</td>
<td>6.0 (2.7–13.4)</td>
</tr>
<tr>
<td>Parent visited fruit orchard*</td>
<td>29/95 (31%)</td>
<td>16/99 (16%)</td>
<td>2.3 (1.1–4.8)</td>
</tr>
<tr>
<td>Absence of evening meal*</td>
<td>76/98 (78%)</td>
<td>51/88 (58%)</td>
<td>2.2 (1.2–4.3)</td>
</tr>
<tr>
<td>Socioeconomic index below poverty line</td>
<td>57/104 (55%)</td>
<td>49/104 (47%)</td>
<td>1.4 (0.8–2.4)</td>
</tr>
<tr>
<td>Routinely wash vegetables and fruits</td>
<td>32/99 (32%)</td>
<td>58/83 (70%)</td>
<td>0.13 (0.05–0.4)</td>
</tr>
</tbody>
</table>

mOR=matched odds ratio. *In 24 h before symptom onset.

**Figure:** Acute neurological illness by date of hospital admission in Muzaffarpur, May 29–July 17, 2014

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no pesticide residue was detected in 14 litchi samples. Additionally, butyrylcholinesterase activity levels was detected in the US CDC. No abnormality in acetylcholinesterase or was identified in 80 case-patient specimens examined at US CDC. No pattern of excessive pesticide or metal exposures was observed in glutaric acidemia type II, an inherited metabolic disorder with a panethnic prevalence that is less than 1:100,000. However, the temporal and spatial concentration of case-patient observed in this outbreak is inconsistent with this specific genetic cause. Furthermore, similar abnormal urinary organic acid profiles (increased ethylmalonic acid, glutaric acid, and adipic acid) have been reported in ackee fruit encephalopathy outbreaks, and this is the first confirmation that this recurring outbreak in Muzaffarpur is associated with litchi consumption and both hypoglycin A and MCPG toxicity. This conclusion is supported by clinical findings consistent with a acute toxic encephalopathy, significant epidemiological association between litchi consumption and illness, laboratory results that show, for the first time to our knowledge, the presence of hypoglycin A and MCPG metabolites, and evidence of resultant metabolic derangement in the biological specimens of cases but not controls, and the confirmation of these toxins in litchi fruits. The absence of clinical, epidemiological, or laboratory findings to support infectious pathogen, pesticide, and heavy metal related causes of illness suggest the observed protective association of routinely washing fruit or vegetables was not directly related to a toxin or infectious agent.

Findings of organic acid and acylcarnitine analysis showed evidence of disruption of several dehydrogenase enzymes involved in fatty acid oxidation, similar to profiles observed in glutaric acidemia type II, an inherited metabolic disorder with a panethic prevalence that is less than 1:100,000. However, the temporal and spatial concentration of case-patient observed in this outbreak is inconsistent with this specific genetic cause. Furthermore, similar abnormal urinary organic acid profiles (increased ethylmalonic acid, glutaric acid, and adipic acid) have been reported in ackee fruit encephalopathy outbreaks, further supporting that the changes observed in patients in Muzaffarpur are a result of disrupted fatty acid metabolism due to hypoglycin A and MCPG toxicity. The acidity resulting from accumulation of certain fatty acids might

### Table 3: Analysis of acylcarnitine, organic urinary acids, and metabolites of hypoglycin A and MCPG in cases and controls in Muzaffarpur, 2013–14

<table>
<thead>
<tr>
<th></th>
<th>Cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abnormal acylcarnitine profile</td>
<td>72/80 (90%)</td>
<td>NA</td>
</tr>
<tr>
<td>Abnormal urine organic acid profile</td>
<td>67/75 (89%)</td>
<td>0/15 (0%)</td>
</tr>
<tr>
<td>Urinary metabolite for hypoglycin A</td>
<td>47/73 (64%)</td>
<td>0/15 (0%)</td>
</tr>
<tr>
<td>Urinary metabolite for MCPG</td>
<td>33/73 (45%)</td>
<td>0/15 (0%)</td>
</tr>
</tbody>
</table>

Data are n/N (%). NA=not available. MCPG=methylenecyclopropylglycine.

### Table 4: Analysis of hypoglycin A and MCPG in litchi fruit arils in Muzaffarpur, 2014

<table>
<thead>
<tr>
<th>MCPG (μg/g dry weight)</th>
<th>Hypoglycin A (μg/g dry weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Ripe</td>
<td>66·4</td>
</tr>
<tr>
<td>Unripe</td>
<td>220·0</td>
</tr>
<tr>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Ripe</td>
<td>68·0</td>
</tr>
<tr>
<td>Unripe</td>
<td>112·0</td>
</tr>
<tr>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Ripe</td>
<td>44·9</td>
</tr>
<tr>
<td>Unripe</td>
<td>82·1</td>
</tr>
</tbody>
</table>

MCPG=methylenecyclopropylglycine. Both the ripe and unripe groups contained 6 homogenates.

Discussion

Although an association with MCPG has been previously proposed, and MCPG has been detected in the seed and aril of the litchi, this is the first confirmation that this recurring outbreak in Muzaffarpur is associated with litchi consumption and both hypoglycin A and MCPG toxicity. This conclusion is supported by clinical findings consistent with a acute toxic encephalopathy, significant epidemiological association between litchi consumption and illness, laboratory results that show, for the first time to our knowledge, the presence of hypoglycin A and MCPG metabolites, and evidence of resultant metabolic derangement in the biological specimens of cases but not controls, and the confirmation of these toxins in litchi fruits. The absence of clinical, epidemiological, or laboratory findings to support infectious pathogen, pesticide, and heavy metal related causes of illness suggest the observed protective association of routinely washing fruit or vegetables was not directly related to a toxin or infectious agent.

Findings of organic acid and acylcarnitine analysis showed evidence of disruption of several dehydrogenase enzymes involved in fatty acid oxidation, similar to profiles observed in glutaric acidemia type II, an inherited metabolic disorder with a panethic prevalence that is less than 1:100,000. However, the temporal and spatial concentration of case-patients observed in this outbreak is inconsistent with this specific genetic cause. Furthermore, similar abnormal urinary organic acid profiles (increased ethylmalonic acid, glutaric acid, and adipic acid) have been reported in ackee fruit encephalopathy outbreaks, further supporting that the changes observed in patients in Muzaffarpur are a result of disrupted fatty acid metabolism due to hypoglycin A and MCPG toxicity. The acidity resulting from accumulation of certain fatty acids might
have further contributed to clinical encephalopathy, which could explain symptoms and signs observed even among patients without documented hypoglycaemia, an event that has also been reported in ackee fruit encephalopathy.46 Limitations in the ability to provide aggressive critical care, including closer respiratory monitoring and mechanical ventilation, probably contributed to mortality among affected children, despite the administration of dextrose supplementation. Our findings support the need to strengthen clinical intensive care capacity at the treating hospitals in Muzaffarpur.

Our analyses indicate that the absence of an evening meal modified the association between litchi consumption and illness. Parents in affected villages report that during May and June, young children frequently spend their day eating litchis in the surrounding orchards; many return home in the evening uninterested in eating a meal. Skipping an evening meal is likely to result in night-time hypoglycaemia, particularly in young children who have limited hepatic glycogen reserves, which would normally trigger β-oxidation of fatty acids for energy production and gluconeogenesis.39,40 However, in the setting of hypoglycaemia, particularly in young children who have been reported from litchi-growing areas of Bangladesh and Vietnam.44,45 These outbreaks have not been similarly comprehensively investigated. The investigation in Bangladesh focused on the possibility that pesticides used seasonally in litchi orchards might be involved, but no specific pesticide was implicated. The investigation in Vietnam focused on possible infectious agents that might be present seasonally near litchi fruit plantations, but found none to explain the outbreak. Our investigations also thoroughly explored the possibilities of pesticide and heavy metal related toxicity but found no clinical, epidemiological, or laboratory evidence to support this. Detailed assessments of infectious causes, including for viral pathogens known to cause encephalitis in the region as well as for potential novel infectious agents, were also consistently negative. The findings of our investigations might help to shed light on the cause of illness in the Bangladesh and Vietnam outbreaks.

At a broader level, the Muzaffarpur outbreak is illustrative of unexplained public health threats in resource-constrained settings, whether localised or regional, that are frequently under-investigated. The application of a comprehensive multisectoral investigation in Muzaffarpur, with the combined inputs of clinicians, epidemiologists, laboratory scientists, environmental specialists, and medical toxicologists enabled the methodical exclusion of infectious pathogens, the consideration of potential environmental causes that had not previously been systematically assessed, and the comprehensive testing of both environmental and human specimens to investigate and confirm a postulated association between litchi fruits, hypoglycin A/MCPG, and illness that led to timely public health recommendations to prevent illness and reduce mortality.* Using similar systematic investigation methods, both in other countries affected by similar outbreaks as well as in other settings of unexplained illness has major potential to contribute toward improving public health response.

Quantitative evaluation of a small number of litchi arils (edible fruit) collected in Muzaffarpur indicated approximately twice the level of detected hypoglycin A, as well as MCPG in unripe versus ripe fruits. This finding is in contrast with what is seen in ackee fruit, where the concentration of hypoglycin A in unripe fruits is more
than 20 times higher than that observed in ripe fruits.4,14 A larger quantitative evaluation of hypoglycin A and MCPG concentrations in different cultivars as well as several stages of maturation is needed to better evaluate this question. If substantial differences in the concentrations of these compounds are consistently detected in different stages of litchis, public health prevention recommendations regarding litchi fruit consumption can be further refined.

This study was subject to two major limitations. First, determination of whether litchi fruit had been consumed before symptom onset relied upon reported information from the parent or caregiver of the child, who might not have been with the child during consumption. However, both cases and controls were ill in hospital and queried about exposures before admission to hospital; we, therefore, expect that both groups would have been equally likely to report exposures such as food consumption, thereby minimising the potential for differential misclassification. Additionally, the absence of difference in socioeconomic status, as well as the overall homogeneity observed throughout the 16 blocks of Muzaffarpur district, suggests that both groups would be presented with equal opportunity for exposure to variables of interest. Second, although our protocol required control interview and specimen collection within a 7 day window of the matched case, this was not always possible and some delays occurred.

In conclusion, to the best of our knowledge, this is the first comprehensive confirmation that this recurring outbreak of acute encephalopathy is associated with both hypoglycin A and MCPG toxicity from litchi consumption. This illness is also associated with absence of an evening meal. To prevent illness and save lives in Muzaffarpur, we recommended minimizing litchi consumption among young children, ensuring children in the area receive an evening meal throughout the outbreak season, and implementing rapid glucose correction for children with suspected illness. Application of a similar comprehensive and systematic approach to the assessment of both infectious and non-infectious causes of unexplained illness outbreaks in other parts of the world can contribute greatly toward identifying interventions that can reduce morbidity and mortality.

References


