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SUMMARY REPORT OF THE INTERNATIONAL CONFERENCE ON DENGUE HAEMORRHAGIC FEVER AND NATIONAL BRAIN STORMING SESSION ON DENGUE

There is ample evidence that India has experienced in recent years major epidemics of dengue and dengue haemorrhagic fever. However, information regarding these outbreaks has generally not been well documented or formally presented in the scientific literature. Further, indications are that (wide) divergence exists in local treatment practices of severe dengue infection, and that local clinicians may not be fully aware of proven successful procedures for the clinical management of these patients. Recognising the need for providing more information on the current status on dengue, and the need for a coordinated approach to address this emerging problem, the Indian Council of Medical Research, in collaboration with the Rockefeller Foundation, New York, the World Health Organisation, Headquarters and South East Asia Regional Offices, and the Department of Biotechnology, New Delhi, organised an International Conference on Dengue Haemorrhagic Fever and a National Brain Storming Session on Dengue. The meeting held between February 7-8, 1994, involved interaction between 144 international and national scientists and clinicians to share experiences and formulate a plan of action for India. A total of 43 papers were presented at the meeting.

Background

Dengue has been known to exist in India for over a century. Indeed, two strains of dengue 1 virus were first isolated in India by Sabin in 1954, both from humans infected in Calcutta. Several references had been made to "7 day fever" outbreaks near Delhi and Patna during World War II, and the clinical description leave little doubt that these were outbreaks of dengue. Since 1956, all 4 dengue serotypes have been isolated from India, frequently from humans and from vector mosquitoes — *Aedes aegypti* and occasionally from *Ae. albopictus*.

Despite frequent outbreaks of dengue, no report of haemorrhagic fever associated with dengue was made until 1963, when outbreaks occurred in Calcutta followed by Visakhapatnam, and several cities of the eastern coast of India. The outbreaks appeared to be associated with both dengue and chikungunya viruses. In Calcutta, several thousand cases were estimated to have occurred, and dengue as well as chikungunya viruses were isolated. The epidemic lasted from 1963 to 1965, and an estimated 100,000 cases of dengue may have resulted. Dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS) were both documented during the epidemic.

A systematic study of dengue was carried out at Vellore during 1956 to 1967. Several strains of virus belonging to all serotypes were isolated from men as well as mosquitoes. All cases presented with classical clinical manifestations.

Division of Publication & Information, ICMR, New Delhi - 110 029

More recently, serious outbreaks of dengue occurred in Delhi in 1988, and dengue and DHF was diagnosed in 30 per cent of the reported cases in Madras in 1989, including many fatalities ascribed to DHF. A large outbreak of dengue 3 occurred in Calcutta in 1990, including cases of DHF, and another outbreak occurred in Delhi in 1991, but little information is available regarding the serotypes of dengue involved, or the attack rates.

Dengue is not a reportable disease in India, and no active surveillance system exists. Consequently, outbreaks of dengue and DHF are not often documented, and only those which are reported are likely to be investigated. There is a consensus that the incidence of dengue and DHF, which was primarily an urban disease in the 1970s, is now increasingly affecting the rural population.

Objectives of the Meeting

In view of the current situation regarding dengue in India, the following objectives were formulated for this meeting:

1. To have experts in DHF and DSS meet and interact with Indian communicable disease epidemiologists, both national and from the states where DHF has been reported (Tamil Nadu, Gujarat, Uttar Padesh, Maharashtra, West Bengal and Andhra Pradesh).
2. To gather preliminary data on recent occurrences of DHF/DSS in India.
3. To meet and interact with key members of the Indian medical community, especially paediatricians who have cared for DHF/DSS patients.
4. To disseminate information on case definition and case management to Indian clinicians.
5. To discuss and propose recommendations on various topics, including: (i) Enhanced surveillance for DHF/DSS in India; (ii) methods of dissemination of technical information on DHF/DSS throughout the Indian public health and clinical channels; and (iii) publication of articles on DHF/DSS in Indian medical journals.
6. To formulate vector control measures.

Summary of Presentations

The meeting was organised into various sessions which focussed on epidemiology, medical entomology, clinical aspects, immunology of dengue infection and strategies of dengue control; and a round table discussion. Following the formal presentations, the National Brain Storming Session on Dengue was held, during which the group broke into four sections to discuss clinical considerations of dengue, surveillance, medical entomology, and research.

Two general themes dominated the meeting; the increasing number of dengue outbreaks, and the spread of the primary vector of dengue, *Ae. aegypti.*

Outbreaks of dengue fever have been known to occur in India for more than a century, but it has only been since the early 1960s that DHF/DSS have been seen. Over the past decade, a dramatic increase in number of DHF/DSS cases has occurred, and during the meeting information was presented on recent DHF/DSS outbreaks in nine India states (Maharashtra, Gujarat, Karnataka, Andaman and Nicobar Islands, Assam, Nagaland, Haryana, Uttar Pradesh, and Tamil Nadu). All four serotypes of dengue virus have been isolated from acutely ill patients in India, and there was a strong suggestion that dengue was now present in nearly all states of India, and that the risk of DHF/DSS was present and growing. The age of the typical DHF/DSS patient appears to be increasing, with most patients being children. Outbreaks are increasing in frequency and magnitude, but continue to go unrecognised due to the lack of appropriate diagnostic facilities in most parts of the country. Dengue and DHF/DSS are not reportable in India, and when outbreaks are recognised, it is often based on clinical presentation alone. Few outbreaks are properly investigated, and when studied, it is often as transmission is waning and the epidemic nearly over. Treatment of the severe forms of dengue, DHF and DSS is not standardized, and it is clear that the general Indian medical community could benefit from provision of guidelines for the proper management of these disease.

With regard to the status of vectors of dengue, several species of mosquitoes are present in India that may transmit dengue, but the principal vector species is *Ae. aegypti.* The species has traditionally been associated with urban centres in India, and is found virtually throughout the country. During the meeting, however, new information was presented to indicate that *Ae. aegypti* is increasingly abundant in rural settings, thus placing much more of the country at risk of dengue and DHF/DSS. Every presentation made that had data on mosquito densities collected over a period

of years found that *Ae. aegypti* population densities were now high, with indications that they were getting higher. In some areas of India, where human migration to urban centres has led to over burdened housing, water supply, and refuse management, concomitant increase in vector mosquito populations has been especially dramatic. In spite of recognition of the increasing densities of vector mosquitoes, no nation-wide campaign exists for vector control, and when present, vector control is often limited to emergency measures, with no sustained programme of control. Thus, it appears that the conditions are ripe for explosive epidemic dengue transmission.

Recommendations of the Brain Storming Session

1. *Recommendations from the clinical group*

(i) An educational campaign should be developed to raise the level of awareness for dengue and DHF/DSS among all segments of the Indian population.

(a) Guidelines encompassing the signs, symptoms and diagnostic procedures for dengue, DHF/DSS should be distributed to medical teachers, practicing clinicians and health care workers. WHO should be approached to assist in the development and distribution of these guidelines.

(b) More stress should be placed on providing information on dengue, DHF/DSS in medical colleges, in both undergraduate and graduate level courses.

(c) Continuing Medical Education (CME) courses should be developed and offered to practicing physicians to update their knowledge of dengue case management, epidemiology, prevention and control.

(d) Practicing clinicians should be taught how to exclude dengue in any febrile patient with flushed face and without signs or symptoms of upper respiratory tract infection, and proven not to have malaria. Antibiotic treatment should not be started unless indicated.

(ii) Treatment of dengue and DHF/DSS

(a) Torniquet tests should be done on all patients suspected to have dengue fever, DHF/DSS.

(b) A flow chart to guide in the treatment of patients with DHF/DSS should be made available to all hospitals and primary health centres.

(c) Administration of drugs such as aspirin, ibuprofen, steroids and heparin should be avoided when treating dengue fever and DHF/DSS patients.

(d) Minimum diagnostic facilities for dengue, such as haemagglutination inhibition tests or ELISAs, should be available in all medical colleges. Antigens for this test should be of high quality and supplied through central source. The National Institute of Virology (NIV), Pune, should coordinate and provide quality control for dengue diagnostic reagents.

(e) Multicentric research on dengue, DHF/DSS should be encouraged.

2. *Recommendations from the surveillance group*

(i) Dengue haemorrhagic fever and dengue shock syndrome should be reportable diseases.

(a) WHO case definitions should be used.

(b) Existing reporting networks *ie* for poliomyelitis should be used whenever possible.

(c) Each state should define its own areas of surveillance.

(d) The National Institute of Communicable Diseases (NICD), Delhi, should present a coordinated reporting system to national authorities.

(ii) Each state should have laboratory diagnostic capabilities for dengue, DHF/DSS.

(a) The NIV should serve as a national reference centre to assist with technical problems and to maintain quality assurance and proficiency testing of local testing of the peripheral groups.

(b) Other laboratories should be used as surveillance centres.

(c) The IgM capture ELISA is the test of choice for routine diagnosis of dengue, DHF/DSS.

(d) WHO should be requested to assist with the initial acquisition of reagents and for training.

(iii) Entomological surveillance should be initiated and linked with vector control efforts.

(a) Entomological surveillance data (*ie* malaria control, *Ae. aegypti*, others) should be shared at the district level.

(b) National summaries could be collated, distributed and stored at the Vector Control Research Centre, Pondicherry.

(iv) Outbreak investigations should be linked to surveillance activities.

(a) NICD should prepare guidelines for surveillance and outbreak investigations.

(b) States should organise their own investigation teams.

(v) A training component should be included in all surveillance activities.

3. *Recommendations from the epidemiology and research group*

(i) Improve Indian research capacity: Apart from improving funding of research in the area through various funding agencies, there is an urgent need to develop and standardize research tests and reagents, and this in turn requires the training of scientists and designation and strengthening of present training centres. The WHO Collaborating Centre at the NIV can assist in standardizing research test systems in India with those of the international community.

(ii) Collaborative research should be stressed: Research test protocols and reagents can best be developed through high quality research projects that have reagent production and training as defined components. Such collaboration could be especially rewarding in the area of biotechnology, where modern techniques could be focussed on reagent production.

(iii) Clinical research should be linked with virological laboratory support through a multicentric approach.

(iv) Epidemiological research should focus on development of several sites where prospective epidemiological studies on DHF/DSS may be undertaken. Both clinical and epidemiological studies will generate research specimens that will require characterization and contribute to virological, immunological and pathogenesis research.

(v) Basic research on pathogenesis should be supported to contribute to human clinical pathogenesis studies.

(vi) To strengthen the prevention and control strategies of dengue, reports on the clinical burden of DHF/DSS should be plublished in the media.

(vii) To assist clinicians and public health workers, video films would be prepared highlighting the clinical features of dengue, DHF/DSS, treatment and vector control strategies.

4. *Recommendations from the vector control group*

(i) A nation-wide vector surveillance system based on existing institutions should be established to map and publish the present distribution of *Ae. aegypti*, and be used to restrict its spread to new areas.

(ii) The ecological factors that encourage the increase or decrease of vector population densities should be investigated.

(iii) An institute or agency should be designated to maintain database of *Ae. aegypti*, including distribution, population densities, insecticide resistance and vector incrimination information.

(iv) To ensure uniform reporting and utilization of information collected, standardized reporting of vector levels and indices should be used.

This report has been prepared by Dr. Kalyan Banerjee, Director, National Institute of Virology, Pune.

ABSTRACTS

Some Research Projects Completed Recently

Susceptibility of vectors of Japanese encephalitis in Karnataka to insecticides.

The study was carried out to test the susceptibility of Japanese encephalitis (JE) vectors of Karnataka to synthetic pyrethroids, organophosphate and organochlorine insecticides.

Both adult and larval population of *Culex tritaeniorhynchus*, *Cx. gelidus* and *Cx. fuscocephala*, from Mysore and Mandya districts of Karnataka were tested for their susceptibility to insecticies. Adult susceptibility tests were conducted with propoxur (0.1%), DDT (4%), malathion (5%), deltamethrin

(0.025%) and cyfluthrin (0.05%). Deltamethrin was found to be the most effective adulticide as it produced 100 per cent mortality in 15 min. DDT required 120 min to kill over 90 per cent mosquitoes compared to 30 min by malathion and cyfluthrin. *Cx. tritaeniorhynchus* was found to be more tolerant compared to the other two species.

The larval tests revealed differential tolerance of the vectors with regard the LC_{50} values of insecticides tested. LC_{50} values for cypermethrin, deltamethrin, fenthion, fenitrothion, temephos and malathion against larvae of *Cx. tritaeniorhynchus* were 0.000254, 0.000424, 0.039472, 0.075500, 0.025930 and 0.061908 respectively, compared to 0.000063, 0.000063, 0.002343, 0.011647, 0.001868 and 0.044426 respectively against larvae of *Cx. fuscocephala*. Results revealed high tolerance against insecticides in *Cx. tritaeniorhynchus* during larval stage also.

Experiments conducted to compare the effects of continuous and intermittent exposures with cypermethrin showed that multipulse exposure of two, 1 h duration exposures with a 6 h insecticide free period was more effective compared to continuous 2 h exposure in all 3 vector species tested.

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Publications:

Vijayan, V.A., Revanna, M.A., Vasudeva, K.S., Pushpalatha, N. and Poornima. Comparative susceptibility of two Japanese encephalitis vectors from Mysore to six insecticides. *Indian J Med Res 97:* 215, 1933.

Metabolic fate of membrane proteins in rat brain during chronic ethanol treeatment.

The study was carried out on Wister strain albino rats of both sexes (age 30 ± 2 days) to identify the changes in morphological, macromolecular and membrane architectural properties of neurons and astrocytes and to ascertain specific cell type vulnerability in brain following chronic *in vivo* ethanol treatment. Rats in the experimental group were given ethanol (5% V/V) as their only oral fluid upto the third month of age. Control animals were given only tap water. Another set of control animals were also maintained on tap water containing isocaloric sucrose substituted for ethanol.

Ethanol treated rats did not show any overt pathological or behavioural symptoms, excepting that their sleeping time was slightly increased and the animals were flaccid. Ethanol consumption induced a 9 per cent decrease in the body weight of rats, however, it did not cause much variation in the pattern of food intake.

Scanning electron microscopy revealed no change in the appearance of neurons from control and ethanol treated rats. However, astrocytes from ethanol treated rats displayed a characteristic smoothening of cell surface and lacked protruding elements which were seen on the surface of astrocytes from normal rats.

There was a decrease in DNA, RNA and protein contents in both neurons and astrocytes from ethanol fed rats, but the changes in RNA and protein were more conspicuous than those in DNA. Protein synthesis as determined by incorporation of ^{3}H-leucine was markedly diminished in both neurons and astrocytes. The enolase activity (cell specific marker) in the neuronal fraction from ethanol treated rat brain did not show any change but glutathione synthetase activity in the astrocytes was decreased by 35 per cent compared to normal rats. There were alterations in membrane protein composition and many of the membrane proteins disappeared in both neurons and astrocytes. Very few proteins seemed to have been synthesized after chronic ethanol exposure. N-terminal glycoproteins showed an increase in astrocyte cell membrane.

Studies using monoclonal antibody for neurofilamental protien-160 and polyclonal antibody for glial fibrillary acidic protein as well as identification of changes in these proteins revealed no significant effect of ethanol treatment suggesting that ethanol was probably not acting on the cytoskeletal elements at the concentration used in the study. The changes that were observed in the membrane fraction of astrocytes and neurons following chronic ethanol exposure might be relevant to the chemical basis of alterations in specific neuronal circuits during memory formations.

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Publications:

1. Prakash Babu, P. and Vemuri, M.C. Liver plasma membrane proteins in chronic ethanol intoxication. *Biochem Int 20:* 517, 1990.

2. Prakash Babu, P., Nagaraju, N. and Vemuri, M.C. Differences in plasma membrane proteins of chronic alcoholic rat brain. *Mem Biochem 9:* 227, 1991.

Metabolism of lipids and glycoconjugates in alcohol treated animals.

The study was carried out on Sprague-Dawley strain male albino rats to ascertain the effect of different concentrations of ethanol on the metabolism of lipids and glycoconjugates, effect of paracetamol on normal and alcohol treated rats as also the protective effect of testosterone and N-acetyl-D-cysteine on the alcohol and paracetamol treated rats. Comparative studies of the hepatotoxic effects of alcohol and paracetamol as also histopathological study of the changes in the liver tissue of the alcohol and paracetamol treated rats were also undertaken.

Serum glutamate oxalacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) and serum acid and alkaline phosphatases activities increased after alcohol and paracetamol treatment. SGOT, SGPT, alkaline phosphatase and acid phosphatase activities were further increased when paracetamol was administered to the alcohol treated rats.

Liver and kidney alcohol dehydrogenase (ADH) activities decreased in alcohol and paracetamol treated rats. This was further decreased after paracetamol administration in alcohol fed rats. Changes were also observed in the levels of liver GOT and GPT as also in the metabolism of lipids, lipid peroxidase and glycoconjugates in the liver and brain. As a result the level of malondialdehyde increased in the liver and brain of both alcohol and paracetamol treated rats. Alcohol administration also increased the deposition of glycolipids in the brain. Alterations in the metabolism of lipids, lipid peroxides and glycoconjugates were also observed in extraneural and extrahepatic tissues (*eg* heart, kidney and aorta).

Testosterone and N-acetyl-D-cysteine offered some protection against the toxic effects of alcohol and pracetamol singly or together. This protective effect was evident from the decrease in SGOT, SGPT and serum acid and alkaline phosphatase activities as also reduction in the levels of triglycerides, peroxidation products, serum lipid and glycolipids.

Hepatic histopathology of alcohol and paracetamol treated rats showed changes in the form of high degree of nuclear disintegration, chromatolysis, cytoplasmic and hydropic vacuolation as also necrosis, necrobiosis and central venous dilation. Liver cells of rats treated with alcohol alone showed appearance of binucleate cells, hyaline bodies, necrobiosis, portal inflammation and kupffer cell hyperplasia. Administration with only paracetamol produced nuclear disintegration, chromatolysis and necrosis. These changes confirmed the hepatotoxic effect of alcohol and paracetamol. Paracetamol produced synergistic effect when administered to alcohol fed rats.

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Publications:

Jaya, D.S., Augustine, J. and Menon, V.P. Role of lipid peroxides, glutathione and antiperoxidative enzymes in the alcohol and drug toxicity. *Indian J Exp Biol 30:* 453, 1993.

ICMR NEWS

The following meetings of various technical groups/committees of the Council were held:

Meetings of Task Forces (TFs)/Expert Groups (EGs)/Scientific Working Groups (SWGs)/Project Advisory Committees (PACs)/Scientific Advisory Committees (SACs) held at New Delhi:

Inter-Departmental meeting of TF on Product Development for Indigenization of Copper T 200 B Materials.	April 18, 1994
EG on Neem Oil for Contraception	April 19, 1994
SWG on Cardiovascular Diseases	April 27, 1994
PAC on Long-term Epidemiological Study on Gas Exposed Victims at Bhopoal	May 3, 1994

SAC of the Bhopal Gas Disaster Research Centre, Bhopal	May 3, 1994
PAC on Coronary Heart Diseases	May 10, 1994

Participation of ICMR Scientists in Scientific Events:

Dr. Kalyan Banerjee, Director, National Institute of Virology, Pune, participated in a WHO meeting on Emerging Diseases at Geneva (April 25-26, 1994).

Dr. C.P. Puri, Dy. Director, Institute for Research in Reproduction, Bombay, participated in the Steering Committee meeting of the Task Force on Post-ovulatory Methods for Fertility Regulation, as also in the Scientific Group meeting on Medical Methods for Inducing Abortion at Geneva (April 20-22 and 25-27, 1994 respectively).

Dr. Puri delivered a lecture on Antiovulatory Activity of RU 486 and related Substance at the Institute of Hormone and Fertility Research at Hamburg (May 2, 1994).

Dr. M.D. Gupte, Dy. Director (Senior Grade), CJIL Field Unit at Avadi, Madras and Dr. T. Shanta Devi, Dy. Director (Senior Grade), Tuberculosis Research Centre, Madras, participated in the joint meeting of the Steering Committees on Immunology (IMMYC) and Chemotherapy (THEMYC) of Mycobacterial Diseases and also in the third meeting of the Steering Committee on the Chemotherapy of Mycobacterial Diseases (THEMYC) at Geneva (April 27-30, 1994).

Dr. G.V. Satyavati, Director-General (Designate), participated in the meeting of the Task Force on Biotechnology of Medicinal and Aromatic Plants at the Department of Biotechnology, New Delhi (May 9, 1994).

Appointments:

Dr. V.K. Srivastava took over as Director of the Council's Regional Medical Research Centre, Dibrugarh w.e.f. April 28, 1994.

Workshops:

Workshops were organized for the staff of the Human Reproduction Research Centres on "Improving Health Education, Counselling and Technical Skills in Delivery of Contraceptives through Cafeteria Approach" at Guwahati nd Belgaum on April 21-22 and 24-25, 1994 respectively.

COUNCIL'S TRAINING PROGRAMMES FOR 1994-95

Virology

At the National Institute of Virology, Pune:

... Diploma in Medical Virology (June 1994-May1995).

Reproductive Biology

At the Institute for Research in Reproduction, Bombay:

... Workshop on Gynaecologic Cytology and Immunocytochemistry (September 26-October 1, 1994).

Endocrinology

At the National Institute of Nutrition, Hyderabad:

... Annual Training Course on Endocrinological Techniques and their Applications (August 1-September 16, 1994).

Nutrition

At the National Institute of Nutrition, Hyderabad:

... M.Sc.in Applied Nutrition (June 1,1994-February 28, 1995).

... Annual Training Course in Nutrition (December 1, 1994-February 28, 1995).

Occupational Health

At the National Institute of Occupational Health. Ahmedabad:

... Orientation Course on Occupational Health for Industrial Medical Officers (September 19-24, 1994).

... Training Course on Air Pollution Monitoring and Risk Assessment (October 19-25, 1994).

... Training Course in Pesticide Residue Analysis (December 5-9, 1994).

Medical Entomology

At the Vector Control Research Centre, Pondicherry:

... M.Sc.in Medical Entomology (from August 1994: for 2 years).

... Short-term Course on Malaria, Filariasis and Urban Vector Control for in-service candidates (June 1994).

Laboratory Animal Technology

At the Laboratory Animal Information Service Centre, National Institute of Nutrition, Hyderabad:

... Training Course for Laboratory Animal Technicians (June 15-July 31, 1994).

... Training Course for Laboratory Animal Supervisors (September 12- December10, 1994).

Haematology

At the Institute of Immunohaematology, Bombay:

... Training Course in Blood Group Serology and Blood Bank Methodology for Technicians (August 9-September 8, 1994).

... Training Course in Blood Group Serology and Blood Bank Methodology for Medical Officers (August 9-October 7, 1994).

INDIAN COUNCIL OF MEDICAL RESEARCH

The following areas have been identified for research in tuberculosis and other chest diseases: :

- Pharmacokinetics of antitubercular drugs in the young and elderly.
- Operational research studies in tuberculosis.
- Studies to evolve epidemiological tools/indicators.
- Diagnostic criteria for tuberculosis of organs other than the lungs.
- Bacteriology of community acquired pneumonia.
- Nontubercular chest diseases.
- Use of bronchoalveolar lavage in the diagnosis of obscure pulmonary pathology.

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