Outbreaks of Hypoglycemic Encephalopathy in Muzaffarpur, India: Are These Caused by Toxins in Litchi Fruit?

THE POINT

In his President's page write-up, Yewale [1] lamented that the annual outbreaks of acute encephalitis syndrome in Muzaffarpur remained a mystery in spite of investigations by experts from National Centre for Disease Control, New Delhi and Centers for Disease Control, Atlanta (USA).

While they had failed to solve the mystery, we showed clearly that the disease is an encephalopathy, associated with hypoglycemia, hence metabolic, and not infectious [2]. We found strong epidemiological association of the disease with *litchi* fruit [2]. The manifestations are similar to hypoglycemic encephalopathy caused by methylene cyclopropylalanine (MCPA) present in ackee fruit; ackee and *litchi* belong to the same family, Sapindacae [2]. Litchi seed contains the analogue methylene cyclopropyl-glycine (MCPG) [2]. Both MCPA and MCPG induce hypoglycemic encephalopathy in experimental animals [2]. The striking spatial and temporal association with litchi harvesting indicated high probability that encephalopathy was triggered by MCPG. Yewale cited our paper but remained unconvinced that the diagnosis was metabolic encephalopathy, without stating the reason, and continued to call it a mystery [1,2].

Vashishtha [3] called the disease 'encephalitis' in the very title of his paper. He wrote: "Already, a few IAP members are involved in the investigations of the ongoing recurring outbreaks in Muzaffarpur, in their own personal capacity" [3]. He was probably referring to our studies in which encephalitis was categorically rejected as the diagnosis [2,4]. Vashsishtha did point out that we had "hinted towards a toxin contained in the *litchi*, a locally grown fruit" [3]. However, his skepticism was expressed as: "the current scenario is a bit murky and resembles like five blind people describing an elephant" [3].

Thus, two IAP stalwarts had rejected our findings – even though well supported with clear evidence and logical arguments – without stating the reasons. They wrote that the disease remained undiagnosed even after we had conclusively proved the diagnosis [1,3]. This situation illustrates the need to teach how to read evidence in publications. Opinion is subjective and need not be supported with evidence. Evidence is objective and if one feels that it is weak, the deficiency has to be identified before rejecting it. It is important to learn to discriminate between well-argued conclusions and ill-supported opinions [1-4]. Members of IAP must learn how to evaluate evidence in papers and presentations.

We have now detected MCPG in Muzaffarpur-grown litchi – in seeds, semi-ripe fruit pulp, and ripe fruit pulp [5]. Since the part of the fruit consumed by humans contains MCPG, and experimentally it has been shown to cause hypoglycemia in starved animals, the biological plausibility of causality by MCPG is thus confirmed [2,4,5]. It stands to the credit of Indian scientists that we solved a long-standing mystery that killed hundreds of children every year, and had continued to baffle national and international disease control experts [1,2,4,5].

***T JACOB JOHN AND #MUKUL DAS**
**439 Civil Supplies Godown Lane, Kamalakshipuram, Vellore, TN; and*
#Food, Drug and Chemical Toxicology Group, CSIR- Indian Institute of Toxicology Research, MG Marg, Lucknow, UP; India.
**tjacobjohn@yahoo.co.in*

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COUNTERPOINT

Dr T Jacob John has criticized the views expressed in two communications published in Indian Pediatrics on the recurrent outbreaks of a mystery disease in Muzaffarpur district of Bihar [1,2]. In one of these communications,

the approach adopted by different experts to investigate these outbreaks is compared to five blind men describing an elephant [2]. Dr John claims that he and his team have conclusively solved the mystery behind these outbreaks and the IAP experts have failed to read and interpret the evidence.

Probably he has misunderstood the objective of these publications which was primarily to draw attention to the malaise afflicting the process of outbreak investigations in India. For example, in the second letter, it is pointed out that there is no coordination and cohesion amongst the experts involved in the investigations of the Muzaffarpur outbreaks [2]. Just to elaborate, the Muzaffarpur outbreaks are investigated by different researchers and investigating agencies in recent times with altogether different findings and conclusions. Sahni, *et al*. [3] termed these outbreaks caused by heat stroke; Dinesh *et al*. [4] suspected role of some 'bat viruses', Samuel, *et al*. [5] suspected some non-JE viruses as the probable etiology, the CDC team called the outbreaks as 'unexplained neurological illness caused probably by a toxin contained in *litchi*. [6], and John, *et al*. [7-9] concluded the these as 'hypoglycemic encephalopathy' caused by a toxin called 'methylene cyclopropyl-glycine (MCPG)' [9]. The special Task Force constituted by Bihar Government to manage these outbreaks in Muzaffarpur is following a syndromic approach of management without establishing an exact diagnosis. In fact, Dr John had himself acknowledged this in the opening paragraph of his first correspondence published in the journal Current Science [7]. Different players are playing their own tunes without any coordination and cohesion.

Both the groups, the CDC team and the investigators led by Dr John, have described the illness as acute hypoglycemic encephalopathy (AHE) and pointed to the toxin, MCPG contained in litchi as the putative agent responsible for the genesis of these recurring outbreaks [6,7-9]. Whereas, the CDC team has stated their investigations incomplete and acknowledged some doubt on the exact etiology, Dr John is quite convinced about the role of MCPG and has claimed that he had adequate evidence to confirm its role [8,9]. However, on a dispassionate analysis, it would be obvious that the MCPG theory as put by John, *et al*. [8,9] is still just a hypothesis. He has so far published his findings in a science magazine in form of scientific letters to editor which usually are exempted from peer review.

There is now good amount of data supporting the notion that these outbreaks are caused probably by a toxin involved with litchi cultivation. However, before the scientific community accepts MCPG theory, it needs solid evidence. Dr John wants us to believe that his hypothesis is infallible and should be accepted as such without even undergoing any scientific validation. Much of the evidence linking MCPG to these outbreaks has been largely circumstantial. There are several missing links/issues that need to be sorted out first.

MCPG *VERSUS* MCPA AS TOXINS

Dr John claims that his team's finding of MCPG in the pulp of litchi fruit and a 3-fold higher concentration in the semi-ripe litchi pulp [9] are the clinching evidence of toxin-mediated acute hypoglycemic encephalopathy (AHE). He postulates that poor children eat these semi-ripe litchis, fallen on the ground during early morning, and develop features of encephalopathy [9]. However, no epidemiological study is done to demonstrate higher risk of the disease to children who had consumed the fruit.

It is known that hypoglycin-A (methylene cyclopropyl-alanine, MCPA), a higher analogue of MCPG incriminated in ackee-fruit poisoning in Africa and West Indies, causes hypogycemic encephalopathy in a dose-dependent manner [10]. In ackee fruit, the concentration of Hypoglycin A is 300-10,000 folds higher in unripe than in ripe ackee [11]. The minimum lethal-dose (LD50) of hypoglycin A was found to be 98 mg/kg for the rat following oral administration [12]. On the other hand, the LD 50 of the MCPG is not known. Only seeds are considered toxic so far, which have around 3 to 10 folds higher concentration of the toxin than a semi-ripe and ripe fruit, respectively [9]. Hypoglycin A is present in the unripe arilli at levels of over 1000 ppm (=1000 µg/g) [11] whereas in semi-ripe *litchi* as per the study of Das. *et al*. [9], MCPG is 0.57 µg/g in a fresh fruit [9]. Is the amount of MCPG present in semi-ripe and ripe litchi sufficient to cause toxicity and deaths of children who consume it? The fatality rates of the illness caused by hypoglycin A (MCPA) in Africa and West Indies (80%) [13] were much higher than the reported figures in Muzaffarpur (26%-44%) [4,6,8]. It denotes that probably the hypoglycin A (MCPA) is a much more potent toxin than MCPG. Even if it is assumed that MCPG is as toxic as MCPA and has the same LD50, a child weighing 10 kg would need to consume at least 1.72 kg (approx 120 litchis) pulp of semi-ripe litchi or 5.4 kg pulp (approx 360 litchis) of ripe litchi to develop full blown toxicity! Is it feasible for an undernourished child to binge on such a huge amount of fruit pulp?

The hypoglycin A usually leads to dysfunction of mitochondria in many tissues and organs, but particularly in liver, brain, and kidney [14]. Many

illnesses that primarily affect mitochondria like Reye's syndrome, medium-chain acyl-CoA dehydrogenase deficiency, acute hepato-myo-encephalopathy syndrome due to cassia toxicity, usually presents with profuse vomiting, hepatic derangement and altered level of consciousness. However, in AHE cases encountered in Muzaffarpur outbreaks, no evidence of hepatic dysfunction was found [3,6,8]. Surprisingly, vomiting, the key symptom of hypoglycin A poisoning, was also not reported as a major presenting feature in all the reports published so far [3,6,8]. The hepatic transaminases were normal and histopathological examination was not done to demonstrate microscopic changes like fatty degeneration of hepatic parenchyma.

Even the seasonality of the disease also does not favor the occurrence of children eating the semi-ripe litchi and falling ill since the disease peaks during first two weeks of June, a time when the entire crop is mature, and ready for plucking and harvesting. The timing of ripening of litchi in Muzaffarpur is usually 3rd to 4th week of May [15].

Finally, no attempt was made to confirm exposure to MCPG by assessing specific metabolites of the putative toxin in the body fluids of the cases. A recent study presents an analytical approach to identify and quantify specific urine metabolite such as methylenecyclopropylformyl-glycine (MCPF-Gly) for exposure to MCPG [16].

The investigators have to quantify the toxic dose of the toxin, need to demonstrate morphological evidence of ill-effects of the toxin, and in the last, need to confirm the presence of its metabolite in any of the biological fluids of the cases before a cause-and-effect theory is established. Mere presence of minute amount of the toxin in the litchi, its similarity with a known toxin, hypoglycin A, and biological plausibility are not enough to prove causality.

HYPOGLYCEMIA AS A KEY SIGN

Both the CDC report [6] and John, *et al*. [8,9] have placed extraordinary emphasis on hypoglycemia as the key sign. They have used serum glucose level of 70 mg/dL as cut-off for hypoglycemia. However, in most of the illnesses presenting as Reye-like encephalopathy, there is profound hypoglycemia and single digit serum glucose levels are encountered. Even, CSF also has very low glucose level. Some degree of hypoglycemia is commonly encountered in many pediatric illnesses like acute encephalitis, sepsis, pneumonia.

The investigations done by CDC team found hypoglycemia (blood glucose <70 mg/dL) in only 21% of 94 children admitted in 2013, and in 2014 they reported hypoglycemia in 52% of 327 patients with the median blood glucose level was 48 mg/dL [6]. John, *et al*. [8] reported moderate to severe hypoglycemia (serum glucose <30 mg/dL) in 13 out of 26 children studied. Whereas others [3-5] did not mention hypoglycemia at all in their reports! So, two out five reports point toward mild to moderate hypoglycemia in the cases; whereas the profound hypoglycemia [13] encountered usually with hypoglycin A- induced ackee fruit poisoning is missing.

Both John, *et al*. [9] and CDC team [6] have reported decline in the mortality following rapid correction of hypoglycemia in affected children with intravenous dextrose to the ill children [6,9]. However, it should be noted that in cases of hypoglycin A toxicity, intravenous dextrose infusions have failed to prevent deaths. Experiments have shown the action of hypoglycin is unlike that of insulin [17].

In conclusion, from the study of published reports on Muzaffarpur outbreaks, it is evident that the disease in question is not acute viral encephalitis but a form of acute encephalopathy probably mediated by a toxin related closely to the *litchi* cultivation in the region. Much of the evidence linking MCPG to these outbreaks has been largely circumstantial due to the lack of properly conducted epidemiological studies, histopathological demonstration of toxic effects of the toxin, and inability to perform analysis for any specific metabolites in the body fluids of the ill children. The experts involved in the investigations should urgently look into these aspects before claiming success in conclusively diagnosing the illness.

VIPIN M VASHISHTHA
Consultant Pediatrician,
Mangla Hospital and Research Center,
Bijnor, Uttar Pradesh, India.
vipinipsita@gmail.com

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