

SEATO MEDICAL RESEARCH STUDY ON ENCEPHALOPATHY AND FATTY DEGENERATION
OF THE VISCERA IN THAI CHILDREN.

Coordinator: Curtis H. Bourgeois, MAJ, MC

Principal Investigators: Marcus R. Beck, COL, MC
Curtis H Bourgeois, MAJ, MC
Pramukh Chandavimol, M.D.¹
Dhira S. Comer, M.D.
Kamnuan Dhiensiri, M.D.¹
Hilary Evans, MAJ, MC
Richard A. Grossman, MAJ, MC
Supha Harikul, M.D.³
Chaiyan Kampanart-sanyakorn, M.D.²
Niyom Keschamras, M.D.³
Azorides R. Morales, LTC, M.C.⁴
Lloyd C. Olson, MAJ, MC
Pratoom Potifong, M.D.⁵
Rattana Rattanawongsa, M.D.⁶
Nuam Settachar, M.D.⁵
Thomas J. Smith, LTC, MC
Phillip W. Winter, LTC, MC

Assistant Investigators: Robert W. Dewey, SFC
Damri Chawalitrujiwong
Arporn Siriwangchai
Suriyont Trapukdi
Edward Williams, SFC

Period of Report: 1 August 1968 — 31 March 1969

- 1 Khon Kaen Hospital, Khon Kaen, Thailand.
- 2 School of Public Health, Rajavithi Road, Bangkok, Thailand.
- 3 Udorn Provincial Hospital, Udorn, Thailand.
- 4 31 Field Hospital, APO San Francisco 96233
- 5 Korat Hospital, Korat, Thailand.
- 6 Childrens Hospital, Bangkok, Thailand.

Title: Encephalopathy and Fatty Degeneration of The Viscera in Thai Children.

Principal Investigators: Marcus R. Beck, COL, MC
Curtis H. Bourgeois, MAJ, MC
Pramukh Chandavimol, M.D.
Dhira S. Comer, M.D.
Kamnuan Dhlensiri, M.D.
Hilary Evans, MAJ, MC
Richard A. Grossman, MAJ, MC
Supha Harikul, M.D.
Chaiyan Kampanart-sanyakorn, M.D.
Niyom Keschamras, M.D.
Azorides R. Morales, LTC, MC
Lloyd C. Olson, MAJ, MC
Pratoom Pottitong, M.D.
Rattana Rattanawongsa, M.D.
Thomas J. Smith, LTC, MC
Phillip W. Winter, LTC, MC

Assistant Investigators: Robert W. Dewey, SFC
Damri Chawalitrujiwong
Arporn Siriwangchai
Suriyont Trapukdi
Edward Williams, SFC

Period of Report: 1 August 1968 to 31 March 1969

OBJECTIVE

A systematic study of the epidemiology, etiology, pathology and clinical features of "Encephalopathy and Fatty Degeneration of the Viscera in Thai Children"

BACKGROUND

Reye, Morgan and Baral (Lancet 2:749,1963) have described a rapidly fatal disease of young children which presents the following clinical features: "profoundly disturbed consciousness, fever, convulsions, vomiting, disturbed respiratory rhythm, altered muscle tone, and altered reflexes". The pathologic changes in Reye's cases were strikingly similar and included marked cerebral edema without inflammation, marked diffuse fatty degeneration of the liver, fatty degeneration of the proximal renal tubules and fat droplets in the myocardium, pancreas and endothelial cells.

Although the condition which Reye et al named "Encephalopathy and Fatty Degeneration of the Viscera" was described as early as 1929, it did not receive widespread attention until after Reye's paper. Since Reye's report from Australia more than 150 cases have been reported from such widely separated geographic areas as Canada, Czechoslovakia, England, New Zealand, Scotland, South Africa, and the United States

In August 1968, the Udorn Provincial Hospital and the SEATO Medical Research Laboratory undertook the joint study of a syndrome which has been recognized by Udorn physicians for a number of years and is known locally as "Udorn Encephalitis". Characteristically the disease is most common during the rainy season, selectively affects young children and begins with the sudden onset of coma or convulsions. There is rapid progression of the condition with deepening coma, irregular respirations and an early (within 72 hours) fatal outcome. The cerebrospinal fluid is customarily negative. One hundred and thirty-nine such cases were hospitalized at the Udorn Provincial Hospital between Jan 1967 and Dec 1968. Eighty-six

percent of the patients were under 7 years of age, 70–75% of the cases occurred between the months of June and November, no definite geographic pattern was recognized and the mortality rate was 81%. After review of the available autopsies from this series it was apparent that "Udorn Encephalitis" was identical to the condition described by Reye et al.

A preliminary report concerning the work at Udorn is now in press.

Since August 1968, a total of 74 cases have been studied in the Udorn, Khon Kaen and Korat Provincial Hospitals, 42 of these cases have been autopsied.

DESCRIPTION

I. Clinical and Epidemiological Aspects (Udorn cases, 1969)

Since January 1969, a field team has been stationed at the Udorn Provincial Hospital. All patients admitted to the Udorn Hospital with diagnoses of encephalitis, encephalopathy, coma or convulsions have been studied. Out of a total of 33 patients studied, 7 were eliminated because either clinical or autopsy findings established a diagnosis other than "Udorn Encephalopathy". The following data are taken from the records of the remaining 26 patients, 12 of which have been autopsied.

Sex and Age: The case material includes 8 males and 18 females. Ages ranged from 1–11 years; median age 3.5 years, with 90% aged 5 or less.

Seasonal Distribution: Three cases occurred in January, 8 in February, 8 in March, 5 in April and 2 in the first week of May.

Geographic Distribution: There has been no evidence to date of clustering of cases in time or space. Five cases lived within a 5 kilometer radius of Udorn city and the remainder came from widely scattered small villages throughout the rural, rice farming areas. The true incidence of disease for this area and time span cannot be adequately gauged. Most cases have lived close to transportation lines or near Udorn city. The ability of the parents to bring their child to the hospital is probably limited too by the rapid progress of the disease and their knowledge and acceptability of hospital treatment. It is assumed that other cases have occurred.

Occurrence in Siblings: In this series of cases there was no concurrent disease in siblings. Two cases each had a history of occurrence of a similar syndrome in a sibling, 3 & 6 years previously.

Fatality Rate and Duration of Illness: Data are available for 24 patients. Nineteen patients died (case fatality rate 79%). Time from onset to death was within 72 hours for 17; duration was 4 and 5 days for the other 2 patients; 5 patients were discharged, apparently well, after periods of 1–5 days hospitalization.

Clinical History: The sudden onset of either vomiting, fever or convulsions was the most common initial complaint. Non-specific prodromal symptoms consisting of abdominal pain, mild upper respiratory infection or headache were occasionally recorded. In one case a mild skin rash was present.

Symptoms: In the 26 cases studied: 25 had convulsions, 24 developed coma, 18 had fever, 16 vomited, 11 had diarrhea, 10 experienced anorexia, 9 developed respiratory distress and 6 had headache.

Vital Signs: During the course of the disease temperatures varied from 36.2° to 40°C. In 14 instances the temperature was normal or subnormal, temperatures above 38°C were recorded in 19 instances. Five patients who expired had normal temperatures. Tachycardia was consistently present. Respiratory rates ranged from 28–30/min. on the average. Blood pressures were recorded in 8 patients and all were within normal limits.

Physical Findings: The pupillary response was recorded as sluggish or absent in 14 of 18 patients tested. Reflexes were present in 14 of 19 patients. Hepatomegaly was recorded in 5 of 7 patients. Muscle tone varied from intense rigidity to flaccidity.

LABORATORY DATA

White Blood Counts varied from 8,200 to 42,200 with 10 to 14 patients having counts of 12,000 or more.

Differential counts were unremarkable.

Hematocrits varied from 28–45% with 9 of the 12 being below 35%.

Urine pH was acidic in all cases tested, specific gravity varied from 1.010 to 1.032. Trace glycosuria was recorded in 3 of 8 patients.

Serum glucose was determined in 16 patients and was found to be below 50 mg.% in 10 patients. In 6 patients the glucose was below 33 mg.%. In two cases elevated glucose levels were recorded.

SGOT and SGPT levels were determined in 19 cases, in 10 cases there was mild elevation (60–80 units), in 4 patients there was moderate elevation (150–250 units) of the transaminase levels.

Bilirubin was found to be within normal limits in all 9 patients tested.

Serum Electrolytes were performed in 16 patients, in virtually every patient there was a decrease in sodium (120–135 mEq./L.), chloride (80–97 mEq./L.) and CO₂ with an increase in the potassium (5–7 mEq./L.).

Hospital Course: Therapy has included large doses of procaine penicillin, streptomycin, 5% dextrose in water and steroids. In some of the later cases, mannitol infusion and tracheostomy with assisted respiration were added to the regimen. In no case did therapy appear to influence the course of the disease. Patients invariably exhibited a progressive, rapid, downhill course with deepening coma, decerebration and increasing respiratory difficulty.

II. Overall Geographic Distribution

Field trips to Khon Kaen, Korat, and Chiangmai and consultation with the physicians at the Bangkok Childrens Hospital have provided the following data regarding the overall distribution of the disease in Thailand.

A. Khon Kaen: The records of the Khon Kaen Provincial Hospital and Health Office indicate that a disease clinically and epidemiologically similar to that seen in Udorn has been observed in Khon Kaen. The number of cases admitted to the Khon Kaen Hospital is at least equal to that in Udorn. In 1968, 27 cases were admitted during October, 22 in November and 15 in September. During the dry season cases were infrequent. Autopsies were performed on four of the cases that occurred during October.

B. Nakorn Rajasima: Cases similar to those found in Udorn and Khon Kaen have been seen at the Korat Provincial Hospital for a number of years. Initial findings indicate that the disease is less common in Korat than in Udorn or Khon Kaen. However, an epidemic affecting some 30 children occurred in an area South of the city of Korat during late February and March of 1969. Autopsies were performed on 13 of these cases.

This outbreak represents a distinct departure from the epidemiologic features previously observed. The large number of cases occurring in a discrete area over a very short period of time is the first evidence of epidemic behavior. In one village 3 children from one family were simultaneously affected and all died. Finally the prevalence of cases during the dry season is in sharp contrast to the usual association with the rainy season in other provinces.

C. Chiangmai: Two fatal cases autopsied at McCormick Hospital, Chiangmai, proved to be Encephalopathy and Fatty Degeneration of the Viscera. The impression of physicians in this northern city is that prevalence of this disease is much lower in Chiangmai than in the northeast. The picture is also confused by the large number of cases of encephalitis which occur in children of this area. On the basis of serological evidence many of these appear to be due to Japanese B encephalitis virus.

D. Bangkok: Since September 1968 more than 20 cases have been observed at the Bangkok Children Hospital, 9 cases have been autopsied. The existence of the disease in Bangkok is of interest, in view of the rural origin of cases in the Northeast. Because of the short period of time observation has been carried out, the relatively small number of cases, and the immense size of the population at risk, epidemiological features have yet to be established.

E. General Distribution: Questionnaires regarding the prevalence of clinical disease resembling that seen in Udorn were sent to the Directors of the Provincial Hospitals through the Director General of Medical Services, Dr. Prathuang Singkhalawanit. Replies were received from 59 hospitals representing all areas of the country. The existence of Encephalopathy and Fatty Degeneration of the Viscera was considered probable in an area if the clinical picture and epidemiological features were consistent with those observed in Udorn. Disease appears to be uniformly absent in the southern peninsular area, present in moderate numbers throughout the central plains and northern region, and heavily prevalent over the entire northeast. Provincial Hospitals reporting the largest number of cases include Chaiyaphum, Buriram, Kalasin, Ubol, Uttaradit, Roi-et, and Udorn.

III. Autopsy Findings

Autopsies have been performed on 44 cases (4 from Khon Kaen, 13 from Korat and 27 from Udorn). The findings in these cases have been remarkably uniform. The following general description includes the findings that have been common to these cases.

General: All subjects have appeared well developed, and well nourished, without evidence of edema, jaundice or significant skin lesions.

Heart: Epicardial petechiae are commonly observed on the otherwise grossly unremarkable heart. The most striking microscopic abnormality is accumulation of varying amounts of small Oil Red O positive droplets within the myocardial fibers. Interestingly the SA and AV nodal fibers are not affected by the fatty change, whereas the fibers of the bundle of His are strongly positive with the Oil Red O stain.

Lymphoid Tissue: Invariably the abdominal lymph nodes are increased in size and number. There is moderate enlargement of the thymus and spleen. Microscopic examination of the lymphoid tissues shows an increase in histocytes, most of which contain Oil Red O positive material. An interesting but unexplained finding is marked phagocytic activity by the histocytes in the thymus and in Peyer's patches.

Brain: In all cases the brain is markedly edematous with flattening of gyri, compression of the ventricles, uncal grooving, and tonsillar pressure cones. In no case was there gross or microscopic evidence of inflammation. Microscopically the cerebral edema is manifested by perivascular and pericellular clear spaces. Fat vacuoles within the walls of small cerebral vessels and accumulation of yellow brown, PAS positive material in the perivascular connective tissue are commonly seen.

Kidneys: The kidneys are generally swollen and pale displaying a slightly thickened cortex. The tubular epithelial cells contain varying amounts of Oil Red O positive droplets. The proximal tubules are most severely affected but in some cases all portions of the nephron are involved. Glomerular and vascular lesions are not seen.

Livers: Characteristically the liver is increased in weight and displays smooth slightly rounded edges. The cut surfaces are oily, pale grey with a yellow tinge. Microscopic examination reveals preservation of the general hepatic architecture but diffuse almost complete replacement of the hepatocyte cytoplasm by small droplets of Oil Red O positive lipid. Inflammation and necrosis are rarely observed.

SPECIAL STUDIES

Histochemistry: A variety of histochemical techniques have been applied to the liver and kidneys from these cases. No histochemical demonstrable changes have been observed in oxidative enzymes, acid mucopolysaccharides, phospholipids, lipase and alkaline phosphatase. PAS stains show a mild decrease in demonstrable glycogen, Oil Red O stains show marked increase in hepatic and renal lipids. Chromatographic studies on hepatic, renal and brain lipids show preservation of normal phospholipid patterns and marked increase in renal and hepatic neutral lipids.

Fluorescent Microscopy: Reveals orange–yellow autofluorescent material in the liver, kidney and the walls of small cerebral blood vessels.

Electron Microscopy: Electron microscopy of selective cases show numerous fat droplets in liver cells associated with mitochondrial changes and dilatation of the endoplasmic reticulum. Renal changes are confined to fatty deposition in epithelial tubular cells without additional subcellular alterations. Fat accumulates in the heart along clusters of mitochondria without fibrillar disruption. Sinus and atrioventricular nodes show little or no fatty deposition while a large amount of fat is present in the bundle of His and bundle branches. No virus particles were observed but similar subcellular alterations were seen in the organs of a monkey after administration of aflatoxin.

IV. Etiology:

Mycotoxins and viruses are being investigated as possible etiologic agents, to date some evidence in support of each has been established.

A. Mycotoxins:

Using standard techniques for the extraction, identification and isolation of mycotoxins, specimens of foodstuffs and tissues obtained at autopsy have been examined for the presence of mycotoxins.

1. A blue fluorescent compound with chromatographic properties similar to Aflatoxin B₁ was isolated, in high concentrations, from a sample of cooked rice a portion of which had been eaten by a patient proved at autopsy to have Encephalopathy and Fatty Degeneration of the Viscera. Although the compound was chromatographically similar to Aflatoxin B₁ in 3 solvent systems; analyses by Dr. Ronald Shank (MITTHAI Project, Ramathibodi Hospital) indicate that the chemical structure of the compound is somewhat different from Aflatoxin B₁.

2. Cultures of the rice sample mentioned above grew Aspergillus flavus. Extracts of the fungus contained high concentrations of the blue fluorescent compound. One hundred milligrams of the crude extract given orally, suspended in peanut oil, were lethal for two young (1500 gm.) monkeys in 3 days. Both experimental animals, as well as appropriate controls, were kept on a high carbohydrate low protein diet simulating the standard diet in Northeastern Thailand. At autopsy both experimental animals showed hepatic, renal, and cerebral lesions identical to those seen in Encephalopathy and Fatty Degeneration of the Viscera. The blue fluorescent compound was extracted from the brain, kidney and liver of both experimental animals. A control animal sacrificed at the end of the experiment showed no lesions.

3. The blue fluorescent compound and its apparent breakdown products have been isolated from 5/9 brain and 4/9 kidney specimens from autopsied cases of Encephalopathy and Fatty Degeneration of the Viscera. Four of four liver specimens have been negative. Two of four urine specimens obtained prior to death have shown trace amounts of the fluorescent compound.

B. Virological Studies:

Paired sera were available from seven patients with highly suggestive clinical illnesses—two from Bangkok, two from Udorn and three from children in Kamphangphet. All were tested for antibody to group A and B arboviruses and all were negative for evidence of recent infection.

Autopsy specimens were available for isolation studies from 14 patients. In addition 18 patients with symptoms consistent with Udorn encephalitis were cultured. Only one of the latter was autopsied so the diagnosis is unknown on 17. A transmissible agent was isolated from ten of the 14 autopsies, and four of the 18 patients. In all, 26 strains have been recovered. One agent differed from the others in that it possessed a hemagglutinin, but otherwise preliminary information suggests they all share other properties in common. The fact that they only irregularly caused ill–defined CPE in monkey kidney tissue culture cells had made their characterization difficult. In addition the possibility of adventitious agents in these cell cultures has led to caution in interpreting the role of these agents in the disease observed, until additional evidence can be accumulated.

SUMMARY

Encephalopathy and Fatty Degeneration of the Viscera has been found to be endemic in North-eastern Thailand. Preliminary data suggest that the disease is uncommon in the southern regions of the country. Since August 1968, more than 75 cases originating in Udorn, Khon Kaen, and Korat provinces have been studied, 44 of these have been autopsied.

The disease is most common during the rainy season, selectively affects young children, begins with the sudden unexpected onset of coma or convulsions and usually eventuates in death 48—72 hours after the onset. Laboratory findings include: normal cerebrospinal fluid, hypoglycemia, mild-moderate elevations of transaminases, mild leukocytosis and normal bilirubin. Autopsy findings include marked cerebral edema without inflammation and generalized fatty degeneration of the viscera.

Viral infection and mycotoxicosis are being investigated as possible etiologies.