

# Study of Acute Encephalitis Syndrome (AES) in relation with litchi fruit in Muzaffarpur, Bihar (India)

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**ABSTRACT:** An Outbreaks of Acute Encephalitis Syndrome (AES) occurred in Muzaffarpur district in the litchi Cultivation season. In Bihar, outbreaks of an acute neurological illness with high mortality among children occur annually in Muzaffarpur. Cases were investigated on children aged 15 years or younger who were admitted to two hospitals in Muzaffarpur. Age-matched control 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 methylene cyclo propyl glycine (MCPG), naturally-occurring fruit-based toxins that cause hypoglycemia and metabolic derangement. 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 (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 control; 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 12.4µg/g. To prevent illness and reduce mortality in the region, we recommended minimizing litchi consumption, ensuring receipt of an evening meal and implementing rapid glucose correction for suspected underscoring the importance of using systematic methods in other unexplained illness outbreaks.

**Keywords:** Outbreaks, Infectious, Pesticides, Toxic, Pathogens etc.

## INTRODUCTION

The term Acute Encephalitis Syndrome (AES) was coined by the world Health Organisation (WHO) in 2008. The Symptoms of AES include an acute onset of fever and associated clinical neurological manifestation such as mental confusion disorientation, delirium, convulsion or coma. Early symptoms include headaches and vomiting, along with sudden hypoglycemia (drop in blood sugar levels). 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 county. 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 characterized by acute seizures and changed mental.

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 (<70mg/dL) on admission, which was also associated with increased mortality. These finding 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 report of toxic hypoglycaemic syndrome in the West Indies<sup>8-10</sup> that was due to the effects of hypogly 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.

### Methods: Hospital-based clinical surveillance

Surveillance was done at the Shri Krishna Medical College Hospital (SKMCH) and the Krishna Devi, Deviprasad Kejriwal Maternity Hospital (KDKMD), 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 KDKMD. 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 generalized 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 hospital in Muzaffarpur were prospectively enrolled.

### Environmental specimen collection

Between May and June, 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  $-20^{\circ}\text{C}$  within 3 h of collection and subsequently transferred to  $-70^{\circ}\text{C}$  until analysis.

### Laboratory testing

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. Blood and urine specimens of case 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 & litchi fruit samples were analysed for pesticide residues using the Quick Easy Cheap Effective Rugged and Safe method. Plasma acylcarnitine and quantitative and qualitative urine organic acid profiles were assessed at the Emory Genetics Laboratory 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  $\beta$ -oxidation.

### 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

	n/N (%)
Men	213/390 (55%)
Age (years)	
<1	8/390(2%)
1-5	280/390 (72%)
6-10	98/390 (25%)
11-15	4/390 (1%)
Mortality	122/386 (32%)
Generalised seizure at presentation	326/348 (94%)
Altered mental status at presentation	345/362 (95%)
Afebrile (<37.5 °C) on admission	219/357 (61%)
Illness onset between 0300-0800 h	224/342 (66%)
Blood glucose <3.89 mmol/L on admission	204/327 (62%)
CSF cytology <0.5×10 <sup>6</sup> WBC/L	52/62 (84%)
CSF protein < 450 mg/L	58/62 (94%)
CSF glucose >2.50 mmol/L	49/62 (79%)
Brain MRI with no focal lesions	16/16 (100%)
EEG consistent with generalized encephalopathy	22/30 (73%)

**Table 1: Characteristics of case patients, Muzaffarpur, May-June, 2014**

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 result 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.

## Results

Between May, and July, 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 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.

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 characterized by intermittent generalized 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 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 generalized encephalopathy in 22 (73%); seven showed epileptiform discharges. Of 62 patients with CSF collected for analysis, 52 (84%) had normal WBC counts (<0.5×10<sup>6</sup> cells per L), 58 (94%) had normal protein (<450 mg/L), and 49 (78%) 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 center 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 cases and 104 age-matched hospital controls were enrolled. Exposures that were significantly associated with illness on matched bivariate analysis included litchi consumption (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 [non-litchi] 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 analysis controlled for age (appendix p 11). Calculated socioeconomic index did not differ between cases and controls (mOR 0.1 [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 [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).

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

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

Other factors, including biting, eating, or chewing the litchi seed and peeling or eating the litchi peel were not associated with illness (data no shown). Similarly, no association was noted between illness and consumption of raw vegetable or medications, drinking water source, or exposure to insecticides or chemicals sprayed in and around the house or nearby fields or orchards (data no shown). For children younger than 5 years, mean Z scores for height for age (-2.85 [cases] vs -2.18 [controls],  $p=0.12$ ) and weight for height (0.00 [cases] vs -1.00 [controls],  $p=0.08$ ) did not significantly differ between cases and controls. Among children older than 5 years, the mean Z score for calculated BMI did not differ significantly between cases and controls (-0.81 [cases] vs -1.90 [controls],  $p=0.08$ ).

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 on 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 27 case-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 metabolites. Creatinine-corrected concentrations of MCPF-Gly and MCPA-Gly were determined for each sample as  $\mu\text{g/g-cr}$ . For MCPF-Gly, the 33 positive samples ranged from 0.289 to  $6.80 \times 10^3 \mu\text{g/g-cr}$ , with a median of  $1.22 \times 10^3 \mu\text{g/g-cr}$ . For MCPA-Gly, the 47 positive samples ranged from 0.0402 to  $1.89 \times 10^3 \times 10^3 \mu\text{g/g-cr}$ , with a median of  $2.63 \times 10^3 \mu\text{g/g-cr}$  (table 3). On assessment, 67 (89%) of 75 specimens showed abnormal urinary organic acid profiles and 72 (90%) of 80 specimens had abnormal plasma acylcarnitine profiles, consistent with severe disruption of fatty acid metabolism. None of the 15 control specimens tested showed abnormal urinary organic acid profiles nor tested positive for reportable concentrations of either the hypoglycin A or MCPG metabolite.

## Discussion

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 panethnic 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 causes. Furthermore, similar abnormal urinary organic acid profiles (increased ethylmalonic acid, glutaric acid, and adipic acid) had 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 acidosis 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. 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  $\beta$  -oxidation of fatty acids for energy production and gluconeogenesis. However, in the setting of hypoglycin A/MCPG toxicity, fatty acid metabolism is disrupted and glucose synthesis is severely impaired, 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 develop this acute encephalopathy. Although litchi fruit 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 causation 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 ackee, 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. In southeast Asia, outbreaks of similar acute neurological illnesses have also been reported from litchi-growing areas of Bangladesh and Vietnam. 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.

At a broader level, the Muzaffarpur outbreak is illustrative of unexplained public health threats in resource constrained settings, whether localized 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 litchi, 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 before 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 minimizing the potential for differential misclassification. Additionally, the absence of deference 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, I 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 outbreak in other parts of the world can contribute greatly toward identifying interventions that can reduce morbidity and mortality.

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