Exploration of Association between Litchi Consumption and Seasonal Acute Encephalopathy Syndrome

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SUMMARY

In this hospital-based surveillance and nested age-matched case-control study, authors performed laboratory investigations to assess potential infectious and non-infectious causes of acute neurological illness in children (age ≤15 y) who were admitted with new-onset seizures or altered sensorium to two hospitals in Muzaffarpur, India. Age-matched controls were residents of same area who were admitted to the same 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 (litchi fruits) 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 hypoglycemia and metabolic derangement. Out of 390 patients meeting the case definition admitted to the two referral hospitals, 122 (31%) died. On admission, 204 (62%) of 327 had blood glucose concentration ≤70 mg/dL. In comparison of 104 cases with 104 age-matched controls, litchi consumption (matched odds ratio [mOR] 9.6; 95% CI 3.6, 24) and absence of an evening meal (mOR 2.2; 95% CI 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 (OR 7.8; 95% CI 3.3, 18.8 without evening meal; and OR 3.6; 95% CI 1.1, 11.1 with an evening meal). Metabolites of hypoglycin A, MCPG, or both were detected in 48 (66%) of 73 urine specimens from cases 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, 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. Authors concluded that outbreak of acute encephalopathy in Muzaffarpur was associated with both hypoglycin A and MCPG toxicity.

COMMENTARIES

Evidence-based Medicine Viewpoint

Relevance: For the past two decades, seasonal outbreaks of an acute encephalopathy syndrome (AES) affecting children, have been reported from a few districts in Bihar, notably Muzaffarrpur [1-3]. The disease is associated with high mortality rate, and has several public health implications. Initial investigations suggested causes such as heat stroke, unidentified viral infection, and toxins present in litchis [1,4]. Investigations into the outbreaks of 2013 and 2014 pointed more firmly towards a hypoglycemic encephalopathy, possibly related to methylenecyclopropylglycine (MCPG) found in litchis [4,5] and previously associated with hypoglycemia in animal experiments. A recent publication [6] attempted to confirm the role of litchi consumption in the seasonal encephalopathy.

Critical appraisal: The study [6] was described as a case-control design, comparing potential exposure factors among children with the acute encephalopathy syndrome (cases) *versus* age-matched children without neurological illness (controls). Besides this component, the authors undertook several additional prospective investigations to identify (or rule out) alternate cause(s) of the syndrome. Thus strictly speaking, this study is a combination of a case-control design and prospective cohort study. The significance of this distinction is highlighted subsequently. ***Table* I** presents a critical appraisal of the case-control component of the study using a criteria derived from an excellent tool designed for the purpose [7].

The observational component focused on five distinct lines of inquiry: (*i*) measurements of hypoglycin A and MCPG metabolites in biological specimens of affected cases, (*ii*) measurement of markers of fatty acid metabolism (since the two toxins act through disruption

TABLE I CRITICAL APPRAISAL OF THE CASE-CONTROL COMPONENT OF THE STUDY

Criteria	*Appraisal*
Did the study address a clearly focused issue?	Yes. The authors outlined their objectives to identify risk factors associated with the seasonal acute encephalopathy syndrome, and also examine whether toxins (specifically hypoglycin and MCPG) could be involved. They also intended to confirm that the syndrome was not infectious in origin.
Did the authors use an appropriate to answer their question?	Yes. The case-control design is an acceptable method to work backwards and identify method associations between exposure to potential risk factors, and the disease condition, especially because prospective observational studies (although methodologically superior) would be time-consuming, prohibitively expensive and logistically challenging.
Were the cases recruited in an acceptable way?	Case was defined clinically as a child (<15 y) with new seizure or sensorial alteration originating within 7 days of presentation to either of the referral two hospitals in the region. In that sense, cases were recruited through passive (rather than active) surveillance. However, only those cases who survived beyond six hours of presentation were enrolled in this study. Thus more severe cases, and those with early fatal outcome were automatically excluded. Further, not all cases were included; for logistic reasons, only every fourth case was included in the study. Thus 104 cases were recruited from a total of 390 affected children. Sample size calculation was done *a priori* to ensure adequate power.
Were the controls recruited in acceptable way?	Yes. Controls were (age-matched) children, admitted to the same referral hospitals (within 7 an days of a case) but without acute neurological illness. Although the authors intended to recruit both community and hospital-based controls for each case, they excluded the former and restricted to hospitalized controls. The authors cited 'over-matching' for excluding community controls; however it is unclear why or how this could be a problem. In a case-control study, it would be ideal if cases and controls were identical in all respects, except the outcome. Hence near-complete matching would be welcome. Further, there is no description of the controls recruited in this study.
Was the exposure accurately measured to minimize bias?	Ascertainment of exposure was done by 'asking' cases and controls about potential risk factors including litchi consumption. Presumably these questions were directed towards attendants/family members of the affected children, raising the possibility of recall bias. Further, since most of the exposure(s) were unwitnessed, some of the responses could at best be speculative or presumptive. Although the methods used were not ideal, alternate approaches would have been difficult.
What confounding factors have authors accounted for?	Although not explicitly stated, the authors attempted to account for obvious confounding the factors such as age, area of residence, nutritional status, etc. Limited statistical adjustments were also performed although multivariate analysis and logistic regression are not described.
What are the results of this study? How precise are the results?	Cases *vs* Controls: Consumption of litchi: matched OR 9.6 (95% CI 3.8, 24.1); Consumption of unripe litchi: matched OR 7.9 (95% CI 1.1, 347.0); Consumption of rotten litchi: matched OR 7.4 (95% CI 1.5, 69.8); Consumption of fallen litchis: (22 cases *vs* no controls); Consumption of partially eaten litchis (17 cases *vs* no controls); Absence of meal after 7 PM: matched OR 2.2 (95% CI 1.2, 4.3); Litchi consumption without evening meal: OR 7.8 (95% CI 3.3, 18.8); Litchi consumption with evening meal: OR 3.6 995% CI 1.1, 11.1); Biting/ chewing/consumption of litchi seed: Data not presented Visiting fruit orchard(s): matched OR 6.0 (95% CI 2.7, 13.4); Parental visit to fruit orchard: matched OR 2.3 (95% CI 1.1, 4.8) Washing fruits / vegetables before consumption : matched OR 0.13 (95% CI 0.05, 0.40); Below poverty line (BPL) status: matched OR 1.4 (95% CI 0.8, 2.4); Data pertaining to consumption of raw vegetables, source of drinking water, exposure to chemicals/insecticides/ pesticides, etc are not presented.
Do you believe the results?	The results are impressive and difficult to ignore. Some issues that are not taken into account are presented below. Appraisal of potentially causal relationship using the Bradford Hill criteria is presented in ***Table* II**.
Can the results be applied to the local population?	Yes.
Do the results of this study fit with other available evidence?	See text for detailed analysis of this point.

TABLE II BRADFORD HILL CRITERIA [8] FOR ASSESSMENT OF CAUSALITY

Criteria	*Assessment*
Strength of association	A very strong association was demonstrated between direct and indirect markers of litchi consumption, and the acute encephalopathy syndrome.
Temporalitya	On the one hand, temporality is clearly demonstrated as the majority of cases presented within 12-18 hours of litchi consumption. On the other hand, litchi consumption by children is unlikely to be a one-time event; hence presuming that the affected children had similar behaviour patterns on other days also, it is unclear why/how they were not affected. In any case, the encephalopathy outbreak coincided with the litchi harvesting season.
Consistency	There is data from other settings (notably Vietnam and Bangladesh) that litchi consumption could be associated with similar acute encephalopathy syndromes [9,10]. Further, another fruit from the same family as litchi was implicated in metabolic encephalopathy in Haiti, Surinam and French Guyana [11,12]. Limited data from the annual Muzaffarpur outbreaks also implicated litchis [4,5].
Theoretical plausibility.	On the one hand, there is theoretical plausibility as (i) two toxins were demonstrated in urine of a few affected patients, as well as randomly analysed fruit samples; (ii) metabolic markers of fatty acid oxidation pathway were deranged in some of the affected cases vs none of the controls, and (iii) alternate explanations including viral meningo-encephalitis, environmental exposure to other toxins, and heat stroke appear unlikely. However, it is unclear why there is no clustering of cases (as litchi orchards are apparently ubiquitous in Muzaffarpur), how/why young infants were affected, and whether siblings and/or parents of index cases (presumably having similar behaviour patterns) were affected.
Coherence	There appears to be coherence between the results of the case-control component of the study and the multiple lines of inquiry pursued in the prospective component; although it can be argued that there were methodological limitations with respect to the number of cases and/or controls studied.
Specificity in the causes.	There are threats to specificity including: (i) absence of clustering of cases, (ii) lack of involvement of family members, (iii) lack of data on relationship between toxic levels and clinical outcome, (iv) protective effect of washing fruits/vegetables when the toxin acts through ingestion, rather than contact etc.
Dose response relationship.	This component has not been demonstrated.
Experimental evidence.	There is experimental evidence in animals supporting the hypothesis that MCPG can cause a metabolic encephalopathy, but no direct demonstration through litchi consumption. Further, the levels of hypoglycin A and MCPG demonstrated in a few litchi samples in this study have not been correlated with toxicity levels in previous studies [13].
Analogy	Previous reports of metabolic encephalopathy from ackee fruit [11,12] are considered analogous.

of this metabolic pathway), (*iii*) confirmation of the absence of viral etiology through detailed CSF analysis and/or cerebral imaging techniques, (*iv*) demonstration of elevated concentrations of hypoglycin and MCPG in representative litchi samples, and (*v*) exclusion of environmental metabolic encephalopathies by measurement of plasma and RBC cholinesterase activity, as well as analysis of litchi samples for pesticide levels. These efforts and the procedures used within each line of inquiry are indeed laudable. However, three points should be noted: (*i*) not all the affected children were uniformly subjected to all the clinical tests (thereby creating an inadvertent element of selection bias), (*ii*) the control group were either not subjected to the complete extensive clinical workup, or the data are not shown, and (*iii*) the methods for selecting fruit samples for analysis are not described. ***Table* III** presents data from the prospective component of the study.

Critical appraisal of this report [6] raises several issues that could have been addressed in this otherwise excellent study. First, it is safe to assume that children visit litchi orchards (and consume fruit), accompanied by siblings and friends. Therefore, if the exposure occurs as described by the authors, family and community clustering of cases would be evident. It is surprising why this was not observed. Second, it would have been interesting to see the age-stratified data of affected cases, to determine whether toxin levels were distributed similarly across all age groups. Presumably older children have greater capacity for consumption, and this could be reflected in clinical data as well as laboratory measurements of toxin levels. Third, it would be interesting to learn whether adolescent girls were affected

TABLE III ADDITIONAL ANALYSES IN THE PROSPECTIVE COMPONENT OF THE STUDY

Line of inquiry	*Summary of results*
Presence of Hypoglycin A and MCPG metabolites in urine	Cases *vs* controls: Hypoglycin A metabolite: 47/73 *vs* 0/15; MCPG metabolite: 33/73 *vs* 0/15
Markers of fatty acid metabolism	Cases *vs* controls: Abnormal acylcarnitine analysis: 72/80 *vs* not mentioned; Abnormal urinary organic acid analysis: 67/75 *vs* 0/15
Confirmation of the absence of viral or alternate etiology	17 CSF samples from cases were negative for JE and West Nile viruses (by PCR);12 CSF samples were negative for 11 (unspecified) viruses by PCR; Sequencing for viruses in 40 CSF and 40 serum samples were negative in 39 each; CSF was examined in 62 cases, but hypoglycorrhachia was detected in only 79%. Cerebral imaging (MRI) was done in only 16 cases. No specific abnormalities were detected. EEG was done in only 30 cases. No pattern(s) suggesting a specific etiology were observed.
Exclusion of environmental metabolic encephalopathies	Neither pesticide nor metals were detected in 80 case samples. No abnormal cholinesterase activity was detected in 27 cases. No pesticide residue was found in 14 litchi samples
Hypoglycin and MCPG in representative litchi samples	36 litchi arils were tested from Muzaffarpur, although samples were not taken from homes of cases and controls. Hypoglycin A levels varied from 12.4-152.0 µg/g. MCPG level varied from 44.9-220.0 µg/g. Data comparing unripe *vs* ripe fruit are presented for only 3 arils and showed that the former had higher levels of both compounds.

similarly, as younger girls and/or age-matched boys, as it is unlikely that adolescent girls would visit orchards alone. Fourth, assuming that adults also consume litchi in excess during the harvesting season, and there was a strong association with parental visits to orchards, it is important to learn whether any adults (or household contacts) in the family of affected children, were similarly afflicted. Further, the exposure is likely to occur for several days/weeks rather than a single day. In such a scenario, what tips the balance towards a potentially fatal disease on a given day, but not on other days? Last, but not the least, the disease could have occurred by consumption of litchis at home also; hence laboratory testing of samples from homes of cases and controls would have added value.

The authors emphasized that the absence of an evening meal was associated with disease. They attributed this to a relative fasting state where hypoglycemia induced fatty acid oxidation could not occur due to the toxins. However, the reasons for skipping the evening meal have to be understood. If children are 'too full' with litchis as the authors propose, it is only an indirect indicator of a large(r) quantity of litchis consumed (hence greater amount of toxin in the system). If it is because of hypoglycemia caused by the toxins, cases should have occurred as frequently during the daytime also as children could consume litchis during the morning hours and skip lunch. This conundrum could have been resolved by comparing the blood glucose level, toxin levels and clinical outcomes in those who did and those who did not have the evening meal.

It is surprising that washing of fruits/vegetables in households was protective, as the toxin is present within the fruit (especially within seeds) and not the surface. Further, the hypoglycemia hypothesis necessitates consumption of the toxin, rather than contact; hence it is unclear how washing could help. On the other hand, each year, the onset of rains is associated with abatement of the epidemic, suggesting that either consumption declines dramatically, or the fruit (surface) is (naturally) washed.

This study had an excellent opportunity to compare survivors among the recruited children with those who died. This would help to determine risk factors for adverse outcome, a possible dose-response relationship with the toxin levels and/or markers of abnormal fatty acid oxidation, and/or other clinical or biochemical markers. Unfortunately, no data on fatal cases have been presented.

Finally, it should be emphasized that the association between litchi consumption and the acute encephalopathy in this study is not derived solely from the case-control component, but also the multiple additional lines of investigation. In fact, the Bradford-Hill criteria are not entirely fulfilled from the data presented.

Further, the contribution of previous studies in excluding alternate cause(s) for the clinical syndrome [1,4,5,14,15], has to be emphasised. It is commendable that the authors themselves described their impressive findings as an association, rather than a causal relationship.

Extendibility: The study was conducted in the epidemiological source of the clinical condition; hence the data are easily applicable. It would be interesting to observe whether similar observations are made in other litchi growing areas of the country.

Conclusion: This well-designed study strongly suggests that the seasonal acute encephalopathy syndrome occurring in Muzaffarpur India, is associated with toxins present in litchi fruits.

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Pediatric Infectious Disease Specialist's Viewpoint

This paper is an important contribution, re-confirming the discovery of a new disease in India by a team of Indian investigators [1-3]. Additionally, it provides information on a few missing details of previous reports. Re-confirmation by independent investigators is how science progresses. In August 2014, then President of Indian Academy of Pediatrics had highlighted the failure of prestigious agencies such as National Centre for Disease Control (NCDC, New Delhi) and US Centers for Disease Control and Prevention (CDC), to diagnose the disease that occurred seasonally every year and took a heavy toll of lives of young children, in Muzaffarpur [4]. He was skeptical of the veracity of the diagnosis of the disease as hypoglycemic encephalopathy and its cause as Litchi fruit consumption by malnourished children who missed the evening meal [1-3]. Therefore, independent re-confirmation of the nature of the disease and its cause is welcome news [1-3].

Muzaffarpur district in Bihar, famous for Litchi orchards, has had annually recurring acute brain disease of children with high mortality, believed by most pediatricians, health ministry officials and NCDC/CDC scientists as viral encephalitis. As no virus was found in spite of repeated search, the disease was called 'acute encephalitis syndrome' (AES) by professionals and 'mystery disease' by the public [1,4]. Disappointed by lack of help from NCDC/CDC for saving the lives of children, a pediatric infectious disease specialist was called in by Bihar health ministry officials [1]. During the 2013 outbreak season, he led a team (hereafter referred to

as the Bihar team) that identified the disease as 'hypoglycemic encephalopathy', a form of metabolic coma that is non-infectious [1-3]. This was received by other investigators and health ministry officials as total surprise, some with scepticism [4]. Until then, clinical diagnosis according to International Classification of Diseases had not been attempted in Muzaffarpur, the thrust of NCDC/CDC being sophisticated laboratory tests for possible viral and pesticide causes of AES [5]. Searching for the cause before making clinical diagnosis is like tying the bullock to the back of the cart; the cart will not move forwards [5].

Sporadic cases of metabolic coma are due to inborn errors of metabolism, such as acyl-CoA dehydrogenase deficiency, blocking gluconeogenesis (fatty acid β-oxidation), which itself is triggered by prolonged fasting [6]. The close similarity, including early morning onset of encephalopathy, was obvious to the trained eye. The Bihar team had to explain seasonal increase and temporal/spatial restriction of cases to Litchi harvests. Ackee fruit in Jamaica is a known extrinsic causative factor of blocked fatty acid β-oxidation and hypoglycemic encephalopathy [7]. The Bihar team found that Ackee and Litchi belonged to one plant family and that Litchi seeds had been reported to contain methylene cyclopropyl glycine (MCPG), an analogue of the Ackee Hypoglycin A [1]. They once again confirmed the disease to be hypoglycemic encephalopathy prospectively, and demonstrated the presence of MCPG in the edible fruit pulp of Litchi [2,3].

Armed with all this information, widely shared with Bihar health ministry, Bihar Task Force on AES, NCDC and the media, the NCDC/CDC investigators changed tactic and carried out a quick detailed study in 2014, involving 51 scientists from various fields in USA and India, and re-confirmed all the earlier published findings, as described in this Lancet paper. However, it is disturbing to note that no staff of the two institutions in which they carried out the study – Sri Krishna Medical College Hospital and Krishnadevi Deviprasad Kejriwal Maternity Hospital – is included among the 51 authors, and neither institution is named among the 13 agencies conducting the case-control study. Such brazen impropriety would not have been tolerated elsewhere; in Bihar, NCDC and in India CDC are guests, and we in India tolerate such imperious attitudes of some guests. Indian co-authors should have defended fairness to Indian colleagues.

The metabolic products accumulating due to blocked fatty acid β-oxidation cycle are amino acids and fatty acids that are toxic to brain cells [6]. Organic acidemia and aciduria are thus tell-tale signals of blocked fatty acid β-oxidation cycle, and the Lancet paper has convincingly shown both in many children. This gap in the earlier study by the Bihar team has now been filled by the CDC/NCDC study. Once organic acidemia is established for many hours, brain cells are apparently permanently damaged, resulting in death of children or brain function defects in those who survive; hypoglycemia is not the only or the major cause of the metabolic coma [6].

The Indian team had recommended early intravenous infusion of 10% dextrose and documented its protection of brain functions [2]. The Lancet authors repeated this recommendation as 'rapid glucose correction' without realizing that 'glucose correction' requires only 5% dextrose; then why did the Bihar team recommend 10% dextrose? The purpose is to establish higher than normal blood glucose concentration to stimulate insulin secretion for turning off the fatty acid oxidation cycle [6]. The consequences of the absence of a competent clinical pediatrician among NCDC/CDC investigators were missed diagnosis for many years, delay in conducting focused investigations, lack of discriminative case definition to separate encephalopathy from all other diseases and poorly understood therapeutics.

The Bihar team had recommended that no child should be allowed to go to sleep without a cooked meal, and that parental supervision was needed to minimize small children eating Litchi fruits [1-3]. The Lancet paper repeated these recommendations without acknowledging that the health ministry had already instituted them long before the Lancet paper was published. There was a meeting of the Task Force on AES (Indian Council of Medical Research) in Patna on 29 November 2016, to review AES/JE situation in Bihar. A senior Bihar health ministry official thanked the Bihar team for solving the mystery expeditiously and mentioned that by applying the three recommendations, the disease incidence had been drastically reduced and death almost completely prevented. Some of the authors of the Lancet paper were listening, as was myself!

It is a common error to make a broad case definition for sensitivity, when investigating outbreaks of unknown etiology, as the Lancet paper illustrates. The broad case definition would allow the inclusion of encephalitis, encephalopathy, viral and bacterial meningitis, and cerebral malaria among the study subjects. There cannot be one etiology for such medley of maladies. Being a quickly put-together study, and rushing for submission for publication, apparently the investigators could not sift all cases to include only acute encephalopathy and exclude all others. The Lancet paper clearly shows that

only a subset of study cases was confirmed with hypoglycemic encephalopathy, the recurrent outbreak disease. Had they been discriminative, they need not again have looked for viruses and pesticides in metabolic coma cases as they had done repeatedly in previous years – obviously even in 2014, the investigators were not sure if the 'outbreak disease' was infectious or non-infectious. The outbreak was exclusively encephalopathy. The clinical features were quite typical of acute encephalopathy (sudden onset, overnight development of severe disease, rapid progression within hours, absence of CSF pleocytosis and death or recovery within a few days); they did not at all fit with acute viral encephalitis or pesticide poisoning [1-3]. When investigating outbreaks for etiology, the case definition should aim for high specificity, not sensitivity.

The Bihar team had found that a large majority of children with hypoglycemic encephalopathy was from litchi-harvesting labourers' families. During harvest season, they camp in the orchards with children. While fruits harvested in bunches are marketed, single stray fallen fruits belong to children for collection and hoarding. Harvesting is during early morning, starting at 4 am. Thus the family routine is disturbed and children may sleep off without night meal. They are the ones affected during the night. All cases had consistently early morning onset, a feature missed by all earlier investigators.

Litchi is a popular and safe fruit. It is a commercial crop in Muzaffarpur and Litchi orchards provide livelihood employment for many poor families. Giving Litchi a bad name is inappropriate and unnecessary, and Bihar Government had advised all investigators to avoid sensationalizing the Litchi-connection (unpublished). The MCPG in Litchi is inconsequential in healthy well-nourished children. What it does is to block the fatty acid β-oxidation cycle when neo-glucogenesis is demanded on account of failed glucogenesis by glycogenolysis, for which undernutrition is a predisposing factor [1]. The Lancet paper flouted the State Government's directive. If Litchi market declines, the worst affected would be the very same families of vulnerable children, pushing them further down the poverty scale.

Unfortunately the authors of the Lancet paper use language to imply that the diagnosis, risk factors and preventive recommendations are their own original contributions. How could the authors claim they were the first to diagnose the disease and identify its cause when they had already been published? Their literature search ended in 2013, while the first two papers of the Bihar team were published in 2014, and the third in 2015 [1-3]. So they missed them, or probably timed the literature search deliberately to miss them.

However, they did cite the original papers, which is proof that they knew of them, but still claimed they diagnosed the disease first and offered the very same recommendations made by the Bihar team. Their claims are untenable. But to their credit, they applied good laboratory analytical methods, much of which was done overseas. Is not the very purpose of housing CDC scientists in India to improve the investigative facilities within the country?

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