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-neurologic 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 – 24·1]) and absence of an evening meal (2·2 [1·2 – 4·3] 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.

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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. Described cases or outbreaks of ackee fruit poisoning which implicated hypoglycin toxicity; an additional 11 studies described the pathophysiology of hypoglycin A in animal models, and five described methods to characterise hypoglycin A in ackee fruits. An ecological study from 2012 from Vietnam indicated an association between litchi plantation surface area and acute encephalitis incidence. A study from 1962 described the isolation of MCPG in litchi seeds, and two studies from 1989 and 1991 described the hypoglycaemic effect of MCPG in animal studies. No studies implicated a direct epidemiological association between litchi consumption in affected individuals and encephalopathy. No studies showed hypoglycin or MCPG or their metabolites in affected individuals.

Research in context

Evidence before this study

We searched PubMed between Jan 30, and April 30, 2013, before our 2013 field investigation, for any studies related to the acute unexplained neurological illness in Muzaffarpur using the search terms “Muzaffarpur,” AND (“encephalitis” OR “encephalopathy” OR “seizure.”) This identified two articles that suggested potential causes for the outbreak illness varying from Japanese Encephalitis virus, another unknown virus, to heat stroke. Following the results of our 2013 investigation, which suggested hypoglycaemia might be an important factor in illness, and raised the possibility of a toxic origin, we repeated a PubMed search in December, 2013, using terms (“ackee hypoglycin” OR “ackee encephalopathy” OR “glycine analog AND litchi” OR “litchi encephalopathy” OR “litchi methylenecyclopropylglycine” (MCPG), OR “Japanese vomiting sickness”) for studies describing an association between litchis, ackee fruit, hypoglycin, MCPG and acute neurologic illness published between Jan 1, 1954, and Dec 1, 2013. We found 61 studies; 11 described cases or outbreaks of ackee fruit poisoning which implicated hypoglycin toxicity; an additional 11 studies described the pathophysiology of hypoglycin A in animal models, and five described methods to characterise hypoglycin A in ackee fruits. An ecological study from 2012 from Vietnam indicated an association between litchi plantation surface area and acute encephalitis incidence. A study from 1962 described the isolation of MCPG in litchi seeds, and two studies from 1989 and 1991 described the hypoglycaemic effect of MCPG in animal studies. No studies implicated a direct epidemiological association between litchi consumption in affected individuals and encephalopathy. No studies showed hypoglycin or MCPG or their metabolites in affected individuals.

Added value of this study

This study, to the best of our knowledge, is the largest investigation of the Muzaffarpur outbreak and the first comprehensive confirmation that this recurring outbreak illness is associated with litchi consumption and toxicity from both hypoglycin A and MCPG. We confirm the presence of MCPG and hypoglycin in litchis, and, for the first time, our data show the metabolites of these toxins in human biological specimens, the biological impact of these toxins on human metabolism, and the modifying effect of the lack of an evening meal on the impact of these toxins.

Implications of all the available evidence

Based on the results of our investigation, public health and clinical recommendations targeted at preventing illness and reducing morbidity and mortality from the Muzaffarpur outbreak illness were provided to state and national health authorities. This included recommendations to minimise litchi consumption among young children in the affected area, to ensure that children receive an evening meal throughout the outbreak period, and to rapidly assess and correct hypoglycaemia in any child suspected of having the outbreak illness. Evaluation of other potential factors, including missed evening meal, poor nutritional status, and as yet unidentified genetic differences, may provide further insights into additional risk factors for this outbreak illness. Application of a similar comprehensive and systematic approach to the evaluation of both infectious and non-infectious aetiologies of unexplained illness outbreaks in other parts of the world has the potential to contribute toward identifying interventions that can reduce morbidity and mortality.

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 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 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 β-oxidation of fatty acids and gluconeogenesis. 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 aetiological 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 and have continuous enrolment throughout the outbreak period. For each case, we initially enrolled one community control and one hospital control within 7 days of case enrolment. In view of the overall homogeneity of rural Muzaffarpur and the ubiquitous nature of the variables of interest, including litchi orchards and litchis, we were concerned about the possibility of overmatching. To prevent this, both community (adjacent village) and hospital controls (any other village) were selected from villages other than the case-patients; the community controls were subsequently assessed to still be overmatched and were thus dropped from the analysis. A hospital control was defined as a resident of Muzaffarpur district who was admitted to one of the surveillance hospitals for a non-neurological illness within 7 days of the date of admission of the case. Children who had a history of altered mental status or seizures in the previous 3 months were excluded as controls. For case-patients younger than 5 years, hospital controls were age-matched to within 6 months of age; for case-patients who were 5 years or older, controls were age-matched to within 12 months of age. Informed consent was obtained from parents or guardians.

Cases and controls were asked about consumption of food items, food washing, water sources, and other exposures, including time spent in agricultural fields. Standardised data for household characteristics, ownership of household assets or goods, and land were collected to calculate a socioeconomic index (SEI) according to the methods of the National Family Health Survey, a large-scale, multiround survey undertaken throughout India by the Ministry of Health and Family Welfare. Data for both case and controls were systematically collected using standardised questionnaires. Bodyweight (kg) and body height or length (cm) were measured for each enrolled case and control. A child was defined as wasted if the Z score was more than 2 SD below WHO Child Growth Standards of calculated body-mass index (BMI; children ≥5 years of age) or weight for height (children <5 years of age), and stunted if the Z score was more than 2 SD below the same standards of calculated BMI (children ≥5 years of age) or height for age (children <5 years of age). Additionally, urine and blood 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 entroviruses.
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; appendix p 4). Blood and urine 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 qualitative and quantitative 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°]). 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 (<2·5 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-care 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 age-matched hospital controls were enrolled. Exposures that were significantly associated with illness on matched bivariate analysis included consumption of litchi (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 meal before 1900 h; mOR 2·2 [95% CI 1·2–4·3]) in the 24 h preceding illness onset (table 2), and were similar to what was noted in unmatched bivariate analyses controlled for age (appendix p 11). Calculated socioeconomic index did not differ between cases and controls (mOR 1·4 [95% CI 0·8–2·4]); routinely washing vegetables and fruits (mOR 0·1 [0·05–0·4]) could be protective. Among those who consumed litchis, cases were more likely to eat unripe litchis (mOR 7·9 [95% CI 1·1–47·0], eat rotten litchis (7·4 [1·5–69·8], report eating litchis from the ground versus from the tree (22 cases vs no controls), and report eating partially eaten litchis (17 cases vs no controls).

Other factors, including biting, eating, or chewing the litchi seed and peeling or eating the litchi peel were not associated with illness (data not shown). Similarly, no association was noted between illness and consumption of raw vegetables or medications, drinking water source, or exposure to insecticides or chemicals sprayed in and around the house or nearby fields or orchards (data not shown). For children younger than 5 years, mean Z scores for height for age (z̄ = 2·85 [cases] vs –2·18 [controls], p=0·12) and weight for height (z̄ = 0·00 [cases] vs –1·00 [controls], p=0·08) did not significantly differ between cases and controls. Among children older than
Among 73 case-patient urine specimens assessed, no pesticide residue was detected in 14 litchi samples and 32 (44%) specimens contained both MCPA-Gly, 33 (45%) contained metabolites of MCPG, 47 (64%) contained metabolites of hypoglycin A as assessed at NIOH. Additionally, no abnormal acetylcholinesterase or plasma acylcarnitine profiles nor tested positive for reportable concentrations of either the hypoglycin A or MCPG metabolite. Of 36 litchi arils analysed from Muzaffarpur, observed concentrations ranged from 12.4 μg/g to 152.0 μg/g MCPG and 44.9 μg/g to 220.0 μg/g hypoglycin A and 44.9 μg/g to 220.0 μg/g MCPG.

5 years, the mean Z score for calculated BMI did not differ significantly between cases and controls (−0.81 [cases] vs −1.90 [controls], p=0.08).

On stratified analysis controlled for age, the absence of an evening meal in the previous 24 h significantly modified the relation between litchi consumption and illness (OR 7.8 [95% CI 3.3–18.8], without evening meal; OR 3.6 [95% CI 1.1–11.1] with evening meal).

At NCDC, laboratory diagnostic testing of 17 CSF specimens for Japanese encephalitis virus and West Nile virus by PCR, and an additional 12 CSF specimens with an 11-virus multiplex PCR platform assay were negative. Pan-viral family or genus PCRs and sequencing of 40 CSF and 40 serum samples at US CDC showed one CSF sample and one serum sample (from two different patients) were positive for Adenovirus 41. A separate CSF specimen tested positive for a divergent rhabdovirus. All other samples were negative for all assays tested.

No pattern of excessive pesticide or metal exposures was identified in 80 case-patient specimens examined at US CDC. No abnormality in acetylcholinesterase or butyrylcholinesterase activity levels was detected in the specimens of 27 patients examined at NIOH. Additionally, no pesticide residue was detected in 14 litchi samples assessed at NIOH.

Among 73 case-patient urine specimens assessed, 47 (64%) contained metabolites of hypoglycin A (MCPA-Gly), 33 (45%) contained metabolites of MCPG (MCPF-Gly), and 32 (44%) specimens contained both MCPG and hypoglycin A than did the ripe fruit.

### 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, 2013–14

<table>
<thead>
<tr>
<th></th>
<th>MCPG (μg/g dry weight)</th>
<th>Hypoglycin A (μg/g dry weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ripe 66.4</td>
<td>Unripe 220.0</td>
</tr>
<tr>
<td></td>
<td>Ripe 68.0</td>
<td>Unripe 112.0</td>
</tr>
<tr>
<td></td>
<td>Ripe 44.9</td>
<td>Unripe 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,²³,³⁴ and MCPG has been detected in the seed and aril of the litchi,¹⁰,³⁷ 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 an 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 academia type II, an inherited metabolic disorder with a panethnic prevalence that is less than 1:100 000.³⁶ 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,³⁷ 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 acidosis resulting from accumulation of certain fatty acids might...
have further contributed to clinical encephalopathy, which could explain symptoms and signs observed among patients without documented hypoglycaemia, an event that has also been reported in ackee fruit encephalopathy.39 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 hypoglycin A/MCPG toxicity, fatty acid metabolism is disrupted and glucose synthesis is severely impaired,11,12,41 which can lead to the characteristic acute hypoglycaemia and encephalopathy of the outbreak illness. The association between illness and the absence of an evening meal could explain the early morning onset of symptoms noted in most patients, and supports recommendations to ensure that children receive a night-time meal throughout the outbreak period. The important interaction between litchi consumption and the absence of an evening meal also contributes toward an understanding of why only some children in Muzaffarpur developed this acute encephalopathy. Although litchi fruits are ubiquitous in the orchards surrounding the villages in rural Muzaffarpur, typically only one child in an entire village develops this acute illness. The synergistic combination of litchi consumption, a missed evening meal, and other potential factors such as poor nutritional status, eating a greater number of litchis, and as yet unidentified genetic differences might be needed to produce this illness.

Although our findings show an association between hypoglycin A/MCPG toxicity, litchi consumption, and this outbreak illness, causality is considerably more difficult to establish. Assessment of our results using the Bradford Hill criteria for causation42 showed that seven of nine criteria are met: 1) strength of association (large ORs for consumption of litchi, modified by presence or absence of evening meal); 2) consistency (clinical findings shown in both 2013 and 2014, and MCPG detected in litchi fruit previously); 3) specificity (specific population, primarily young children, at a specific location, Muzaffarpur, affected, and no clear evidence for any other cause; 4) temporality (illness follows the litchi harvest season); 5) plausibility (biological mechanism for MCPG/hypoglycin A toxicity leading to the observed metabolic derangements and clinical manifestations); 6) coherence between the laboratory and epidemiological findings; and 7) analogy (similar reports and findings in outbreaks of toxic encephalopathy due to, a fruit in the same botanical family as litchi). Based on these observations, we conclude that our findings reflect a plausible, but not necessarily sufficient, causal pathway between litchi consumption and illness.

Within India, an outbreak of a similar acute neurological illness with hypoglycaemia and seizures was reported in June, 2014, among young children in Malda, a litchi cultivation district in West Bengal.41 In southeast Asia, outbreaks of similar acute neurological illnesses have also 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. 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 and 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.

Contributors
AS, PS, KL, and JJS conceived the study design. AK, JT, JS, AC, LSC, and KE contributed to the study design. AS and PS led the field investigation. KL, JJS, AK, GB, MD, RY, AV, MP, PS, DS, AP, KG, RP, MK, SK, RS, and RSS facilitated and did the field epidemiological investigation. JJS did the nutritional assessments. JD, MDC, SLJ, RCJ, JLP developed, did, and interpreted assays to evaluate and quantify the presence of hypoglycin A and MCPG metabolites as well as pesticide metabolites in human biological specimens, and assays to detect hypoglycin A in fruit samples. JDS and PLHI did and interpreted assays to evaluate the profiles of urinary organic acids and acylcarnitines in biological specimens. MC, VM, SK did and interpreted testing for potential viral causes of illness. AC, SP, and RR did and interpreted testing for pesticide metabolites in environmental specimens, and acetyl cholinesterase levels in human biological specimens. LAG, TPF, DJ, LV, KLC, and [MJ did and interpreted testing for toxic elements and pesticide metabolites. LAH and GRT did and interpreted testing for hypoglycin A and MCPG in litchi fruit samples. ST, KQ, CP, and AW did and interpreted tests for novel infectious pathogens. PS, AS, KL, AK analysed the clinical and epidemiological data. JJS, JGSG, AC, and SV supported analysis and interpretation. JJS and DH did the nutritional analyses. PS wrote the first draft of the report. PS, AS, KL, JJS, JD, JLP, MDC, SLJ, ST, GRT, LAH, and SV wrote the report. All authors discussed the results and contributed to revision of the final manuscript.

Declaration of interests
We declare no competing interests.

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