Acute encephalitis syndrome in children in Muzaffarpur: hypothesis of toxic origin

One among three long-standing mystery diseases listed in Wikipedia is acute encephalitis syndrome (AES) in Muzaffarpur, Bihar[1]. This disease has remained for over two decades without determining a cause for it; hence it is called mystery disease. It occurs as annual seasonal outbreaks during the months of April–July, affecting hundreds of children with 40–60% mortality, according to local physicians. It was thought that Muzaffarpur AES is Japanese encephalitis (JE), which is widely prevalent in India; however, JE was ruled out in a recent study by Samuel *et al.*[2]. Another study explored several possibilities of causation of the disease and again excluded JE[3]. These studies documented that cases coincided spatially and temporally with lychee cultivation. The investigators noted colonies of fruit-eating bats and the tendency of children eating fruits fallen to the ground and suggested the possibility of a bat virus (through saliva contamination on fruits) as a cause of the disease[2]. Other studies concluded that the disease was not due to infection, but was due to heat stroke[4,5].

One of us (T.J.J.) visited Muzaffarpur in the 2013 season, examined children with AES and held extensive conversations with healthcare personnel, parents, family members and neighbours of the affected children. Thereafter, we searched the literature and after mutual discussions developed a hypothesis about the cause of the disease, which is presented below.

Clinical features in patients are stereotype – sudden onset without prodromal phase, inconsistent presence of fever, brain oedema, absence of inflammatory cell response in cerebrospinal fluid (CSF) and hypoglycaemia. These clinical features and preliminary epidemiological findings of tightly restricted seasonality and geographic distribution as well as sparing of children below 2 years support the diagnosis of acute non-infectious encephalopathy as against viral encephalitis[6]. Children are quite well until evening, but early next morning they are found seriously ill with brain function derangement and seizures. Undernutrition (short and underweight for age) has been observed as a consistent associated factor.

Association of AES with lychee is important, interesting and challenging. In Vietnam and Bangladesh, outbreaks of AES have been reported in lychee cultivation areas and during lychee harvesting season[7–9]. Investigators in Vietnam believed that the disease was caused by some unknown virus, while in Bangladesh the disease was not thought to be infectious but was attributed to pesticides used in the orchards. Curiously, both these studies and the one from Muzaffarpur showed positive correlation between number of cases and amount of lychee harvest[5,7–9].

Lychee (*Litchi sinensis*) belongs to the family Sapindaceae (soapberry). Another soapberry member, ackee (*Blighia sapida*), commonly cultivated in Jamaica, is the cause of a childhood (under 15 years) acute encephalopathy disease called Jamaican vomiting sickness (JVS), also referred to as toxic hypoglycaemic syndrome[10]. The clinical features of ackee poisoning and Muzaffarpur AES have many close similarities, including early morning onset, encephalopathy, hypoglycaemia and high case fatality. A toxic substance methylenecyclopropylalanine (MCPA), also called hypoglycin A, is present in ackee unripe fruits. Ripe ackee has a small concentration (0.1 ppm), which is less by a factor of 10,000 compared to unripe fruit[11]. Studies related to

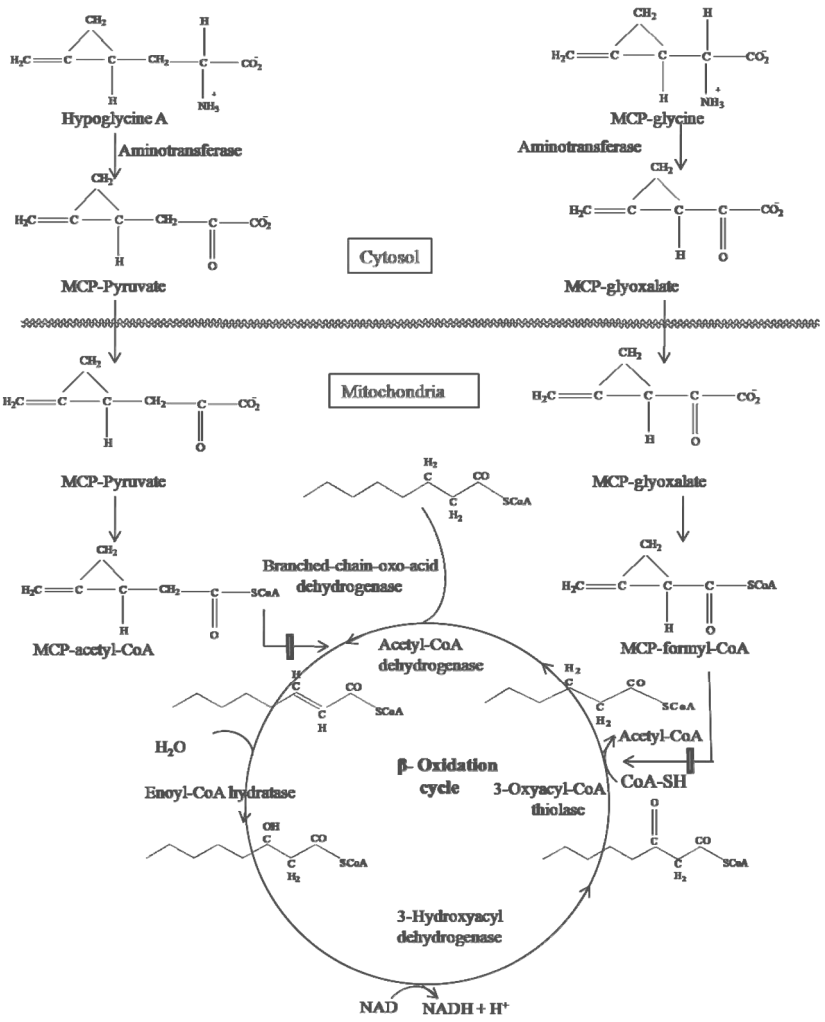

Figure 1. Conversion of MCPG and hypoglycine into active metabolites and their sites of inhibition of β-oxidation[16].

Table 1. Similarities and differences between hypoglycin A and MCPG in undernourished population or animals

Ackee fruit (undernourished population/animals)	Litchi (undernourished rats)
Sapindaceae family	Sapindaceae family
Hypoglycaemia	Hypoglycaemia
Inhibition of β-oxidation of fatty acid	Inhibition of β-oxidation of fatty acid
Methylenecyclopropyl-alanine	Methylenecyclopropyl-glycine
Methylenecyclopropyl-acetyl CoA is a toxic metabolite	Methylene cyclo propyl-formyl CoA is a toxic metabolite
Glucose (↓), lactate (↑), non-esterified fatty acid (↑)	Glucose (↓), lactate (↑), non-esterified fatty acid (↑)
Dicarboxylic acidurea (↑)	Not known
Hyperketonemia (ketone bodies ↑)	Hypoketonemia (mechanism not known)

hypoglycin A toxicity revealed that the actual causative agent of JVS is a metabolite of hypoglycin A called methylenecyclopropane-acetyl CoA (MCPA-CoA)[12]. MCPA-CoA exerts its effect by inhibiting several coenzyme-A dehydrogenases, which are essential for gluconeogenesis[13]. Depletion of glucose reserves (the result of under-nutrition) and the inability of cells to regenerate glucose through neoglucogenesis lead to hypoglycaemia. However, hypoglycaemia alone may not be able to explain encephalopathy, which usually persists in spite of infusion of glucose. The toxin affects mitochondrial functions in the liver. Since brain cells require constant supply of glucose, hypoglycaemia triggers mitochondrial β-oxidation of fatty acids. The putative toxin blocks this reaction with accumulation of back-up intermediates which are toxic to cells. This we surmise will explain the functional abnormalities and the acute brain oedema recorded in many subjects of JVS.

Lychee seeds are known to contain a lower analogue of hypoglycin A, namely methylenecyclopropyl-glycine (MCPG)[14]. MCPG has not been analysed in the ripe or unripe lychee fruit, but has been shown to cause hypoglycaemia and derangement of fatty acid β-oxidation in liver cell mitochondria in experimental animals[15]. The mechanism of toxicity of MCPG is related to the formation of MCPF-CoA, which in turn inhibits several dehydrogenases responsible for gluconeogenesis, causing depletion of glucose reserve in the body[16]. Comparative action of hypoglycin A from ackee fruit and MCPG from lychee on metabolic conversions and their sites of inhibition of β-oxidation of fatty acid are depicted in Figure 1 (ref. 16).

In animal experiments, MCPA and MCPG have been shown to induce encephalopathy and hypoglycaemia. The encephalopathy is explained by the mitochondrial inhibition of fatty acid β-oxidation and accumulation of toxic metabolites. Our hypothesis is that the Muzaffarpur AES is caused by MCPG in lychee. However, we do not know if it is present only in the seed or also in the edible fruit flesh (in ackee and lychee, the aril is transformed as the edible part) and if unripe lychee has more MCPG than ripe fruits.

The similarities and differences between hypoglycin A and MCPG in undernourished population or animals are given in Table 1, which indicates the possibility of association of Muzaffarpur AES with lychee fruit. This hypothesis ties up several observed features of Muzaffarpur AES. Early morning onset is after several hours of fasting and points to a metabolic disease; hypoglycaemia points to inhibited gluconeogenesis and malnutrition is associated with depleted glycogen/glucose store in the liver. Well-nourished children are not affected since their glycogen/glucose store in the liver is sufficient to maintain normal glucose levels and presumably gluconeogenesis is not triggered.

This hypothesis is testable and we have proposed to the Ministry of Health in Bihar precisely to do that in the 2014 season. Moreover, knowing that fatty acid metabolism is deranged, treatment modalities are possible to save lives. Ensuring adequate nutritional status in young children will prevent this disease. If and when the lychee connection is confirmed, children's behaviour modification can further help prevention.

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