Ann Natl Acad Med Sci (India), 42(2): 85-93, 2006

National Standards for Medical Education
*A focus on quality enhancement**

Prof. J.S. Bajaj
Emeritus Professor, National Academy of Medical Sciences
President, Indian Association for the Advancement of Medical Education
Honorary President, International Diabetes Federation
Former Member, Planning Commission, Government of India

Introduction :

India has a rich heritage of medical and health sciences as is reflected in the antiquity of health care and medical education, practiced since the pre-historic times. Since the time India attained independence, there has been a rapid expansion in the education and training of practitioners of all systems of medicine. However, it is being increasingly realized that there has been a dichotomous growth of health services and manpower, each developing in isolation and without proper linkages in termporal and spatial dimensions (1).

With the dawn of new millennium, one cannot but agree with the observation that advances in biomedicine during last century have produced greater impact on human health than all the cumulative knowledge since the dawn of history. These exciting developments while unraveling the enormous potential of scientific creativity, also raise issues which are more fundamental. How well have these basic advances in bio-medical knowledge been translated into their practical applications to problems of human health and national welfare. How far have the physicians succeeded in fulfilling the expectations of the society, and in particular of the patients?(2)

Situation Analysis :

A set of following profound statements may act as a strong motivating force :

(i) 'Health is fundamental to national progress in any sphere. In terms of resources for economic development, nothing can be considered to be of higher importance than the health of the people which is a measure of their energy and capacity as well as of the potential of man-hours for productive work in relation to the total number of persons maintained by the nation. For the efficiency of industry and of agriculture, the health of the worker is an essential consideration'.

Correspondence : Prof J.S. Bajaj, Chief Consultant and Director, Diabetes, Endocrine and Metabolic Medicine, *Batra Hospital & Medical Research Centre, New Delhi-110062.* Based on the Inaugural Keynote Address delivered at the National Symposium on Quality of Medical Education at the 45th Annual Conference of the National Academy of Medical Sciences, 17-19 December, 2005 at Ahmedabad.

(ii) 'The output of the industrial worker in India is low compared with that of the worker in other countries. The productive capacity of the agricultural worker is comparatively low. The loss caused by morbidity in working time is enormous. To this must be added the expenditure to the individual and to the State in the provision of medial care.'

The statements have a most contemporary resonance and reflect the contour and content of newly emerging branch of 'Development(al) Economics', widely recognized and amplified since the award of Nobel Prize to Dr. Amartya Sen. However, these statements were recorded several years prior to the time of university graduation of most of modern messiahs of economics, and are from the First National Five Year Plan signed by the then Prime Minister of India, Sh. Jawahar Lal Nehru, on December 7, 1952 (3).

A major link in the nexus between ill-health, low productivity and low economic development is the inequitable, inefficient and poor quality of health services(4). The existing vast health infrastructure for health care delivery suffers from suboptimal performance. We must recognize that the quality of health services, their efficacy, efficiency, accessibility, sustainability and accountability depend ultimately on the performance of those who deliver the services. Improving the performance of health system in the final analysis depends on improving the knowledge, skills, motivation, work culture, and availability of the health workforce.

Health Workforce includes all human resources for health. The ILO estimates that approximately 38 million persons are currently employed in the health sector worldwide. Although health expenditure claims an increasingly important share of GDP, wage costs account for between 65% and 80% of the renewable health system expenditure.

Hence the need to optimize the performance of HRH. There are several categories of HRH : their role must be viewed in the context of health-directed tasks and activities. Broadly such tasks include :

A. Delivery of health care to individuals and families :

a. Physicians, Nurses, Pharmacists, Dentists, Physiotherapists and other health professionals and paraprofessionals.

b. Traditional healers, traditional birth attendants.

B. Delivery of Public Health Services :

Preventive and promotive health, environmental health, occupational health, industrial health.

One of the important reasons for the sub-optimal performance of health systems is a major disequilibrium between the quantity and quality of those assigned the task of delivery of services under these two broad categories, with delivery of public health services at a great disadvantage(5). Equally, if not more significant, is the poor quality and inappropriateness of the education and training of health paraprofessionals and allied health personnel. *Thus the need and rationale to enhance the quality of professional education.* What is the scope of such an endeavour?

"We cannot speak of 'quality'; we must speak of qualities. Not only are there different types of qualities, but also there are different aspects of quality : *quality of input, quality of process, and quality of output".*

While *quality of input* includes selection of students, selection of faculty, institutional infrastructure, financial resources and governance, *quality of output* reflects the competence, skills, attitude, motivation, and behavioural attributes of those who qualify as a result of reliable and valid methods of assesment. All these yardsticks provide valid measurements of some aspects of the quality of professional education. However, it is the *quality of process* which needs much sharper focus. Such measurements can only be validated against defined standards. *Hence the need of developing national standards.*

Planning for medical education in the twenty-first century must not only be in the context of contemporary needs and available technology, but must also take cognizance of a rapidly transforming society and of the newly emerging technologies, which are setting the direction of possible paradigm shifts in the near future. While articulating the thoughts and addressing the issues, besides critically examine data, whatever available, one needs to be guided by the wisdom of Bertrand Russell who struck a note of caution by stating, *"for it is not enough to recognize that all our knowledge is, in a greater or lesser degree, uncertain and vague; it is necessary, at the same time to learn to act upon the best hypothesis without dogmatically believing it"* (6). Some of the concepts and contents of medical education have already become a dogma; others are in the process of doing so. The final judgement regarding the soundness of educational policy planning for the twenty-first century shall be measured by one major yardstick: have we succeeded in imparting *relevance* and *excellence* to teaching and learning in medicine?(7)

Relevance of Medical Education

There is an on-going shift in modern health care: a shift towards an interest in the population as a complement to the physicians's traditional concern with the individual. The major health care problems of today cannot be understood without knowing how they occur in large segments of the population. The appropriate goal for the health professional is the enhancement of health, over and above that of preventing the disease or curing the ill.

While everyone would generally agree that the essential prerequisites of quality must be relevance and excellence, what is exactly intended by the use of these terms? The role of human resources of health, both individually and jointly, must be redefined to make them relevant to prevailing and prospective health needs, to acquire problem solving skills that are socially and culturally acceptable, affordable and effective, and to bring out changes in professional attitudes towards greater social responsibility and public accountability. Notwithstanding such an overarching view of relevance, it must also be recognized that at the highest level relevance may aim at collaboration between medical schools and different sectors of society so as to ensure that medical education and research programmes

effectively contribute to sustainable social, and national development.

What is implied by the term excellence? The Higher Education Quality Control Council, UK tends to describe excellence as 'The arrangement by which an institution discharges its corporate responsibility for the quality of the teaching and learning it offers by satisfying itself that its structures and mechanisms for monitoring its quality control procedures are effective and, where appropriate, they promote the enhancement of quality' (8).

The author firmly believes that *quality in medical education is a multidimensional concept,* which should embrace all its facets and functions: teaching and academic programmes, research and scholarship, faculty development, student counseling, academic infrastructure including library, laboratories and equipment, services to the community and above all, the academic environment.

Based on the consensus arrived at the Consultation arranged by the Education Commission for Foreign Medical Graduates (ECFMG) and the WHO in Geneva in 1994, Nancy Gary, past President of ECFMG, USA proposed a Matrix for establishing the quality of professional education (9). It has been suitably modified and adapted by the author to include :

Admission and support of qualified students.

Recruitment and retention of appropriate Faculty.

Institutional mission, aims and objectives.

The curriculum.

Evidence-based professional education.

A programme of quality assurance.

Accountability through academic and social audit.

The components of the Matrix need brief annotation. Selection of students must be based on the stated admission policy, including a clear statement on the process of selection. The policy should be reviewed periodically based on relevant societal and professional data, to comply with the social responsibilities of the institution and the health needs of community and society. As this issue has presently become a matter of state policy, national policy, and also of judicial pronouncements which have become areas of public debate, one must not rush in where wise men fear to tread. However, taking cognizance of contemporary needs, a reference must be made to a paradigm shift in the context of patient care which may have a bearing on student selection. The most significant aspect has been the recognition by the medical profession that today's patients are not prepared to be merely passive recipients of medical care. Today people want to be involved in decision-making process that affects their health; they want to know not only what is wrong with them but also what the choices of treatment are, and what risks are involved. To respond with sensitivity to this new patient care paradigm, we shall require a judicious mix of analytical reasoning and problem-solving skills. To these must be added a blend of empathy, communication skills, and a ready willingness to facilitate participatory management. Some of these behavioural attributes can only be ascertained by an aptitude test or a personal interview. Suffice

it to say that this requires consideration and needs to be discussed thoroughly in the context of prevailing circumstances.

Precise statements of *Institutional mission, goals and objectives* must be considered mandatory. Every medical institution must develop a Mission Statement. The institutional mission must take cognizance of present and future health needs of society, and must ensure a clear congruence between the institutional mission and the learning objectives pursued by each department and each member of the Faculty. In essence, *the Mission Statement of Faculty of Medicine in any institution is the conceptual, operational and moral compass.* It is an essential prerequisite for academic navigation and, as is often necessary, for midcourse remedial actions. As important as the statement of mission, are the statements of goals and objectives. What do we want our students to learn, and how can we express our goals succinctly and in a comprehensible manner? How do we ensure that the broad objectives are precisely understood by those assigned the task of facilitating student learning? This is the rationale of goals and objectives.

Before discussing curriculum in detail, there is a need to comprehend philosophy of learning. Sir William Broadbent has most succinctly described the knowledge dimension : *'there are two kinds of knowledge, one consisting in the accumulation and certification of facts and their natural relation; the other of a more profound character, comprehending the underlying significance of phenomena'*. The emphasis on the latter ensures life-long learning irrespective of the design of curriculum; learning must lead to comprehension of underlying significance of phenomena. It is only through such a philosophy of learning that we aim at true education as defined by George Bernard Shaw : *'Education is what you remember after you have forgotten what you have been taught'*. Finally, whatever be the method of curriculum planning and course design, it must be clearly understood that a curriculum is not a Time Table. It is a vital force in the teaching and learning of medicine and its various constituents need to be understood (10). These include :

- Curriculum definition
- Curriculum purpose and content
  Goals, objectives, context
- Curriculum development
  Principles and Percepts, Style, Structure
- Curriculum organization
  Basic educational concepts
- Curriculum design
  Systematic approach

Furthermore, it has to be continuously reviewed regarding its effectiveness and efficiency through a system of feed-back and evaluation. *'Curriculum is like water; leave it alone and it will seek the lowest level.'*

Academic staff selection, its retention, and continuing development subsequently, is a weak link in the quality matrix in almost every institution in the country (11). Every medical college must have an academic staff recruitment policy. Alternately, every state may have such a policy based on a collective thinking of all institutional heads in the state. Policy must have transparent staff selection criteria including scientific, educational, clinical, and managerial merit (the proportion of each may vary with the

job description/task analysis), as well as a track record of demonstrable attainments that foster a close relationship with the mission, goals and objectives of the institution. While the merit can be graded by assessing formal qualifications, professional experience, teaching experience, student recognition, research output, and peer recognition, the issues of local significance may include gender and SC or ST status. In essence, the policy must ensure recognition of meritorious academic activities including appropriate emphasis on research attainments, teaching qualifications obtained through attendance at workshops aimed at enhancing learning, track record as a teacher and demonstrable competence to plan and implement an educational programme.

It seems that education of educators is the most deficient component in the matrix of quality medical education. It is often said that medical education and politics were the only professions that did not require formal training. Therefore, what is equally, if not more important, is the Academic staff development. A lifelong education requires academic staff to continue updating their knowledge and improving their teaching skills and learning methods. Medical schools must establish appropriate academic staff development structures, mechanisms and programmes. National Academy of Medical Sciences, with a rich pool of unmatched talent, may initiate action to constitute a Task Force assigned the responsibility to develop a national model which may be replicated elsewhere.

Medical pedagogy is being challenged by a wide range of opportunities relating to technologies that are improving the ways in which knowledge can be generated, managed, disseminated and accessed. New information technology does not reduce the need for teachers capable of innovation, but changes their role in relation to the learning process.

A computer can assist learning but cannot impart the basic tenets of medical professionalism. Being a professional is more than being a technician. It is rooted in our moral nature : it is a matter not only of the mind and hand but also of the heart; not only of intellect and skill but also of character. Plato, in the *Meno* poses the question : *Can virtue be taught?*(12) He suggests that all depends on the meaning of 'virtue', and of 'teaching'. In this context, the 'virtue' of medicine is the ethics and ethos of health profession. Students will develop the knowledge and skills necessary to make ethically responsible patient care decision, only when they are in a position to recognize and emulate the teachers as role models(13).

Robotics may enhance motor skills but cannot be surrogate mentors. As Henry Adams aptly put it : *a teacher affects eternity; he can never tell where his influence stops.* A true mentor imparts a traditional perspective, as well as a futuristic potential, to the career choice and professional development. It is nurtured and fostered by intuitive foresight and exceptional devotion to new knowledge. Mentor is, and continues to remain, the trusted friend, the constant and always encouraging advisor, and eternal guide with selfless dedication.

Although a slight deviation from the present narrative, it may be of import to put

mentoring in its historical perspective. Mentor is the name of a Greek legend who is a principal character in Homer's Odyssey which describes the Trojan War and the life and times of Greek King Odysseus, also known as Olysses by Romans. When Odysseus was away from Greece for nearly 20 years, Mentor assumed the role of father, friend, and philosopher to Odysseus' son, Telemachus. Mentor was an exceptionally unselfish man, rich in patience, erudition, scholarship, and wisdom. Mentor gave most of his life to the education and counseling of Telemachus so that he could be a worthy son of Greece. The story of Mentor and Telemachus illustrates that a great and sustained personal investment lies at the heart of mentoring.

Continuing Professional Development (CPD) designates the period of education and training of medical professionals commencing after completion of basic medical education and postgraduate training, thereafter extending throughout their professional working life. The challenge that faces medical educators is to create a mechanism that encourages personal responsibility for maintaining competencies, yet can assure society that this responsibility is being, and has been, fulfilled. Effective CPD is characterized by a clearly felt but unmet need, and requires that structures are in place for reinforcing the learning accomplished.

Inherent in continuing professional development is a learning process which takes cognizance of evidence-based medicine. Nevertheless, it must be recognized and remembered that evidence-based medicine may usher a new era in clinical medicine, but it may best supplement and not supplant logic and reasoning as the basis of sound clinical judgment(13). What is the essence of clinical judgment? The experiential reasoning may not be statistically valid nor may it be purely deductive, yet it can be equally rigorous. In the ultimate analysis, clinical judgment is a blend and balance of experiential, personal and value-based aspects of medical decision-making with the full knowledge, but without the rigid application, of empirical evidence(14).

In the context of present narrative, what would be most appropriately included amongst the proposed methods and measurable indicators of quality in medical education. Quality assurance and appraisal may be *internal* based on self-evaluation which is an indication of the maturity and confidence of an institution. The parameters may include evaluation of incoming *students*, their scholastic qualities, academic qualifications, motivation and attitudes, as well as the assessment of the *faculty* and their qualities. The *academic ambience*, the *curriculum*, the *support infrastructure*, the *financial resources* and the *faculty compensation* constitute additional critical areas of academic audit as a part of continuing appraisal. Quality appraisal may also be *external* wherein universities of health sciences or other national agencies may evolve a regional system of accreditation of all medical schools in a defined geographic area. Such an appraisal may include peer view and site visits, providing a valid external assessment of quality, and assisting medical schools in the *attainment* and *maintenance* of *standards* of

structure, *function*, and *performance*. Finally, there must be a social audit which implies *ability to respond with sensitivity to the legitimate expectations of society*. The requisite attributes of those graduating from an institution may include the following(15) :

- Physicians must be *altruistic*, *compassionate* and *empathic* in caring for patients and must be *trustworthy* and *truthful* in all professional dealings.
- Physicians must be able to feel obliged to *collaborate* with other health care professionals to employ systematic approaches for *promoting*, *maintaining* and *improving* the health of *individuals* and *populations*.

Collaboration and functional cohesion between health care professionals is *sine qua non* for the success of health care delivery team. The essential prerequisite for *Act together* is *Learn together*. Multiprofessional (or interprofessional) education aims at imparting a task-centred, problem-based learning through interprofessional collaboration, based on commonality of learning modules and instructional strategies. It has been extensively reviewed earlier(16,17).

In summary, imparting and enhancing quality of professional education and training shall directly improve the quality of health services, thus transforming the present vicious cycle into a futuristic virtuous cycle. Eventually, the success of curriculum planning and implementation of educational programme directly depends upon those who administer and govern the system. Health is too serious a concern to be left in the hands of bureaucrats, nor can it be safely entrusted to those health professionals who do not exhibit the requisite sensitivity and lack the desired accountability to the people. Only a healthy symbiosis can lead to a paradigm shift(18).

The task is onerous and challenges are enormous. As Bertrand Russel said : *there is strength and weakness in those who stand for status quo : the strength that comes of tradition and the weakness that comes of lack of fresh thought*[6]. There is an urgent and imperative need of fresh thought to transform the mindset of those who have stood for *status quo*, for much too long for national good.

References :

1. Bajaj JS (1997). Report of the Steering Committee on Development of Human Resources for Health, Government of India, Planning Commission, Yojna Bhawan, New Delhi.
2. Bajaj JS (1997). Report of the Steering Committee on Health, Health Education and Biomedical Research. Government of India, Planning Commission, Yojna Bhawan, New Delhi.
3. First National Five-Year Plan (1952). Government of India, Planning Commission, New Delhi.
4. Bajaj JS (2000). Quality of Education in Health Sciences. *Ind. Jour. Med. Ed.* Vol. XXXVIII-IX : 1-2.
5. Bajaj JS (2003). Quality of Professional Education as a Determinant of Health Systems Performance. In : Symposium on Restructuring the Health Care Sector and Medical Education. World Conference on

Global Standards in Medical Education for Better Health Care. Copenhagen, 15-19 March. (Abstract)

6. Bertrand Russell (1993). Sceptical Essays. Routledge Publishers, London (Reprinted).
7. Bajaj JS (1998). Second Radhakrishnan Memorial Lecture. *Education in Health Sciences : Relevance and Excellence.* In : Medical Education and Health Care. Indian Institute of Advanced Study, Shimla, p1- p23.
8. Stewart A (2000). Quality Assurance : A Framework for ensuring the achievement of WHO thrusts in Medical Education over the past 25 years. *Ind. Jour. Med. Ed.* Vol. **XXXVIII-IX** : 8-14.
9. Gary NE (2000). What are the Essentials of Quality Medical Education? *Ind. Jour. Med. Ed.* Vol. **XXXVIII-IX** : 3-7.
10. Lowry S (1993). Medical Education. London : BMJ Publishing Group.
11. Bajaj JS (1999). Planning for Medical Education in the Twenty-First Century. In: Advancing Horizons in Medical Edcuation, Kuwait University. p94- p105.
12. Rouse WD (1961). Great Dialogues of Plato. Mentor Books, New York.
13. Bajaj JS (2004). Ethics in Clinical Practice. In : Pande JN, Bajaj JS (eds.). CME-Monograph, Series VII. *Natl. Acad. Med. Sci.* p1- p16.
14. Bajaj JS (2002). Evidence-Based Medical Education. *Ind. Jour. Med. Ed.*Vol. **XL** : 1-2.
15. Cohen JJ (1998). Leadership for Medicine's Promising Future. *Academic Medicine.* **73** : 132-137.
16. Report of a WHO Study Group on Multiprofessional Education of Health Personnel : The Team Approach (1988), Learning together to work together for Health, Technical Report Series 769, World Health Organization, Geneva.
17. Bajaj JS (1994). Multiprofessional Education as an Essential Component of Effective Health Services. *Medical Education.* **28** : 86-91.
18. Bajaj JS (1997). Public Health and National Economy : An interactive interdependence. In : Nath KJ (ed). Public Health in India, Five Decades. All India Institute of Hygiene & Public Health. p1 – p10.

Ann Natl Acad Med Sci (India), 42(2): 95-108, 2006

Management of severe acute pancreatitis – How far have we come

J.D. Wig
Department of General Surgery
Postgraduate Institute of Medical Education & Research
Chandigarh-160012

ABSTRACT

Background: The management of severe acute pancreatitis has undergone considerable changes.

Methods: This review presents our single centre experience in patients with SAP. Stratification of morphological severity was based on CECT. Patients with clinically suspected IPN routinely underwent image-guide FNA. Indications for operative treatment were FNA proven infection of pancreatic and extrapancreatic necrosis, persisting or deteriorating organ failure and/or abdominal complications. Careful necrosectomy of pancreatic and extrapancreatic necrosis in combination with continuous, postoperative closed lesser sac lavage was performed. We sampled necrotic pancreatic tissue (following necrosectomy), and blood for cytokines such as interleukin (IL-2, 6, 10, 12), tumour necrosis factor (TNF∝), and reactive nitrogen intermediates.

Results: Our principal findings from necrotic pancreas were that stimulated T cells had fewer IL-2 and IL-4 producing cells than controls. Production of IL-2 is less in alcoholic and biliary pancreatitis and may indicate the impaired cellular immunity and increased susceptibility to infection seen in AP. We measured and correlated the percentages of peripheral blood mononuclear cells that contain IL-6 and IL-12 and compared these with APACHE scores. Patients with severe pancreatitis had higher IL-6 values and a correlation was seen between IL-6 value and APACHE III score and based on our results it seems logical to use both APACHE III and IL-6 percentages to assess severity. Monocyte function is affected in AP as shown by reduced HLA-DR numbers and lowered TNF-∝ producing cells. We also studied the role of nitric oxide and showed significantly higher levels of RNI as compared to controls. RNI levels were higher in patients who developed sepsis (199.5 vs. 134.7 n mol/ml) and in nonsurvivors as compared with those of survivors (216.0 vs. 140.1 n mol/ml). Patients with higher serum nitric oxide levels are at a significantly higher risk of sepsis and mortality. Fifty eight patients underwent pancre-

Correspondence : Dr. J.D.Wig, Professor & Head, Department of General Surgery, P.G.I.M.E.R., Chandigarh. 160012. Col. Sangham Lal Oration 2003-2004 delivered at the Annual Meeting of NAMS at Mumbai, 2004.

atic necrosectomy after a median period of 28 days after the onset of illness. Preoperative image-guided aspiration and/or drainage was carried out in 41 patients. The overall mortality was 29%.

Conclusions: The review highlights the challenges posed by SAP. Rapid advances are taking place in the assessment of severity, markers of immune activation, understanding the pathophysiology of the disease and development of anti-inflammatory therapy through targeting of tumor necrosis factor, cytokines, interleukins and other inflammatory mediators. Fine needle aspiration of the necrosis is used to detect microorganisms and IPN is an indication for surgical intervention. Enteral nutrition via the nasojejunal tube or through a feeding jejunostomy tube placed at the time of necrosectomy has become the preferred route of feeding and is feasible, well tolerated and does not exacerbate the disease. Prophylactic antibiotics with good penetration in pancreatic tissue are recommended in SAP. Surgical necrosectomy is combined with continuous closed lesser sac lavage to continuously remove necrosis and debris. There is variability in both the nature and timing of surgical necrosectomy. The role of percutaneous, radiological, endoscopic, and laparoscopic drainage techniques are being defined and show promising results.

Key Words: Severe acute pancreatitis, acute necrotizing pancreatitis, cytokines, organ failure, infective pancreatic necrosis, inflammatory mediators, computed tomography, enteral nutrition, necrosectomy, closed lesser sac lavage.

INTRODUCTION

Acute pancreatitis (AP) is a relatively common inflammatory disease of the pancreas, predominantly caused by symptomatic gallstone disease and excessive alcohol intake and is a lethal disease (1). The mortality of severe acute pancreatitis (SAP) is as high as 30-40%. Its pathogenesis is poorly understood. One of the key questions concerning the pathogenesis is why some patients develop only a limited local inflammatory response whereas others progress to systemic inflammatory response syndrome (SIRS) and multisystem organ failure (MSOF) (2,3). Pulmonary complications have long been recognized to account for a significant number of deaths occurring within the first week of AP (4). The profiles for AP depend upon the degree of pancreatic necrosis (PN) and the intensity of MSOF (5). Several inflammatory mediators have been documented to be present at increased concentrations in the plasma of patients with SAP(6). Interruption of these mediators has the potential to improve outcome in these patients (3). The value of giving antibiotics to patients with SAP remains unresolved (7). Infected pancreatic necrosis (IPN) is a serious complication of AP occurring in 20-40% of patients and one half

of all deaths in AP are attributed to IPN (7,8). Early recognition of IPN by image-guided fine needle aspiration (FNA) or radiological evidence of gas followed by prompt surgical management is the best way to reduce morbidity and mortality (9,10). The main unresolved issues in SAP include who require surgery, what is the optimal time to intervene and what technique should be employed (11-13). In a recent survey, no consensus was reached on optimum timing of surgery, and only 53% would operate on a patient with positive results from FNA (14). A recent study has suggested that patients with IPN and severe disease can generally be managed nonsurgically without compromising prognosis and outcome (15).

Do we know more about the pathogenesis?

The pathogenesis of AP in many ways presents the same dilemmas that have confronted the clinicians since 1889(16). Fitz's (17) contributions was based in large part on the principles of cellular pathology and presented analysis of 53 patients distinguishing between haemorrhagic, suppurative and gangrenous forms of the disease. He proclaimed that an operation in the early stage of the disease is extremely hazardous. He laid the foundation of our present knowledge of the pathology, symptomatology and treatment of this so frequently and suddenly fatal disease. Chiari (18) postulated that the underlying pathophysiological mechanism of the disease was pancreatic autodigestion- the pancreas succumbs to its own digestive properties. Opie (19) proposed that a gallstone lodged in ampulla might occlude both the common bile duct and the pancreatic duct forming a common channel that would allow reflux of bile into the pancreatic duct with activation of pancreatic enzymes and pancreatitis. Mayo (20) described acute fulminating pancreatitis and referred to this as haemorrhagic pancreatitis occurring in fleshy alcoholic males. He recommended that if a patient is seen during the first 48 hours, the abdomen should be opened and free drainage should be furnished. He noted better outcome in patients with subacute pancreatitis and localized septic accumulations that can be opened and drained.

Fitz described the initial injury in AP as one of oedema, white cell infiltration, and microvascular disruption (17). It has recently become clear that the initiating events need to be evaluated at the level of acinar cell (1). The key to mortality in the disease is related not to histological or morphological changes but to distant manifestations in organs such as lung, kidney and cardiovascular system. Patients may develop MSOF within first week of illness, 40% develop it later (21,22). Despite the differences in the initiating triggering factor, the pathophysiological events in the pancreas and systemically follow a common pathway (2). Enormous efforts have been made to unravel the complexities and intricacies surrounding the pathogenesis of pancreatitis and SIRS (5,23). The pathway between the initial pancreatic injury, the systemic response, and organ failure is mediated by a variety of inflammatory mediators in response to local tissue damage (6). It is generally believed that proinflammatory mediators released play an important role in pathogenesis (24).

We have tried to examine possible mediators in patients with SAP. We have tried to define the cytokine phenotype of individual T cells and macrophages obtained from the necrotic pancreas. This has the advantage of avoiding the pitfalls of serum cytokine measurements such as the presence of circulating cytokine inhibitors. The macrophages isolated from the necrotic tissue were examined for intracellular cytokines to scrutinize the recruitment of macrophages at the affected site. In both alcoholic and biliary pancreatitis CD4+T cell subsets produced reduced amount of IL-2. There were significantly fewer CD4+ T cells in alcoholic disease than in controls, but were significantly more in biliary cases. Alcoholic patients had more IL-4 producing cells than the biliary group. There was a significant difference between the number of CD4+ T cells that expressed IL-10 in controls and the alcoholic group. No significant differences were found in the number of cells that expressed IFN-γ in the control and biliary groups, but these were significantly more in the alcoholic group. There were fewer IL-2 producing CD8 +T cells in alcoholic disease. There was a significant reduction of IL-4 positive cells in alcohol group and a pronounced reduction in biliary group. Cells from alcoholic group produced more IFN. Our results indicate that production of IL-2 is less in alcoholic and biliary pancreatitis. This reduction may indicate the impaired cellular immunity and increased susceptibility to infection that is seen in AP. We also detected reduced amounts of IL-4 in alcoholic disease and a pronounced reduction in biliary disease. It is conceivable that during the inflammatory process the expression of IL-4 is down regulated (25). There was increased release of proinflammatory cytokines together with reduced IL-10 activity in alcoholic disease, while those with biliary disease had a pronounced downregulation of their anti-inflammatory response. The variable development of local and systemic complications could be a result of such activities.

Our results have shown that local response of cytokines varies with the aetiology of the disease. Biliary group had less local proinflammatory response than the alcoholic group. IL-6 and IL-12 levels were measured in peripheral blood cells on day of admission. Percentage of positive cells for IL-6 and IL-12 were significantly higher in SAP as compared to mild disease. IL-12 values were high in alcoholic acute pancreatitis as compared to patients with biliary pathology.

We monitored the cytokine concentration from the drainage fluid following closed lesser sac lavage. Increased IL 12 levels were observed in fluid from both groups. IL-6 levels were increased in alcoholic cases as compared to biliary group. Can these observations be used to stop the lavage following necrosectomy is difficult to answer at this stage.

We have made an attempt to understand the role of lymphocyte monocyte system in limiting the destructive process in AP. HLA-DR expression on monocytes was significantly reduced in SAP. Assessment of intracellular cytokines in immunocompetent cells could be a useful tool to study the relationship between the different mediators and their role in cases of dysregulation as seen in AP (26).

We correlated the percentages of peripheral blood mononuclear cells that contain IL-6 and IL-12 and compared with APACHE III score in patients with SAP IL-6 positive peripheral blood mononuclear cells reflect the severity of AP. Cutoff percentage for IL-6 and IL-12 positive peripheral blood mononuclear cells were >25% and >9% respectively. Based on our results it would seem logical to use both APACHE score and IL-6 percentages to assess severity in AP(27).

We have studied adhesion and activation molecules in order to evaluate dysregulation. ICAM_1 in the pancreas is a critical link in the development of tissue injury and organ dysfunction. The adhesion molecules showed a unanimous rise in the blood and tissue samples. Monocyte function is affected in AP as shown by reduced HLA-DR numbers and lowered TNF-∝ producing cells (28).

The role of nitric oxide in AP has been a subject of intense research and controversy. Serum nitric oxide levels represent the extent of cytokine response induced by pancreatic inflammation. We tried to correlate the blood levels of nitric oxide in patients with SAP with computed tomography severity score and APACHE II scores. Patients with high levels of nitric oxide in the blood are at a significantly higher risk of sepsis and mortality. High APACHE score and reactive nitrogen intermediates (RNI) levels on admission were associated with an increasing number of organs failed. RNI levels were higher in those who subsequently developed biliary sepsis. High RNI levels were associated with an increased risk of mortality. RNI levels were higher in nonsurvivor group as compared with the survivor group (29).

Several pathologic responses occur in AP and include oedema, inflammation, parenchymal cell injury and death including disorganization of cellular ultrastructure, necrosis, and apoptosis (1). Ischemia of tissue in the pancreas participates in the mechanism of pancreatitis. A recent study compared the angiographic abnormalities with perfusion abnormalities by contrast enhanced computed tomography (CECT). The correlation between angiography and CECT demonstrated the vasospasm in small and medium sized vessels of the pancreatic bed led to decreased downstream perfusion. These areas of decreased perfusion resulted in necrosis in upto 50% of the patients. The mortality was related directly to the severity of the vasospasm on the initial examination (30). Another experimental study has shown that endothelial nitric oxide synthase activation (e Nos) leads to increased blood flow in the pancreas and in the absence of its activation, the increased blood flow is blocked, resulting in worsening of severity of pancreatitis (31).

Even today the precise means by which diverse elements induce pancreatitis remain unclear. A significant morbidity and mortality associated with the disease reminds us that we are still chasing a destructive path rather than interrupting or controlling the events of the disease. The continuing challenge is to translate the findings into treatment strategies. Based on our results we hope that in future we will witness immune modulation therapy for arresting systemic manifestations of AP and to institute organ support early in the course of the disease.

Have advances in imaging techniques changed the evaluation or management of these patients with SAP?

Advances in imaging techniques have changed the evaluation and management of patients with SAP (32). The imaging modality of choice currently is multidetector row computed tomography (MDCT). The role of imaging in AP is to confirm the diagnosis, to identify necrosis, their topographical location and to determine the presence of complications (fluid collection, and vascular abnormalities). Resolution can only be confirmed by repeat CT study. CT imaging should be performed in patients with SAP with persisting organ failure, signs of sepsis, or clinical deterioration after admission. The early detection of PN signified severe disease and is used as a prognostic indicator in the initial evaluation. Computed tomography severity index (CTSI) grades the severity of pancreatitis on the basis of degree of pancreatic inflammation and necrosis and is a significant advance in the assessment of patients with AP. The anatomical site of necrosis is clearly better than its crude extent in predicting the risk of complications (33). Patients with necrosis in the head of pancreas have a severe course of the disease.

CTSI does not correlate with the development of organ failure or pancreatic complications. The modified CTSI (inflammation, necrosis, extrapancreatic complications) shows improved correlation with organ failure, severity, the occurrence of infection, the need for surgical or radiological intervention, and hospital stay (34).

Other imaging modalities that have been studied include magnetic resonance cholangiopancreatography (35), tissue harmonic imaging(36), and leukocyte scintigraphy(37). Magnetic resonance severity index scores correlated with serum levels of C - reactive protein at 48 hours, duration of hospitalization, Ranson score, and morbidity from local and systemic complications (35).

In our own experience, CTSI ranged from 4-10 in 64.4% of patients. Systemic complications and culture proven infection were higher in these patients ($p<0.05$). A recent study has shown that CTSI is superior to Ranson criteria and APACHE II score in predicting acute pancreatitis outcome, the mortality, length of stay and complications were higher in patients with a CTSI >5 than that in patients with CTSI<5(38).

The extent of PN appears to be a useful determinant of prognosis. Mortality increases markedly in patients with necrosis involving >30% of the gland (39,40).

Early detection of vascular complication by CT is important (41-43). Massive haemorrhage after PN results from a ruptured pseudoaneurysm, severe capillary or venous bleeding may be seen in the immediate aftermath of PN (42).

Infected necrosis is diagnosed by CT guided FNA (9). It is recommended that FNA should be performed 7-14 days after the onset of pancreatitis in all patients with persistent symptoms and greater than 30% PN, and those with small areas of necrosis and clinical suspicion of sepsis (44). Image guided FNA may need to be repeated to detect IPN.

Innovations that have improved outcome.

The treatment of SAP continues to be largely supportive therapy and subse-

quently to treat specific complications. Management has alternated between aggressive intervention and intensive nonsurgical support (45). Treatment currently focuses on three factors of supposed pathophysiologic significance. First it is generally accepted that secondary infection of PN constitutes one of the crucial factors in the progression from SIRS to sepsis in patients with SAP. Second recent data suggest that is possible to diminish SIRS by giving specific anti-inflammatory components. Thirdly the importance of early intensive care therapy and organ support is being increasingly emphasized. The findings of a recent study suggest that these patients can be managed conservatively and surgery can be avoided without compromising prognosis and outcome. Sixteen patients (APACHE II score 18.1 (11-33), Ranson score 5.9 (4-10) were managed with medical treatment alone with a mortality of 12.5%, six patients recovered without further complications, 10 patients developed single or multiple organ failure (15).

The real challenge is the development of a more accurate predictor of severity and organ failure. The determination of the severity is difficult as all methods exhibit a significant uncertainty. Various scoring systems have been used to make the prediction. However their value in everyday clinical practice is limited as they are cumbersome, and requiring multiple measurements (45). Severity is now determined by Atlanta criteria (46), CTSI (33) and modified CTSI (34).

Issue of antibiotic prophylaxis: Infection of PN can lead to local and systemic septic complications which can cause MSOF and account for a mortality of upto 30%. Its incidence tends to peak in the third week of disease. Several randomized controlled trials suggest that prophylactic antibiotics can reduce morbidity and mortality in these patients by preventing pancreatic infections (47). A recent study has concluded that prophylactic antibiotics did not reduce the incidence of IPN in patients with SAP (7). Fungal infections appear in 15 to 30% of cases [50]. In our own experience, Candida infection was observed in 17.9%, and Candida spp. were isolated from pancreatic tissue in 36.7% .There is conflicting evidence regarding association of secondary fungal infections with the widespread use of antibiotic prophylaxis (51,52). However, studies are needed to accurately quantify the incidence and risk of fungal colonization, any association with antibiotic prophylaxis, and association with a significant increase in mortality. A recent study has shown that prophylactic dosage of antifungal agents can reduce the incidence of fungal infections in patients with SAP (53). There is insufficient evidence to recommend antifungal prophylaxis in patients with SAP (10). Our practice is to use prophylactic broad spectrum antibiotics in all patients with SAP which is corroborated by current recommendations (48, 49).

Specific anti-inflammatory therapy: Recent data suggests that it is possible to diminish SIRS by giving specific antiinflammatory components. There is a therapeutic window between onset of symptoms and development of organ failure during which anticytokine therapy may be successful (10). The role of many inflammatory mediators has been investigated (54). However in large multicentre trials the role of platelet activating factor antagonist could not be confirmed (55,56). The failure of beneficial

effect may be due to the fact that patients had already developed MSOF before the beginning of the treatment (10).

Nutritional support: It seems logical to meet the calorie and protein requirement of these patients with SAP to protect intestinal barrier function reducing bacterial translocation from the gut (57). Timely institution of feeding is important to prevent malnutrition and has been demonstrated to be safe (1). Evidence has emerged from clinical trials that enteral nutrition is superior to parenteral nutrition (58,59). Parenteral nutrition is associated with an enhanced systemic inflammatory response and increased septic complications (49). Enteral nutrition by means of a nasojejunal feeding tube helps in preventing atrophy of the intestinal mucosa and loss of barrier function (10). A recent metaanalysis has shown that infection rates, rates of surgical intervention and hospital stay were significantly lower in those fed enterally (60). A recent consensus statement also recommends that enteral nutrition be used in preference to parenteral nutrition after initial resuscitation (10). Enteral nutrition modulates acute phase response, improves immune function, reduces mortality rate and reduces risk of infections (61). Our current practice is to place a nasojejunal feeding tube once the ileus has settled down. Following necrosectomy, we routinely place a feeding jejunostomy tube for enteral nutrition. Postoperative enteral nutrition has been shown to be safe and to decrease infectious complications (62).

Pancreatic necrosectomy

The main contribution to the overall management of SAP is pancreatic necrosectomy (8). Surgery is indicated when there are signs of MSOF, clinical sepsis with no improvement on intensive care treatment, and CT shows extensive areas of PN with confirmation of bacterial infection by FNA. The principles include optimal debridement with a postoperative management concept that maximizes drainage of the residual and ongoing necrosis, and evacuation of retroperitoneal exudates and debris. Necrosectomy should favour an organ preserving approach and should be delayed to permit proper demarcation of pancreatic and peripancreatic necrosis (8). A recent consensus conference recommended debridement in those with IPN or abscess confirmed by radiologic evidence of gas or results of FNA (48).

Several approaches described are open transperitoneal approach (11-13), laparoscopic (63), and the extraperitoneal translumbar approach (64,65). Another unresolved tissue is what drainage technique to use- continuous closed lesser sac lavage (CLSL) (12-13), planned staged relaparotomy(11), and the open packing technique (66). Rau *et al* (12) reported an overall mortality of 25% following necrosectomy and CLSL. In our experience of 58 patients who underwent necrosectomy and postoperative CLSL, it was possible to start irrigation in 48 patients, the overall mortality was 29% (13). In another study, planned staged reoperative necrosectomy using an abdominal zipper in the treatment of necrotizing pancreatitis reported 34% hospital mortality (11). Extraperitoneal translumbar approach (64) with periodically programmed retroperitoneal endoscopy enables to explore the retroperitoneal space under direct

visual guidance (avoids contamination of the peritoneal cavity). In a study of 11 patients, there was no technique- related morbidity and no subsequent operations needed, the mortality rate was 27% due to MSOF, and the integrity of abdominal wall was preserved (65).

Laparoscopic technique with transperitoneal infracolic approach as an alternative is also considered in the treatment of PN. Laparoscopic necrosectomy is feasible with dislocation of the infected sequestrum and followed by closed irrigation of the lesser sac. The main difficulty is with evacuation of necrotic material due to its viscous consistency. Out of 13 patients who underwent laparoscopic pancreatic necrosectomy, 11 survived and made a full recovery (63).

Percutaneous necrosectomy has been introduced to remove debris in a minimally invasive way (67,68), and is stated to be more successful later in the course of the disease . This is indicated in patients with organized necrosis after the acute episode, after open surgery to remove residual devitalized tissue thereby avoiding multiple operations, and in patients with devitalized tissue following percutaneous drainage (68). In a study of 6 patients undergoing percutaneous video- assisted necrosectomy, sepsis control was achieved in all patients with no mortality (69).

Endoscopic necrosectomy and lavage has added a new therapeutic dimension to the management of PN and pancreatic abscess (70). The efficacy of endoscopic treatment of pancreatic necrosis and abscess has been demonstrated (71). Seifert *et al* (72) described endoscopic ultrasound directed transmural puncture into necrotizing pancreatitis or abscess followed by tract dilatation and repeated endoscopic debridement of lesser sac. Endoscopic therapy was successful in resolving the infected necrosis or the abscess in 12 of 13 patients with minor bleeding in 4 cases in a recent study (73). They state that this aggressive endoscopic approach expands the potential for endoscopic treatment in these patients. However, the effectiveness of endoscopic therapy needs further trials.

Acute gall stone pancreatitis (AGP)

It is still a matter of controversy whether there is a need for early endoscopic retrograde cholangiopancreatography and endoscopic sphincterotomy (ES) in acute gallstone pancreatitis (74). Pezelli *et al* (75) evaluated the effect of ductal decompression in patients with AGP and common bile duct stones in two groups: ES within 24 hours of admission (n=21) and conservative medical treatment (n=21). AP worsened in one patient in ES group, in contrast to seven patients in the group receiving medical treatment ($p<0.02$). In another study, early ES had no impact on the outcome or period of hospitalization (76). Uhl *et al* (77) advocate that at least 3 weeks should elapse in patients with SAP before undertaking cholecystectomy because of an increased infection risk. Fagniez and Rotman (78) have confirmed that early biliary surgery worsens the prognosis in SAP. Postponing the biliary operation until after the acute attack reduces the need for early exploration and drainage of the pancreas (79). Nealon *et al* (80) in a retrospective analysis of patients with moderate to severe gallstone associated pancreatitis advocate that

cholecystectomy be delayed. Delayed treatment is associated with lesser chances of infecting fluid collections and lesser complications of cholecystectomy. In our experience, ES was carried out in patients with jaundice or cholangitis.

Conclusions

The management of patients with SAP has changed over recent years with readily available imaging and image guided interventions and improvement in intensive care unit management. The degree of necrosis and presence of infection are the crucial determinants of the outcome in patients with PN. The primary objective in the management of patients with PN is supportive therapy and subsequently to treat specific complications. Another objective is to limit the severity of pancreatic inflammation and necrosis and the systemic effects by removing causative factors. Image guided FNA is recommended in patients with SAP, greater than 30% necrosis and those with clinical suspicion of sepsis. A positive FNA result or the presence of gas in a collection is usually an indication for intervention. Patients with SAP require repeated imaging as guidance to progress. With the advent of FNA to diagnose infection, more timely operations can be performed.

The strategy of management of patients with PN has changed dramatically. The main contribution to the overall management of SAP is necrosectomy. Necrosectomy is performed usually at around 3-4 weeks. The standard open technique followed by postoperative lavage, and / or drainage to evacuate devitalized tissue has recently been challenged by various minimally invasive approaches- percutaneous, endoscopic or laparoscopic determined by size and localization of necrotic lesions, sequestra and septa.

Given the advances in care and ongoing challenges, treatment of SAP will continue to be an

area of innovation and discovery. Treatments targeted at specific events may be able to halt development of or progression and may impact disease control.

REFERENCES

1. Pandol S J. (2005). Acute pancreatitis. *Curr Opin Gastroenterol*; **21**: 538-543.
2. Granger J, Remick D. (2005). Acute pancreatitis: models, markers, and mediators. *Shock*; **24**(suppl 1): 45-51.
3. Pezzilli R, Ceciliato R, Barakat B, Corinaldesi R.(2004). Immune manipulation of the inflammatory response in acute pancreatitis. What can be expected? *J Pancreas (JOP)*; **5**: 115-121.
4. Makhija R, Kingsnorth AN. (2002). Cytokine storm in acute pancreatitis. *J Hepatobiliary Pancreas*; **9**: 401-410.
5. Dugernier T, Laterre P-F, Wiltebole X, et al. (2003). Compartmentalization of the inflammatory response during acute pancreatitis. *Am J Respir and Crit Care Med*; **168**: 148-157.
6. Uehara S, Gothoh K, Handa H, Tomita H, Tomita Y. (2003). Immune function in patients with acute pancreatitis. *J Gastroenterol Hepatol*; **18**: 363-370.

7. Isenmann R, Runzi M, Kron M, *et al.* (2004). Prophylactic antibiotic treatment in patients with predicted severe acute pancreatitis: a placebo –controlled double blind trial. *Gastroenterology*; **126**: 997-1004.

8. Leppanienii A, Kemppainen E. (2005). Recent advances in the surgical management of necrotizing pancreatitis. *Curr Opin Crit Care*; **11**: 349-352.

9. Banks PA. (2005). Computed tomographic fine needle aspiration (CT-FNA) is valuable in the management of infected pancreatic necrosis. Debate: Is computerized tomographic fine needle aspiration helpful in the management of infected pancreatic necrosis. *Am J Gastroenterol*; **100**: 2371-2374.

10. Nathens AB, Curtis JR, Beale RJ, *et al.* (2004). Management of the critically ill patient with severe acute pancreatitis. *Crit Care Med*; **32**: 2524-36.

11. Radenkovic DV, Bajec DD, Tsiotos GG, *et al.* (2005). Planned staged reoperative necrosectomy using an abdominal zipper in the treatment of necrotizing pancreatitis. *Surg Today*; **35**: 833-840.

12. Rau B, Bothe A, Beger HG. (2005). Surgical treatment of necrotizing pancreatitis by necrosectomy and closed lavage: Changing patient characteristics and outcome in a 19 year single centre series. *Surgery*; **138**: 28-39.

13. Wig JD, Mettu SR, Jindal R, Gupta R, Yadav TD. (2004). Closed lesser sac lavage in the management of pancreatic necrosis. *J Gastroenterol Hepatol*; **19**: 1440-1446.

14. King NK, Siriwardena AK. (2004). European survey of surgical strategies for the management of severe acute pancreatitis. *Am J Gastroenterol*; **99**: 719-728.

15. Runzi M, Niebel W, Goebell H, Gerken G, Layer P. (2005). Severe acute pancreatitis: nonsurgical treatment of infected necrosis: *Pancreas*; **30**: 195-199.

16. Leach SD, Gorelick FS, Modlin IM. (1990). Acute pancreatitis at its centenary. *Ann Surg*; **212**: 109-113.

17. Fitz RH. (1889). Acute pancreatitis: a consideration of pancreatic haemorrhage, hemorrhagic, suppurative, and gangrenous pancreatitis and of disseminated fat necrosis. *Boston Med J*; **120**: 181-7,205-7, 229-35.

18. Chiari H. (1896). Ulber die Selbestverdauung des menschlichen pankreas. *Z Heilk*; **17**: 69-96.

19. Opie EL. (1901). The etiology of acute hemorrhagic pancreatitis. *Johns Hopkins Hosp Bull*; **12**: 182-8.

20. Mayo WJ. (2005). The surgical treatment of pancreatitis. Surg Gynecol Obstet 1908, VII; 607(quoted by Sarr MG, van Heerden JA, Kendrick ML. William J Mayo's vision a century later) *J Am Coll Surg*; **201**: 324-326.

21. Mentula P, Kylanpaa M L, Kemppainen E, *et al.* (2005). Early prediction of organ failure by combined markers in patients with acute pancreatitis. *Br J Surg*; **92**: 68-75.

22. Johnson CD (2004). Abu-Hilal, members of the British Acute Pancreatitis Study Group. *Gut*; **53**: 1340-1344.

23. Neoptolemas JP. (2003). Compartments that cause the real damage in severe acute pancreatitis. *Am J Resp Crit Care Med*; **168**: 141-142.

24. Manabe T, Okada Y. (2004). Serum markers for predicting the severity of acute pancreatitis. *J Gastroenterol*; **168**: 141-142.

25. Bhatnagar A, Wig JD, Vaiphei K, Majumdar S. (2001). Intracellular cytokines in cells of necrotic tissue from patients with acute pancreatitis. *Eur J Surg*; **167**: 510-517.

26. Bhatnagar A, Wig JD, Majumdar S. (2003). Immunological findings in acute and chronic pancreatitis. *ANZ J Surg*; **73**: 59-64.

27. BidarKundi GK, Wig JD, Bhatnagar A, Majumdar S. (2002). Clinical reference of intracellular cytokines IL-6 and IL-12 in acute pancratitis, and correlation with APACHE III score. *Br J Biomed Sci*; **59**: 85-89. .

28. Bhatnagar A, Wig JD, Majumdar S. (2001). Expression of activation, adhesion molecules and intracellular cytokines in acute pancreatitis. *Immunology Letters*; **77**: 133-141.

29. Mettu SR, Wig JD, Khullar M, Singh G, Gupta R. (2003). Efficacy of serum nitric oxide level estimation in assessing the severity of necrotizing pancreatitis. *Pancreatology*; **3**: 506-514.

30. Takeda K, Mikami Y,Fukuyama S, *et al.* (2005). Pancreatic ischemia associated with vasospasm in the early phase of human acute necrotizing pancreatitis. *Pancreas*; **30**: 40-49.

31. Di Magno MJ, Williams JA, Hao Y, *et al.*(2004). Endothelial nitric oxide synthase is protective in the initiation of Caerulein induced acute pancreatitis in mice. *Am J Physiol Gastrointest Liver Physiol*; **287**: G80-G87.

32. Balthazar EJ,Robinson DL,Megibow AJ,Ranson JH. (1990). Acute pancreatitis: Value of CT in establishing prognosis. *Radiology*; **174**: 331-336.

33. Mortele K J, Mergo PJ, Taylor HM, *et al.* (2004). Peripancreatic vascular abnormalities complicating acute pancreatitis: contrast enhanced helical CT findings. *Eur J Radiol*; **52**: 67-72.

34. Mortele KJ, Wiesner W, Intriere L, *et al.* (2004). A modified CT severity index for evaluating acute pancreatitis: improved correlation with patient outcome. *AJR*; **183**: 1261-1265.

35. Arvanitakis M, Delhaye M, De Maertelaere V, *et al.* (2004). Computed tomography and magnetic resonance imaging in the assessment of acute pancreatitis. *Gastroenterology*; **128**: 715-723.

36. Garg M, Sandhu M, Sood B, *et al.* (2004). Tissue harmonic imaging in the evaluation of acute pancreatitis. *Australas Radiol*; **48**: 117-122.

37. Moreno-Osset E, Lopez A, de la Cueva L, *et al.* (2005). 99m TC hexamethy-lprophylene amineoxime leucocyte scintigraphy in acute pancreatitis: an alternative to contrast enhanced computed tomography? *Am J Gastroenterol*; **100**: 153-161.

38. Leung T-K, Lee C-M, Chen H-C, *et al.* (2005). Balthazar computed tomography severity index is superior to Ranson criteria and APACHE II scoring system in predicting acute pancreatitis outcome. *World J Gastroenterol*; **11**: 6049-6052.

39. Paulson EK, Vitellas KM, Keogan MT, *et al.* (1999). Acute pancreatitis complicated by gland necrosis: spectrum of findings on contrast enhanced CT. *AJR*; **172**: 609-613.

40. Vitellas KM, Paulson EK, Enns RA, *et al.* (1999). Pancreatitis complicated by gland necrosis; evaluation of findings on contrast-enhanced CT. *J Comput Assist Tomogr*; **23**: 898-905.

41. Mendelson RM, Andorson J, Marshall M, Ramsay D. (2005). Vascular complications of pancreatitis: *ANZJ Surg*; **75**: 1073-1079.

42. Flati G, Andren-Sandberg A, La Pinta, *et al.* (2003). Potentially fatal bleeding in acute pancreatitis: pathophysiology, prevention and treatment. *Pancreas*; **26**: 8-14.

43. Balthazar EJ, Fisher LA. (2001). Hemorrhagic complications of

pancreatitis: radiologic evaluation with emphasis on CT imaging. *Pancreatology*; **1**: 306-13.

44. UK (2005). Working Party on Acute Pancreatitis, United Kingdom guidelines for the management of acute pancreatitis. *Gut*; **54** (Suppl 3): 1111-1119.
45. Dejong CHC, Greve J W-M, Soeters PB. (2001). Nutrition in patients with acute pancreatitis. *Curr Opin Crit Care*; **7**: 251-256.
46. Bradley EL III. (1993). A clinically based classification system of acute pancreatitis: summary of the international symposium in acute pancreatitis, Atlanta, GA, September 11 - 13, 1992. *Arch Surg*; **128**: 586-590.
47. Fretland AA. (2005). Antibiotic prophylaxis in acute pancreatitis- in the evidence good enough. *Tidsskr Nor Laegeforen*; **125**: 1323-1325.
48. Uhl W, Warshaw A, Imrie C, *et al.* (2002). IAP guidelines for the surgical management of acute pancreatitis. *Pancreatology*; **2**: 565-573.
49. Flint R, Windsor J, Bonham M. (2004). Trends in the management of severe acute pancreatitis: interventions and outcome. *ANZJ Surg*; **74**: 335-342.
50. King NKK, Siriwardana PP, Wood B, Siriwardena AK. (2005). Trends in fungal colonization of pancreatic necrosis in patients undergoing necrosectomy for acute pancreatitis. *HPB*; **7**: 120-123.
51. De Waele, JJ, Vogelaers D, Blot S, Colardyn F. (2003). Fungal infections in patients with severe acute pancreatitis and the use of prophylactic therapy. *Clin Infect Dis*; **37**: 208-13.
52. Gloor B, Muller CA, Worni M, *et al.* (2001). Pancreatic infection in severe pancreatitis: the role of fungus and multiresistant organisms. *Arch Surg*; **136**: 592-6.
53. He Y-M, Lv, X-S, Ai Z-L, *et al.* (2003). Prevention and therapy of fungal infection in severe acute pancreatitis: a prospective clinical study. *World J Gastroenterol*; **9**: 2619-2621.
54. Bhatia M. (2002). Novel therapeutic targets for acute pancreatitis and associated multiple organ dysfunction syndrome. *Curr Drug Targets Inflamm Allergy*; **1**: 343-351.
55. Larvin M, Ammorri B, Mc Mahon MJ, *et al.* (2001). A double-blind randomized placebo- controlled multi-centre trial to evaluate efficacy and safety of two doses of lexipafant in acute pancreatitis therapy. *Pancreatology*; **1**: 279-280.
56. McKay CJ, Imrie CW. (2004). The continuing challenge of early mortality in acute pancreatitis. *Br J Surg*; **91**: 1243-1244.
57. Abou-Assi S, Craig K, O'Keofe SJD. (2002). Hypocaloric jejunal feeding is better than total parenteral nutrition in acute pancreatitis: results of a randomized comparative study. *Am J Gastroenterol*; **97**: 2255-62.
58. .Gupta R, Patel K, Calder PC, *et al.* (2003). A randomized clinical trial to assess the effect of total enteral and total parenteral nutritional support on metabolic, inflammatory and oxidative markers in patients with predicted severe acute pancreatitis (APACHE II >or =6). *Pancreatology*; **3**: 406-13.
59. Zhao G, Wang CY, Wang F, *et al.* (2003). Clinical study on nutrition support in patients with severe acute pancreatitis. *World J Gastroenterol*; **9**: 2105-2108.
60. Marik PE, Zaloga GP. (2004). Meta-analysis of parenteral nutrition versus enteral nutrition in patients with acute pancretitis. *BMJ*; **328**: 1407-1409.

61. Olah A, Pardavi G, Belagyi T, *et al.* (2002). Early nasojejunal feeding in acute pancreatitis is associated with a lower complication rate. *Nutrition*; **18**: 259-262.

62. Braunschweig CL, Levy P, Sheean PM, Wang X. (2001). Enteral compared with parenteral nutrition: a metaanalysis. *Am J Clin Nutr*; **74**: 534-542.

63. Cuschieri SA. (2005). Laparoscopic surgery: current status, issues, and future developments. *The Surgeon*; **3**: 125-138.

64. Castellanos G, Pinero A, Serrano A, *et al.* (2002). Infected pancreatic necrosis: Translumbar approach and management with retroperitoneoscopy. *Arch Surg*; **137**: 1060-1063.

65. Castellanos G, Pinero A, Serrano A, *et al.* (2005). Translumbar retroperitoneal endoscopy. *Arch Surg*; **140**: 952-955.

66. Tsiotos GG, Luque-den leon E, Soreide JA, *et al.* (1998). Management of necrotizing pancreatitis by repeated operative necrosectomy using a zipper technique. *Am J Surg*; **175**: 91-98.

67. Horvath KD, Kao LS, Ali A, *et al.* (2001). Laparoscopic assisted percutaneous drainage of infected pancreatic necrosis. *Surg Endosc*; **15**: 677-682.

68. Cheung M-T, Ho C N-S, Siu K-W, Kwok PC-H. (2005). Percutaneous drainage and necrosectomy in the management of pancreatic necrosis. *ANZJ Surg*; **75**: 204-207.

69. Risse O, Auguste T, Delannoy P, *et al.* (2004). Percutaneous video-assisted necrosectomy for infected pancreatic necrosis. *Gastroenterol Clin Biol* ; **28**: 868-871.

70. Lochan R, Manas DM, Gould K, *et al.* (2005). Minimal access techniques in the management of infected pancreatic necrosis. *HPB*; **7**(Suppl1): 66-67.

71. Baron TH, Thaggard WG, Morgan DE, Stanley RJ. (1996). Endoscopic therapy for organized pancreatic necrosis. *Gastroenterology*; **111**: 755-764.

72. Seifert H, Webrmann T, Schmitt T, Zeuzem S, Caspray WF. (2000). Retroperitoneal endoscopic debridement for infected peripancreatic necrosis. *Lancet*; **356**: 653-5.

73. Seewald S, Groth S, Omar S, *et al.* (2005). Aggressive endoscopic therapy for pancreatic necrosis and pancreatic abscess: a new safe and effective treatment algorithm. *Gastrointest Endosc*; **62**: 92-100.

74. Neuhau H. (2000). Therapeutic pancreatic endoscopy. *Endoscopy*; **32**: 217-225.

75. Pezzilli R, Billi P, Barakat B, *et al.* (1998). Effect of early ductal decompression in human biliary acute pancreatitis. *Pancreas*; **16**: 165-168.

76. Hammarastrom LE, Andersson R, Stridbeck, *et al.* (1999). Influence of bile duct stones on patient features and effect of endoscopic sphincterotomy on early outcome of edematous gallstone pancreatitis. *World J Surg*; **23**: 12-17.

77. Uhl W, Muller CA, Krahenbuhl L, Schmid SW, Scholzel Z, Buchler MW. (1999). Acute gallstone pancreatitis. Timing of laparoscopic cholecystectomy in mild and severe disease. *Surg Endosc*; **13**: 1070-1076.

78. Fagniez PL, Rotman N. (1998). Effect of the date of surgery on the prognosis of severe acute biliary pancreatitis. Associations of Surgery research. *Chirurgie*; **123**: 368-372.

79. Runkel NS, Buhr HJ, Herfath C. (1996). Outcome after surgery for biliary pancreatitis. *Eur J Surg*; **162**: 307-313.

80. Nealon WH, Bawdunaik J, Walser EM. (2004). Appropriate timing of cholecystectomy in patients who present with moderate to severe gallstone induced pancreatitis with peripancreatic fluid collections. *Ann Surg*; **239**: 741-749.

Ann Natl Acad Med Sci (India), **42(2)**: 109-134, 2006

Virus - Host Interplay During Japanese Encephalitis Virus Infection

Asha Mathur
Professor & Head, Department of Microbiology,
Saraswati Medical and Dental College, Lucknow.

ABSTRACT

Our studies of an extensive epidemic of encephalitis at Gorakhpur (U.P.) in 1978, involving large segment of population with high mortality, revealed that the epidemic was due to Japanese encephalitis virus infection. Etiological diagnosis was based on virus isolation from brain of a fatal case and by serology. Large number of cases had polymorphonuclear leucocytosis. Our further studies had demonstrated the association of production of antigen-specific macrophage derived chemotactic factor (MDF) with polymorphonuclear leucocytosis during JEV infection in patients and in experimental mouse model. MDF is a 10 KD protein with plethora of biological effects. Immunological potentialities of purified MDF e.g. ability to activate neutrophils, regulate granulocytosis, increase capillary permeability with leakage of plasma protein, erythrocytes and cellular infiltrate in brain, lowering of serum iron levels with accumulation of iron in the spleen, degrade virus via triggering respiratory burst, generation of toxic oxygen radicals and production of nitric oxide has been discussed and is postulated that this may be one of the important mechanisms of natural immunity in controlling the initial stage of infection.

Our in vitro studies suggest the development of good humoral as well as cell mediated immune response to JEV experimentally in mice. Following infection, IgM antibodies, having neutralization and haemagglutinating activities appear first followed by IgG antibodies. JEV also triggers development of cell-mediated immunity (CMI) simultaneously. The leucocyte migration inhibition test was taken as an index of CMI. Passive transfer of JEV-specific antibodies or immune spleen cells provided short-term protection against challenge infection with JEV. Primary infection in few is followed by establishment of latent infection both experimentally in mice and in human cases. Latency is associated with depressed cell-mediated immune response. We demonstrated for the first time human transplacental transmission of JEV and developed the mouse model. Our studies had revealed an interesting observation of transmission of virus in consecutive pregnancies.

Correspondence : Asha Mathur, Professor & Head, Department of Microbiology, Saraswati Medical and Dental College, Lucknow. Glaxo Oration 2004-2005 delivered at the Annual Meeting of NAMS at Ahmedabad, 2005.

Japanese encephalitis (JE)

JE is the principal mosquito-borne viral encephalitis in Asia. It is caused by Japanese encephalitis virus (JEV), a member of the family Flaviviridae. It is one of the most important examples of zoonotic viral encephalitis, affecting all age groups with highest incidence of disease among children. The vast majority of JE infections are inapparent, only 1 in 25 to 1 in 1000 infections result in symptomatic illness. The variation could be due to number of factors including endemicity, exposure to mosquitoes, pre-existing antibodies to flaviviruses and virus strain differences (1). The virus is found in vast geographic area and a rising trend in JE cases and spread in new habitats and environment has been observed. It is endemic throughout Far East and South East Asia and recently, has spread to other non-Asian regions e.g. Papua New Guinea and the Torres Strait Islands of Australia (2). Approximately 3 billion people live in endemic regions and about 50,000 cases of JEV infection and 10,000 deaths are reported each year. Approximately 50% of survivors have permanent neuropsychiatric sequelae (3). Virus persistence in the human nervous system has been reported in 5% of patients with JEV associated encephalitis (4).

JEV is transmitted to humans by rice-field breeding mosquito, mainly Culex tritaeniorhynchus. Virus exists between mosquito and pigs or water birds. Man is a "dead-end" host and plays no role in perpetuating the virus. Variation of JE transmission patterns occur within individual countries and from year to year. In subtropical and tropical endemic areas, risk is present throughout the year with occurrence of sporadic cases, but is accentuated during rainy season and early dry season when mosquito populations are higher. In temperate regions of Asia and the northern tropical regions, JEV is transmitted seasonally.

Presence of JEV has been shown serologically in different parts of India since the mid fifties (5). The virus was first isolated in 1958 from three sporadic cases of encephalitis in Tamil Nadu (6). The first major epidemic occurred in 1973 in Bankura and other districts of West Bengal (7). Subsequently, number of JE epidemics have been reported from states of Karnataka, Uttar Pradesh, Bihar, Assam, Goa, Kerala and Haryana (8-10). At present, JE is not only endemic in many areas, it is also spreading to naïve non-endemic areas.

The Virus :

JEV is an enveloped; plus-sense single stranded RNA virus, approximately of 11 kilobase, and is antigenically related to other flaviviruses including dengue. It contains several structural (capsid - C, premembrane - prM and envelop-E) and non-structural (NS1 to NS5) polypeptides, which are encoded by a single, long open-reading frame (ORF). Nucleotide sequence analysis of prM and E gene has suggested that there are five JEV genotypes (11,12). Though JEV isolates are grouped in distinct clusters within each genotype, but no clear distinct geographical boundaries appear to exist between the distinct clusters (11). Complete genomic sequence of the JE strains GP78, isolated at Gorakhpur (8) in Northern India and P20778 isolated from

Vellore in the Southern India are phylogenitically closer to the Chinese SA14 isolate (13) than to the Nakayama strain from Japan.

Pathogenesis:

Following an infective mosquito bite, the viral replication may occur locally and in regional lymph nodes or vascular endothelial cells. The incubation period is about 5-15 days. After hematogenous spread in the host, JEV replicates in number of organs and generates a rapid inflammatory response including peripheral neutrophil leucocytosis and mononuclear and polymorphonuclear leucocytosis infiltration in extra neural tissue (14). Clinically, the infection of JEV results in increased levels of cytokines such as macrophage derived chemotactic factor (MDF), TNF-α and interleukin (1L-8) in the serum and cerebrospinal fluid (15-17). The low levels of inflammatory mediators appear to play a protective role (18) where as increasing concentrations of cytokines are related to severity of illness (16). Viral invasion of central nervous system (CNS) occurs probably via vascular endothelial cells (19). Certain neurotransmitter receptors are involved in the binding of JE virion to the cells in CNS. The response to cerebral infection with JEV in mice is characterized by significant recruitment and extravasation of immuno inflammatory cells, predominantly macrophages, T cells and neutrophils, to sites of viral replication in the brain (14,19). Immuno histochemistry of JE infected human brain tissue indicates the presence of viral antigen in the thalamus, hippocampus, substantia niagra and medulla (20). The host response to infection is central to the effective control and ultimate clearance of invading pathogens.

JEV infection ranges from a febrile headache syndrome to an acute and possibly fatal encephalitis. Neurological sequelae is present in about 30 – 50% of the survivors (21). Viral persistence in the human nervous system has been reported in approximately 5% of patients with JEV associated encephalitis (4,22).

Clinical Features:

JEV being a neurotropic virus, targets the CNS and the clinical picture varies according to the severity of CNS involvement age, nutritional status of the affected individual and on the degree of neuronal maturity as well as of the presence of intercurrent infections (23). In endemic areas children below 15 years of age are most commonly affected. The incubation period in man varies between 1 to 15 days. The course of JEV infection can be divided into 3 stages. The prodromal stage (1-5 days), acute encephalitic stage (6-10 days) and convalescent stage. The prodromal stage is marked by abrupt onset of fever, malaise, anorexia, headache, nausea and vomiting without involvement of the CNS. Although spontaneous recovery from this stage is known, the disease may progress to the acute encephalitic stage which is characterized by signs of involvement of CNS. Onset of this stage is rapid with fever, headache, nuchal rigidity, convulsions, altered consciousness progressing to coma. This is followed by appearance of focal meningeal and extrapyramidal signs, such as dull mask like face, muscular rigidity and cranial nerve palsies. During convalescent

stage neurological signs tend to improve and patient either becomes normal or may develop sequelae. Features suggesting of Parkinsonism (24) or Guillen-Barre Syndrome (25) may occur.

Immune Response:

The host defence in JEV infection is mediated by the cooperative activity of various components of phagocytic cells and different subsets of B and T effecter cells. Though the relative contribution of individual components has not been well understood, the innate immune response, after primary JEV infection, plays an important role in the restriction of infection or clearance of invading pathogens. JEV infection stimulates macrophage derived neutrophil chemotactic factor (MDF) production (15), which is involved in early host defence (18,26). Humoral immunity is an important component of immune response to JEV. First, there occurs IgM response usually within 7 days of infection. Early appearance of IgM antibodies in CSF has been correlated with favourable outcome in JE (27). This is followed by appearance of IgG antibodies. Experimental studies in mice have shown that passive transfer of anti JEV polyclonal as well as monoclonal antibodies provide protection against JEV infection (28,29), while presence of JEV specific immune complexes in CSF has been associated with fatal outcome (20).

The development of cell mediated immunity has been demonstrated experimentally in mice and linked with recovery from JEV infection as adoptive transfer of immune spleen cells provides protection against JEV challenge (28). Induction of JEV specific memory T cells after primary JEV infection has been demonstrated (30,31). JEV also generate protective cytotoxic T lymphocyte response (32)

Diagnosis:

In JE, during acute encephalitic stage peripheral blood counts show leucocytosis with neutrophilia. There is elevated cerebrospinal fluid (CSF) pressure. CSF examination shows marked pleocytosis (cell count 10 to 980 X 10^6/L), mild elevation in protein level and normal glucose concentrations. The electroencephalogram (EEG) shows diffuse theta and delta waves burst suppression and epileptiform activities, but these features are non specific. Brain computed tomography (CT) and magnetic resonance imaging (MRI) techniques may be helpful in distinguishing JE from herpes encephalitis (33).

Confirmatory diagnosis of JE can be carried out either by virus isolation or demonstration of antigen or by serology in CSF, blood or other specimens. Virus isolation from blood is rare, however virus could be isolated from CSF during acute phase of illness or from brain tissue in fatal cases by inoculation of specimens intracerebrally into infant mice, or various cell cultures or toxohynchites splenders larvae (34). Identification of virus is carried out by neutralization test or indirect immunofluoroscence test using JEV specific monoclonal antibiodies (35) or ELISA test. The isolation of virus is correlated with poor prognosis (27). A number of sensitive tests described for rapid antigen detection in CSF are reverse passive haemagglutination test for detection of soluble antigen (36) on immunofluoroscence tests for detection of cell bound antigen (35).

Conventional serologic tests are haemagglutination inhibition (HAI), neutralization and complement fixation tests. Demonstration of four fold or greater rise in HAI antibody titres in acute and convalescent serum samples is widely used antibody detection method for JE diagnosis. Cross reactive antibodies with other arboviruses make serodiagnosis difficult. IgM antibody capture ELISA (Mac-ELISA) is the method of choice to demonstrate virus specific antibody in CSF / blood in early phase of illness. Nitrocellulose membrane based IgM capture dot enzyme immuno-assay (Mac DOT) and antibody radioimmuno assay are some latest antibody detection methods. A reverse transcriptase polymerase chain reaction (RT-PCR) has been used for rapid detection and identification of JEV (37).

Treatment & Control:

For JE treatment no specific antiviral therapy is available. It is essentially symptomatic. Maintenance of fluid and electrolyte and good nursing care is essential. Control of pyrexia, seizures and cerebral edema is necessary. Control measures are aimed at the vector, vertebrate animal host or susceptible human population. Vector control can be achieved by reducing mosquito breeding which include draining out of stagnant water and spraying or fogging with insecticides such as pyrethrum and malathion.

Vaccines:

The main strategy for control of JE is vaccination of susceptible human population, which involves three main aspects such as age of the individual, cost of the vaccine and immune response to the JE. Three main types of vaccines are currently in use and different approaches for development of new effective vaccines are in progress.

Mouse brain inactivated vaccines:

The inactivated mouse brain derived vaccine is prepared by injection of the JE virus into infant mouse brain. The purified vaccine is in use since 1968. It is effective in practically eliminating the disease. As the two strains of JEV may be in circulation, a bivalent vaccine has been developed in 1984. Subcutaneous injection of vaccine of 1ml in adults and 0.5 ml in children below 3 years of age is recommended. For effective protection three doses are recommended over a period of 30 days (days 0, 7 and 30), followed by a booster one year later and subsequent vaccination every 3-4 years in endemic areas.

Cell culture derived inactivated vaccine:

An effective inactivated cell culture vaccine (Chinese SA-14-2 strain) for the control of JE has been used in China since 1967. Inactivated JE vaccine is produced in primary hamster kidney cells and is administered to children aged 6-12 months in two doses spaced 1 week apart followed by a booster dose in second year (38)

Live attenuated vaccine:

An effective, inexpensive live attenuated JE vaccine has been developed using SA 14-14-2 strain of JEV in primary hamster kidney cell line and is in use in China. It is safe with few untoward reactions. The effectiveness of two doses of vaccine given 1 year apart is 97.5% and that

of one dose is 80%. Protection lasts atleast for 5 years (39). Various studies are directed towards the development of the newer JE vaccines. Pox virus (Canary pox and vaccinia) based JE recombinant vaccine has been constructed (40). In recent years subunit vaccine have been developed to induce protective immune response against JE. Immunization with recombinant plasmid DNA vaccine containing the JEV prM and E genes elicit neutralizing antibodies against JE virus (41). A chimera vaccine (ChimeriVax-JE) in which the structural proteins prM and E of yellow fever (YFV 17D) are replaced with those of JEV SA 14-14-2 vaccine strain is under evaluation as a candidate vaccine against JEV (42). However, all these approaches are in the experimental stage and need further evaluation.

Virus - host interplay during Japanese encephalitis virus infection :

In one of our initial study of an epidemic of encephalitis which occurred in Eastern districts of UP during post monsoon season in 1978, involving large segment of population with high fatality, we investigated large number of the cases admitted in different hospitals of Gorakhpur and Deoria. The etiological diagnosis of JE in these patients was established either by isolation of virus from CSF or brain or by demonstration of antigen in CSF by indirect immunofluoroscence or by the presence of virus specific IgM in serum or four fold or greater rise in antibody titre in serum.

Leucopenia with lymphopenia is a frequent feature of most of the viral infections, while JEV infection, results polymorphonuclear leucocytosis with variable effect on different components of the peripheral blood leucocytes (44). To validate the clinical observations, mouse model was developed. One set of our studies had shown that after JEV entry the host virus replicates in a number of organs and generates a rapid inflammatory response with mononuclear and polymorphonuclear cells infiltration in various tissues (14). Figure-2 shows the total leucocyte counts and percentage neutrophils at different periods in the peripheral blood of JEV inoculated mice (i.p.) JEV infection induced leucocytosis with neutrophilia. The mean count in JE infected mice was 20,000+ 580/mm^3 on day 11 after inoculation, while in normal controls it was 8,400+212/mm^3. There was a significant rise ($p<0.001$) in percentage neutrophils in JEV infected mice (60+1%) compared with controls (21 + 3%) (43).

The inflammatory response within the central nervous system in viral encephalitis is regulated through a network of cytokines and chemokines. Chemokines are a family of small (≈8 to 14 KD), structurally related chemoattractant cytokines that are produced upon activation by different cellular sources like T cells , monocytes, microglia, astrocytes, fibroblasts, epidermal and endothelial cells (46). Chemokines are important regulators of leucocyte trafficking to sites of immune challenge or tissue damage (47,48). The chemokine fall into four categories which are defined by a cysteine motif: CXC, CC, XC and CX_3, where C is cysteine and X is any aminoacid residue. The CXC chemokine sub family includes interleukin-8.

The mechanism of neutrophil leucocytosis in Japanese encephalitis virus infection is not known. Present data demonstrate the production of a previously unrecognized neutrophil chemotactic cytokine secreted by the macrophages in spleen during JEV infection. Figure -3 shows that the maximum production of neutrophil chemotactic activity was obtained on day 6 following inoculation of 0.3 ml of 10 LD_{50} of JEV intraperitoneally (mean migration / hpf = 39+1.9). To delineate the cell type responsible for chemotactic activity in vitro, normal mouse splenic macrophages, T and B lymphocytes ($5x10^6$ cells/ml) were cultured and stimulated with 10^3 LD_{50} of JEV. The chemotactic activity was obtained by JEV stimulated macrophages only while T and B lymphocytes supernatants failed to attract neutrophils. On purification of macrophage derived supernatants (MDF) on Sephacryl S-200 column (Fig. 4) and further on Pep-S column, it migrated as a single band of 10 k Da on polyacrylamide gel (15). The MDF reacted specifically with anti-MDF antisera on Western blot. MDF was found heat resistant and show no change after 4h incubation with proteases. It results distinct leucocytosis with neutrophilia after purified MDF inoculation in mice (Table1).

Further our studies have demonstrated that infection of JEV results in increased level of cytokines such as macrophage derived chemotactic factor and interleukin-8 (IL-8) in serum and cerebrospinal fluid (CSF) of JE patients (17), JEV stimulates human peripheral blood monocytes (hMDF) which secrete a chemotactic cytokine named as hMDF which generates a rapid inflammatory response including neutrophil leucocytosis. Figure-5 shows the presence of virus induced hMDF by immunoblot assay in more than 80% of acute phase sera of JE confirmed patients. The observation revealed that mortality rate increased with increasing concentrations of 1L-8 in the serum and cerebrospinal fluid in JE patients(Figure 6) (17).

In another set of experiments the role of MDF in the pathogenesis of JE and its effect on the integrity of the blood – brain barrier was studied. Our finding demonstrated that JEV along with MDF could cause an alteration in the permeability of the blood brain barrier resulting in the leakage of plasma protein bound Evans blue dye and radiolabelled erythrocytes in brain. Figure 7 shows [^{51}Cr]-labeled erythrocytes and leakage of Evans blue dye in the brain of mice at different periods after JEV inoculation (i.c.). The maximum erythrocyte and dye protein leakage occurred at day 6 after JEV infection, while with MDF the leakage was at 1 hour post inoculation (figure 8), which directly correlated with the maximum production of MDF in vivo. Complete restoration of the integrity occurred by 4h (19). In other set of experiments, the effect of MDF in micro-vasculature during JE infection was studied. Figure 9 shows the intradermal inoculation of MDF in rabbits caused [^{51}Cr]-labeled neutrophil emigration and peak accumulation of neutrophils into the injected sites at 1 hour following MDF inoculation (49).

Further observations had revealed significant fall in serum iron levels during JEV infection in humans (35) and in mice (14). To address the mechanism for

hypoferraemia our studies revealed that JEV induced MDF was associated as possible regulator for the lowering of the serum iron levels in mice. The findings presented in table 2 showed that purified preparation of MDF caused significant depression (42%) in serum iron levels as measured after 24 and 48 hours after inoculation. Further, the iron staining of MDF inoculated mice spleen has revealed, increased iron deposition within splenic macrophages at 24 and 48 hours, which gradually declined at 72 hours after MDF injection (14)

Host defence mechanism during JE infection:

The host response to infection is central to the effective control and ultimate clearance of invading pathogen. The response to JEV infection in mice is characterized at the pathologic level by significant recruitment and extravasation of immuno inflammatory cells, predominantly macrophages, T cells and neutrophils to sites of viral replication in spleen as well as the brain (14,19). The neutrophil and macrophages are key effector cells involved in early host defence. The role of neutrophils in antiviral defence has been scarcely studied. It may act as effector cells in antibody - dependent cell cytotoxicity (ADCC) as seen in herpes simplex virus infection (50) or release of interferon like substances in response to certain viral antigen (51), or degrade the virion (52). One of our studies was undertaken to explore the contribution of neutrophils towards host defence against Japanese encephalitis virus. The ability of neutrophils to degrade the phagocytosed JE virion, via triggering the respiratory burst and generation of toxic radicals had been investigated.

In order to establish the interaction of neutrophils with particulate (virus) or soluble substances (MDF) in activation of oxidative and non-oxidative mechanisms, the phagocytic activity of normal neutrophils, exposed to purified JEV and MDF at different time periods was measured. Figure 10 shows that MDF stimulation increased the phagocytosis of virus at 60 minutes as compared to JEV alone. The phagocytosis triggers the activation of the oxidative signals with generation of superoxide anion. Neutrophils were stimulated in vitro with 5 μg of MDF and superoxide generation was evaluated. Neutrophils treated with MDF elicits rapid (maximum at 30 sec) respiratory burst (mean value = 0.23+ 0.08 nM/min/10^6 cells; $p<0.05$) as compared to control (mean value = 0.12 + .001 nM/min/10^6 cells). A series of experiments showed that subsequent formation of hydrogen peroxide via activation of NADPH with concomitant release of myeloperoxidase which peaked on day 7 post JEV infection coinciding with the maximum production of MDF. The respiratory burst was abrogated by staurosporine (protein kinase C inhibitor) indicating that neutrophil activation and signal transduction by MDF are dependent on protein kinase C (26,53). We have also observed that it acts in Ca-dependent manner. The data indicate that MDF showed significantly increased capacity for induction of respiratory burst and appears to be the central mediator for production of oxygen metabolites of neutrophils during JEV infection (54).

Degradation of viral protein and RNA

Further experiments were planned to explore whether the phagocytosed virus is actually degraded by neutrophils. In studying degradation of viral glycoprotein, neutrophils ($3x10^7$ cells / ml) were incubated with 30 µl of purified (^{35}S) Methionine labeled virus in presence of serum for 1 h, treated with TX - 100 and layered onto 6% (W/V) sucrose. The time dependent degradation of the viral protein in the neutrophils was measured and compared with MDF prestimulated and similarly treated neutrophils. The findings summarized in Figure 11 show that significant degradation of viral protein occurred at 120 min (44 +5%, $P<0.05$). The percent degradation at the same time was higher (62 + 3%) in cells prestimulated with MDF. However, a partial inhibition of viral protein degradation enhanced by MDF was observed following pre treatment of cells with staurosporine. To confirm viral protein degradation SDS-PAGE was performed which showed presence of low MW proteins as compared to normal JEV protein pattern. For studying the degradation of viral RNA the neutrophils ($3X10^7$ cells/ml) were incubated with (^3H) - Uridine labeled virion and TCA precipitable radioactivity was measured 60 and 120 min later. Findings summarized in Table 3 show substantial viral RNA damage at 60 minutes in neutrophils. The percentage viral degradation was significantly higher at 120 minutes of incubation ($P<0.05$) (26).

Antiviral effect of Nitric oxide

Nitric oxide (NO) has emerged as an important intra and intercellular regulatory molecule, which plays important roles in immunological pathways in the mediation of central and peripheral nervous system functions. NO has been implicated as a mediator of anti viral host defence. Many cells (macrophages, neutrophils, neurons etc.) are able to produce NO through the enzymatic conversion of L-arginine to L-citrulline by nitric oxide synthase (NOS), in presence of NADPH. Three isoforms of NOS have been identified. Two are constitutively expressed Ca^{2+} dependent forms (cNOS), found in a variety of cell types, including neurons (nNOS) and endothelial cells (ecNOS). The other isoform, an inducible Ca^{2+} independent form (iNOS) has potential to generate NO for extended periods of time. iNOS expression is significantly induced by lipopolysacchride or cytokines, TNF-α and IFN-γ, in a variety of immuno inflammatory cells, including macrophages (55). iNOS may play a role in the antimicrobial and antitumor functions of the immune system. Cytokine inducible NOS is expressed by microglia and astrocytes, which implies a possible role for the enzyme in central nervous system host responses. A series of experiments were performed to study the ability of JEV and JEV induced MDF to modulate NO activity in brain and the possible antiviral effect of NO during JEV infection. Figure 12 illustrates that splenic macrophages of JEV infected mice produce maximum NO in vivo at day 7 post infection. MDF induced NO production was dose dependent and maximum at 60 minutes after MDF treatment. The response was sensitive to anti MDF antibody treatment and the nitric oxide synthase inhibitor N^G - monomethyl -L-arginine (L-NMMA). In order to ascertain the antiviral

role of NO on JEV infection, mice were pretreated with L-NMMA followed by JEV inoculation. Data summarized in Figure 13 show that L-NMMA treatment significantly increased the mortality in JEV infected mice as compared to control (56).

Nitric oxide synthase in JEV infection

The ability of JEV and JEV induced MDF to modulate nitric oxide synthase (NOS) activity in brain and tumor necrosis factor (TNF-α) and the possible anti viral role of NOS during JEV infection was investigated. The data presented in Figure 14 show that the total NOS activity in brain increased gradually from day 3 and reached a peak on day 6 (176.2+12.2 pmol/min/mg protein) in JEV infected mice as compared to controls ($p<0.001$). There was no significant alteration in the cNOS activity (99.3+ 8.9 p mol/min/mg protein) throughout the study period. The control mice, exhibited total NOS activity of 118.1+ 10.1 p mol/min/mg protein, cNOS activity of 5.2 + 0.6 p mol/min/mg protein. The regulatory role of MDF in the modulation of NOS activity in brain was evaluated and finding revealed that maximum NOS activity in brain was at 60 min p.i. and this increase was mainly due to the induction of iNOS. Figure 15 shows NOS protein expression by immunological analysis at different time intervals. A time dependent increase in NOS protein expression after MDF treatment was observed at 30 min, with maximum expression at 60 min post inoculation. No significant alteration in cNOS protein expression was observed. Pretreatment of JEV infected mice with L-NMMA increased the mortality, as evident from reduced mean survival time. Cytokines are potent modulators of iNOS during viral infections and play a pivotal role in regulating the protective immune response. The enhanced level of TNF-α observed in the early phase of JEV infection which correlated well with the enhanced activity of iNOS (18).

Antiviral effect of DDTC

Diethyldithiocarbomate (DDTC), a low molecular weight dithiol, has been described as an immuno modulator and shown to be effective in several disease conditions. Therefore, we studied the therapeutic aspect of DDTC in providing inhibition of JEV infection. DDTC tested at various doses (10-100 μmol /kg/; i.p.) revealed that administration at low concentration (10 μmol/ kg/; i.p.) prolonged the average survival time (AST) of mice infected with lethal dose of JEV. The low dose also provided >80% survival in sub-clinical (10^5 LD_{50}, i.c.) JEV infection (Figure 16). Administration of DDTC to JEV infected mice enhanced the inducible nitric oxide synthase (iNOS) activity in brain. Thus these studies demonstrate that early non specific protection against JEV is mediated by the co-operative activity of reactive oxygen and nitrogen metabolites (57).

Host defence against viral infection is a complex phenomenon. The antibodies and the cell mediated immune response produced after JEV infection (Figure 17, 18). A series of our studies have shown that in JEV infection the antibody and cell mediated immunity afford specific protection. It triggers transient protective cell mediated immune response and induces delayed type hypersensitivity (28).

The CD4 and CD8 cells are thought to be important. JEV has the ability to establish latent infection in mice (58), which is associated with defect in cell mediated immune response (59). JE virus also stimulates the formation of IgM and IgG antibodies both in human and experimental animals with neutralization and haemagglutinating activity (Mathur et al, 1983a). Passive transfer of anti-JEV polyclonal or monoclonal antibodies provides protection against JEV infection (28,29). The accelerated generation of secondary immune response following JEV challenge in latently infected mice due to the presence of antigen specific memory B and T cells is seen in JEV infection.

Transplacental transmission of virus during JE infection

Few viruses have the ability to establish simultaneous infection in both the pregnant female and the foetus in utero. During an extensive epidemic of JE in Gorakhpur in 1978, the human transplacental transmission of virus has been demonstrated for the first time (60). Recently it has been described for West Nile virus as well (61), therefore ultrasonographic examination of the foetus is now recommended if maternal illness due to West Nile virus occurs during pregnancy (62). We have developed the JEV mouse model to validate the clinical observations. The experimental studies in mice have shown the variable effects of JEV virus infection on the foetus at different periods of gestations. JEV infection during first week of gestation led a significantly higher number of abortions, still births and neonatal deaths, as compared to infection during the third week of gestations (63). Development of hydrocephalous, runting or retarded growth in baby mice has been noted three to four months after birth. JEV can establish persistent and latent infection in humans as well as in mice (58, 22) and could transmit the virus to the foetus in consecutive pregnancies (63). Latently infected mice showed poor cell mediated response (59). Persistence of JEV in cerebrospinal fluid upto 110 days post infection can occur along with high titres of IgM and neutralizing antibodies (20).

Conclusion and future directions

The emerging picture based on our research findings exhibit complex interactions between functional characteristics of virus and host immune system mediated in part by Macrophage – derived factor (MDF). These interactions between virus and host have been the subject of many classical studies that have now progressed to the molecular level as well. However, this model should also be viewed cautiously in light of regional differences in the genetic makeup of the human population and the circulating viral strains. There are many secrets to these interactions that must be discovered. It is hoped that further research in the context of ongoing prospective studies, using newer molecular and immunological techniques will achieve this potential. As a better understanding of the immune response to JEV in the context of disease as well as vaccine-induced protection becomes available, the ability o control the growing worldwide burden of JE will likely be improved.

Acknowledgements

I am grateful to various funding agencies and King George's Medical

College, Lucknow for providing the facilities, environment and support that allowed the accomplishment of the work presented here. I also thank all my coworkers and students for their contribution towards the research.

REFERENCES

1. Mackenzie JS, Gubler DJ and Peterson LR (2004). Emerging flaviviruses; the spread and resurgence of Japanese encephalitis, West Nile and dengue viruses. *Nature Medicine Supplement.* **10**: 98-108.
2. Mackenzie JS, Ritchie CA, Van den Hurk et al (2002). Japanese encephalitis as an emerging virus: the emergence and spread of Japanese encephalitis virus in Australia. *Curr Top Microbiol Immunol.* **267**: 49-73.
3. Burke DS and Leake CJ (1998). Japanese encephalitis, in the arboviruses: Epidemiology and Ecology, Vol 3 (ed. Monath, T.P) 63-92 (CRC, Boca Raton, Florida).
4. Ravi V, Desai AS, Shenoy PK *et al* (1993). Persistence of Japanese encephalitis virus in the human nervous system. *J Med Virol.* **40**: 326-329.
5. Banerjee K and Desai PK (1973). Survey of arboviruses antibodies in South India. *Indian J Med Res.* **61**: 344-350.
6. Webb JKG, Pavri K, George S *et al* (1964). Asian Pediatrics, the scientific proceedings of the first all Asian congress of Pediatrics. Asia Publishing House. Bombay.
7. Chatterjee AK and Banerjee K (1975). Epidemiological studies on the encephalitis epidemic in Bankura. *Indian J Med. Res.* **63**: 1164-1179.
8. Mathur A, Chaturvedi UC, Tandon HO et al (1982). Japanese encephalitis in Uttar Pradesh, India during 1978. *Indian J Med Res.* **75**:161-169.
9. Mohan Rao CVR, Prasad SR, Rodrigues JJ et al (1983). The first laboratory proven outbreak of Japanese encephalitis in Goa. *Indian J Med Res.* **78**:745-750.
10. Prasad SR, Kumar V, Marwah RK et al (1993). An epidemic of encephalitis in Haryana: serological evidence of Japanese encephalitis in few patients. *Indian Paediatr.* **30**: 905-910.
11. Williams DT, Wang LF, Daniels PW et al (2000). Molecular characterization of the first Australian isolate of Japanese encephalitis virus, the F U strain. *J Gen Virol.* **81**: 2471-2480.
12. Uchil PD and Satchidanandan V (2001). Phylogenetic analysis of Japanese encephalitis virus: Envelop gene based analysis reveals a fifth genotype, geographic clustering and multiple introductions of the viruses into the Indian Subcontinent. *Am J Trop Med Hyg.* **65**: 242-251.
13. Vrati s, Giri RK, Razdan A *et al* (1999). Complete nucleotide sequence of an Indian strain of Japanese encephalitis virus: Sequence comparison with other strain and phylogenetic analysis. *Am J Trop Med Hyg.* **61**: 677-680.
14. Mathur A, Bhardwaj M, Kulshreshtha R et al (1988). Immunopathological study of spleen during Japanese encephalitis virus infection in mice. *Brit J Exp Path.* **69**: 423-432.
15. Khanna N, Agnihotri M, Mathur A *et al* (1991). Neutrophil chemotactic factor produced by Japanese encephalitis virus –

stimulated macrophages. *Clin Exp Immunol.* **89**: 299-303.

16. Ravi V, Parida S, Deasi A *et al* (1997). Correlation of tumour necrosis factor levels in the serum cerebrospinal fluid with clinical outcome in Japanese encephalitis patients. *J Med Virol.* **51**: 132-136.
17. Singh A, Kulshreshtha R and Mathur A *et al* (2000). Secretion of IL-8 cytokine during Japanese encephalitis virus infection. *J Med Microbiol.* **49**: 607 -612.
18. Saxena S, Mathur A and Srivastava RC (2001). Induction of nitric oxide synthase during Japanese encephalitis virus infection: evidence of protective role. *Arch Biochem Biophys.* **391**: 1-7.
19. Mathur A, Khanna N and Chaturvedi UC (1992). Breakdown of blood brain barrier in Japanese encephalitis by a virus induced cytokine. *Int J Exp Path.* **73**: 603-611.
20. Desai A, Ravi V, Chandramukhi A *et al* (1995). Proliferative response of human peripheral blood mononuclear cells to Japanese encephalitis virus. *Microbiol Immunol.* **39**: 269-273.
21. Kumar R, Mathur A, Kumar A *et al* (1990). Clinical features and prognostic indicators of Japanese encephalitis in Lucknow [India]. *Indian J Med Res.* **91**: 321-327.
22. Sharma S, Mathur A, Prakash V *et al* (1991). Japanese encephalitis virus latency in peripheral blood lymphocytes and recurrence of infection in children. *Clin Exp Immunol.* **85**: 85-89.
23. Ogata A, Nagashima K, Hall WW *et al* (1991). JEV neurotropism is dependent on the degree of neuronal maturity. *J Virol.* **65**: 880-886.
24. Kalita J, Misra UK (2000). Comparison of C T scan and MRI findings in the diagnosis of Japanese encephalitis. *J Neurol Sci.* **174**: 3-8.
25. Ravi V, Taly AB, Shankar SK *et al* (1997). Association of Japanese encephalitis virus infection with Guillian. Barre Syndrome in endemic area in South India. *Acta Neurol Scand.* **90**: 67-72.
26. Srivastava S, Khanna N, Saxena SK *et al* (1999). Degradation of Japanese encephalitis virus by neutrophil. *Int. J Exp Path.* **80**: 17-84.
27. Burke DS, Lorsomrudee W, Leake CJ (1985). Fatal outcome in Japanese encephalitis. *Am J Trop Med Hyg.* **34**: 203-210.
28. Mathur A, Arora KL and Chaturvedi UC (1983). Host defence mechanisms against JEV infections in mice. *J Gen Virol* **64**: 805-811.
29. Burke DS, Nisalok A, Ussesry MA *et al* (1985). Kinetics of IgM and IgG response to Japanese encephalitis virus in human serum and cerebrospinal fluid. *J Infec. Dis.* **151**: 1093-1099.
30. Kulshreshtha R, Mathur A and Chaturvedi UC (1988). Immunological memory in latent Japanese encephalitis virus infection. *Br J Exp Path.* **64**: 465-471.
31. Aihara H, Takasaki T, Toyosoki-Maedo T *et al* (2000). T cell activation and induction of antibodies and memory T cells by immunization with inactivated JE vaccine. *Virol immunol.* **13**: 179-186.
32. Murlikrishna K, Ravi V and Manjunath R (1994). Cytotoxic T lymphocytes against Japanese encephalitis virus: effector cell phenotype, target specificity and in vitro clearance. *J Gen Virol.* **75**: 799-807.
33. Tiroumourougane SV, Raghava P and Srinivasan S (2002). Japanese viral encephalitis. *Post Grad Med J.* **78**: 205-215.

34. Kabilan L, Rajendran R, Arunachalam N *et al* (2004). Japanese encephalitis in India: An overview. *Indian J Pediatr.* **71**: 609-615.

35. Mathur A, Kumar R, Sharma S *et al* (1990). Rapid diagnosis of Japanese encephalitis by immunofluorescent examination of cerebrospinal fluid. *Indian J Med Res.* **91**: 1-4.

36. Ravi V, Vanajakshi S, Gowda A *et al.* (1989). Laboratory diagnosis of Japanese encephalitis using monoclonal antibodies and correlation of findings with outcome. *J Med Virol.* **29**: 221-223.

37. Meiya F, Hnosheng C, Cuihua C *et al* (1997). Detection of flaviviruses by reverse transcriptase polymerase chain reaction with the universal primer set. *Microbiol Immunol.* **41**: 209-213.

38. Xin YY (1995). Japanese Encephalitis vaccine implementation in China. *Southeast Asian J.Trop. Med. Public Health,* **26** (Suppl. 3): 51-53.

39. Tsai TF, Yong-Xin Y, Li Li j *et al* (1998) Immunogenicity of live attenuated SA 14-14-2 Japanese encephalitis vaccine- A comparison of 1- and 3- month immunization shedules. *J.Infect.Dis.* **177**: 221-223

40. Konishi E,Kurane I, Mason P W. (2000) Japanese encephalitis virus vaccine candidates induce JE virus - specific CD8+ cytotoxic T lymphocytes in mice. *Virology* **227**: 353-360.

41. Chang GJ, Hunt AR and Davis B (2000). A single intramuscular injection of recombinant plasmid DNA induces protective immunity and prevents Japanese encephalitis in mice. *J.Virol.* **74**: 4244-52

42. Arroyo J, Guirakhoo F,Fenner S *et al* (2001). Virus/ Japanese encephalitis virus chimera vaccine (ChimeriVax-JE). OraVax.Inc., Cambridge, Massachusetts 02139, USA.

43. Mathur A, Arora KL and Chaturvedi UC (1981). Congenital infection of mice with Japanese encephalitis virus. *Infection and Immunity.* **34**: 26-29.

44. Chaturvedi UC, Mathur A and Tandon P (1979). Variable effect on peripheral blood leucocytes during JE virus infection of man. *Clin Exp Immunol.* **38**: 492-498.

45. Vrati S (2000). Comparison of the genomic sequence and the phylogenetic analysis of the GP 78 and the Vellore P 20778 isolates of Japanese encephalitis virus from India. *J Biosci.* **25**: 257-262.

46. Westwick J, Li SW and Camp RD (1989). Novel neutrophil stimulating peptides. *Immunol Today.* **10**: 146-150.

47. Schall TJ and Bacon KB (1994). Chemokine leucocyte trafficking and inflammation. *Curr Opin Immunol.* **6**: 865-873.

48. Zlotnik A and Yoshie O (2000). Chemokine: a new classification system and their role in immunity. *Immunity* **12**: 121-127.

49. Khanna N, Mathur A and Chaturvedi UC (1994). Regulation of vascular permeability by macrophage derived chemotactic factor produced in Japanese encephalitis. *Immunol Cell Bio.* **72**: 200-204.

50. Siebens M, Tevethia JC and Babior BM (1979). Neutrophil mediated antibody dependent killing of herpes simplex virus infected cells. *Blood* **54**: 88-94.

51. Rouse BT (1981). Role of neutrophils in antiviral immunity. *Adv Exp Med Biol.* **137**: 263-278.

52. Van Strijp JAG, Miltenberg LAM, Van der Tol ME *et al* (1990). Degradation of herpes simplex virions by human polymorpho-

nuclear leucocytes and monocytes. *J Gen Virol.* **71**: 1205-1209.

53. Khanna N, Srivastava S, Mathur A et al (1993). Stimulation of neutrophil respiratory burst and degranulation by Japanese encephalitis virus induced macrophage derived factor. *Int J Exp Path.* **74**: 339-345.

54. Srivastava S, Khanna N, Mathur A *et al* (1994). Role of calcium in neutrophil activation by Japanese encephalitis virus induced macrophage derived factor. *Acta Virol.* **34**: 111-116.

55. Miller RA and Britigan BE (1997). Role of oxidants in microbial pathophysiology. *Clin. Microbiol. Rev.* **10**: 1-8.

56. Saxena SK, Singh A, and Mathur A (2000). Antiviral effect of nitric oxide during Japanese encephalitis virus infection. *Int J Exp Path.* **81**: 165-172.

57. Saxena SK, Mathur A and Srivastava RC (2003). Inhibition of Japanese encephalitis virus infection by diethyldithiocarbamate is independent of its anti-oxidant potential. *Antiviral Chem. and Chemother.* **14**: 91-98.

58. Mathur A, Arora KL, Rawat S *et al* (1986). Persistence, latency and reactivation of JEV infection in mice. *J Gen Virol.* **67**: 381-385.

59. Mathur A, Arora KL and Chaturvedi UC (1983). Immune response to Japanese encephalitis virus in mother mice and their congenitally infected offspring. *J Gen Virol.* **64**: 2027-2031.

60. Chaturvedi UC, Mathur A, Tandon P *et al* (1980). Transplacental infection in human with Japanese encephalitis virus. *J Inf Dis.* **141**: 712-715.

61. Alpert SG, Ferguson J and Neol LP (2003). Intrauterine West Nile virus: Ocular and systemic findings. *Am J Ophthalmol.* **136**: 733-735.

62. Interim guidelines for the evaluation of infants born to mothers infected with West Nile virus during pregnancy. *MMWR Morb Mortal Wkly Rep.* (2004) **53**: 154-157.

63. Mathur A, Arora KL and Chaturvedi UC (1982). Transplacental Japanese encephalitis virus infection in mice during consecutive pregnancies. *J Gen Virol.* **59**: 213-217.

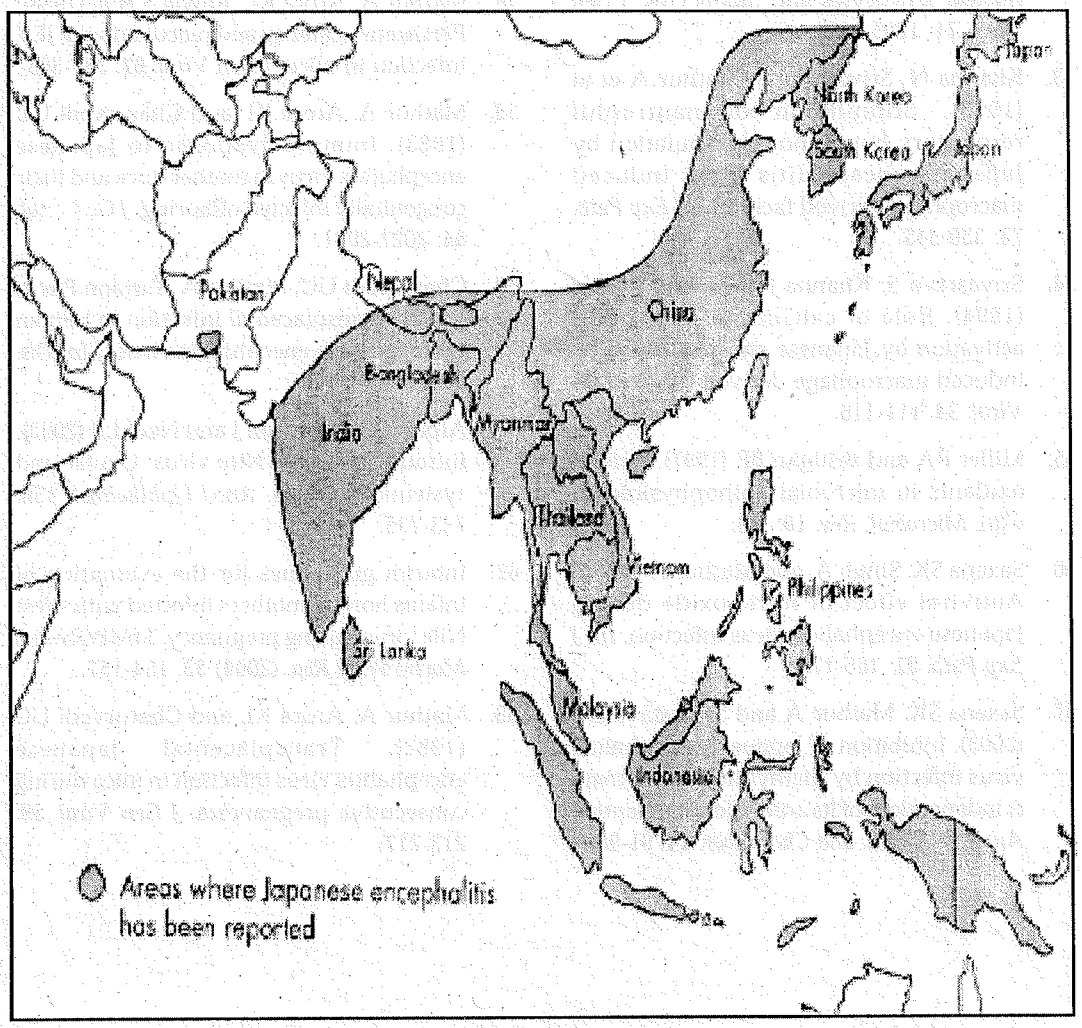


FIGURE 1. Showing areas where JE has been reported
(Courtesy : Tiroumourougane et al, 2002)

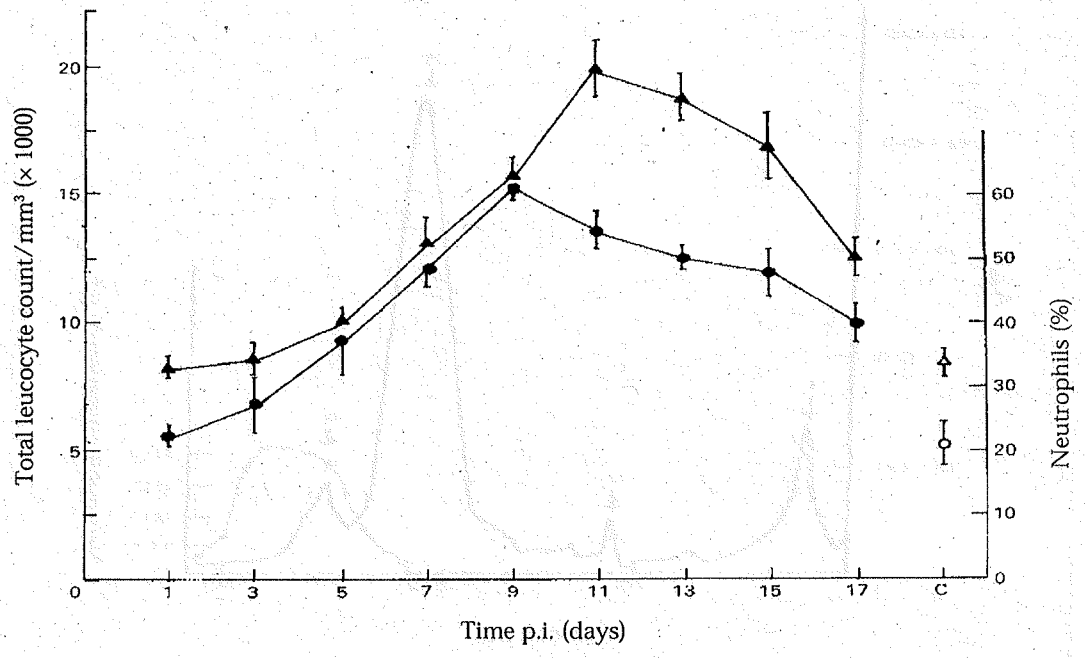


FIGURE 2. ▲ Total leucocyte counts and ● percentage neutrophils at different periods in the peripheral blood of JEV i.p. inoculated mice. Each value represents means ± s.e. from five mice.

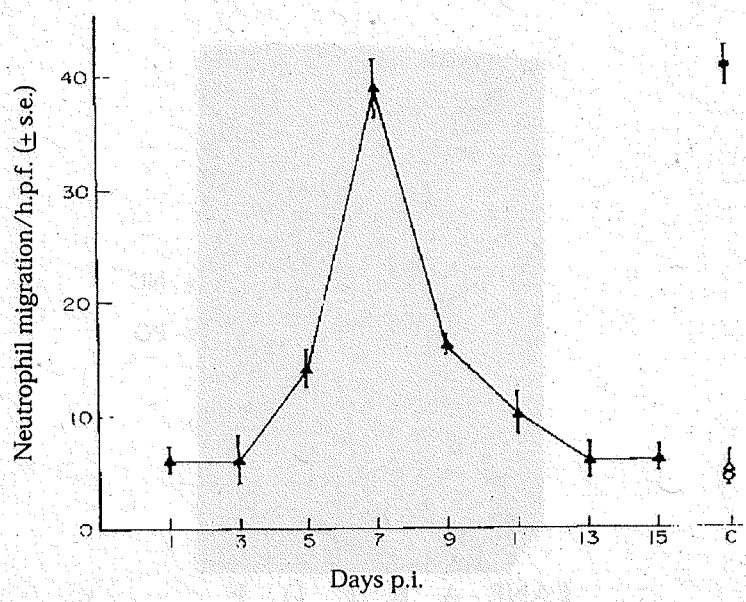


FIGURE 3. Spleen cell neutrophil chemotactic activity at different periods of JEV-primed mice (▲), normal mice (Δ). FMLP (10^{-7}) (●) was used as positive control and MEM (o) as negative control. Each sample was tested in triplicate with neutrophil migration counted in five to seven high power field.

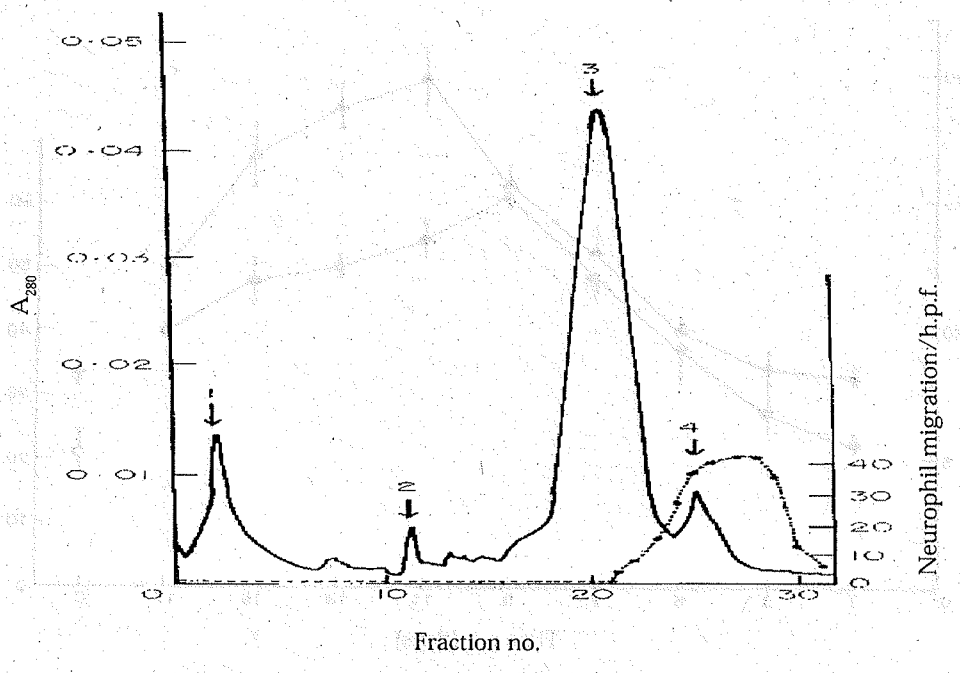


FIGURE 4. ▲ Purification of macrophage-derived factor by low pressure liquid chromatography (—) and neutrophil chemotactic activity (- - -)

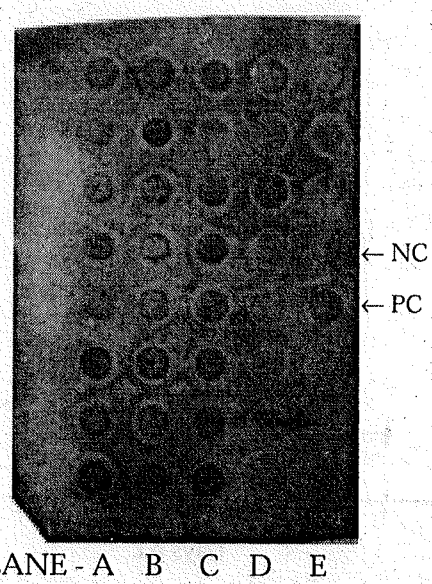


FIGURE 5: Immuno dot blot assay of serum and PBMC culture supernatants of JE confirmed cases.

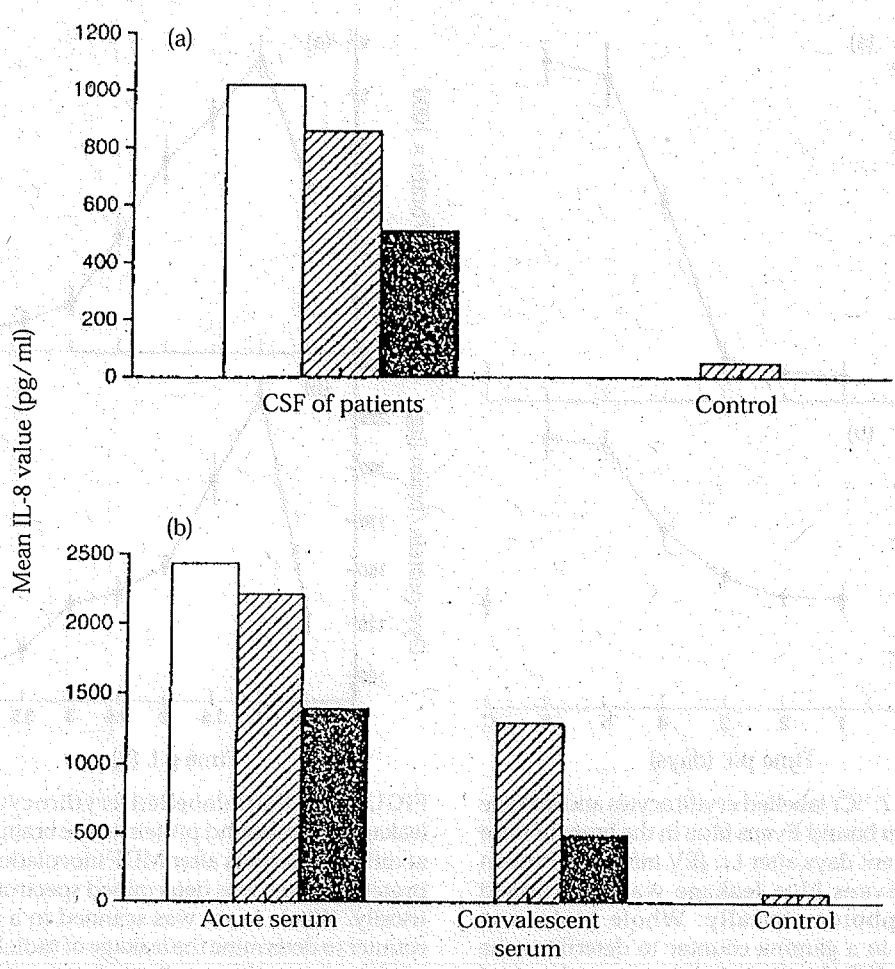


FIGURE 6: IL-8 levels in (a) CSF and (b) serum from patients with JEV infection : fatal cases (□), patients with prolonged illness (▨), and patients who recovered completely (■). Controls consisted of CSF (▨) from patients with symptoms other than acute encephalitis and serum (▨) from normal healthy individuals. Data are expressed as mean and SD.

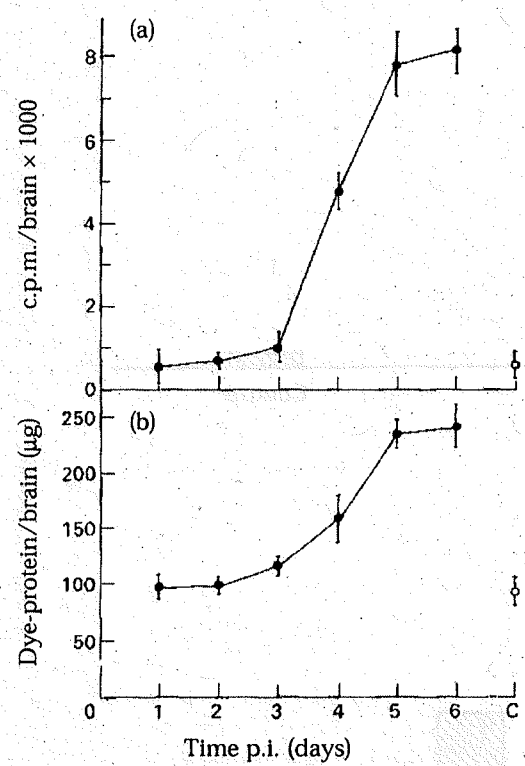


FIGURE 7: ^{51}Cr labelled erythrocytes and leakage of protein bound Evans blue in the brain of mice on different days after i.c. JEV infection. Protein bound Evans blue leakage was determined spectrophotometrically. Whole brain was scanned in a gamma counter to determine the leakage of ^{51}Cr labelled erythrocytes, and c.p.m./brain is presented. Each value represents mean ± s.e. from 12 mice.

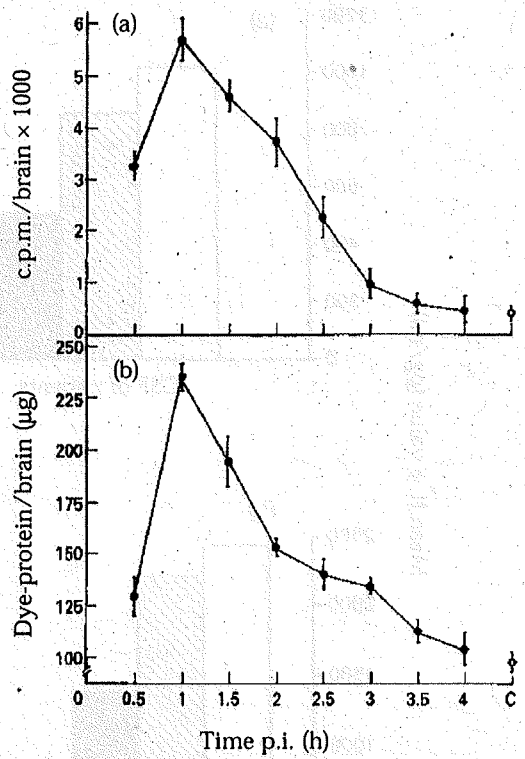


FIGURE 8: Radiolabelled erythrocytes and leakage of dye bound protein in the brain of mice at different periods after MDF inoculation. Dye-protein leakage was determined spectrophometrically. Whole brain was scanned in a gamma counter to determine the leakage of radiolabelled erythrocytes, and c.p.m./brain is presented. Each point represent mean ± s.e. from 12 mice.

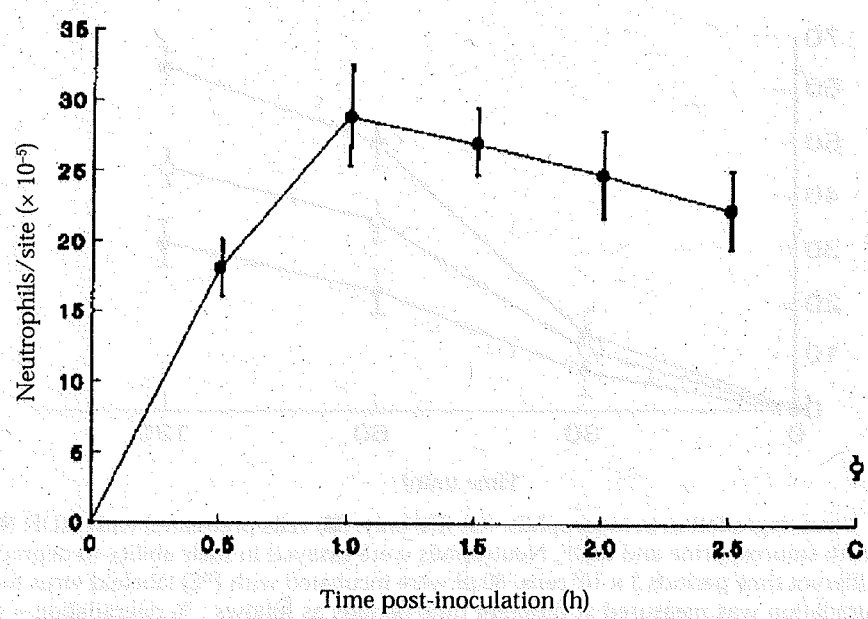


FIGURE 9: [^{51}Cr]-labeleld neutrophil accumulation at different periods after intradermal injection of MDF in rabbit

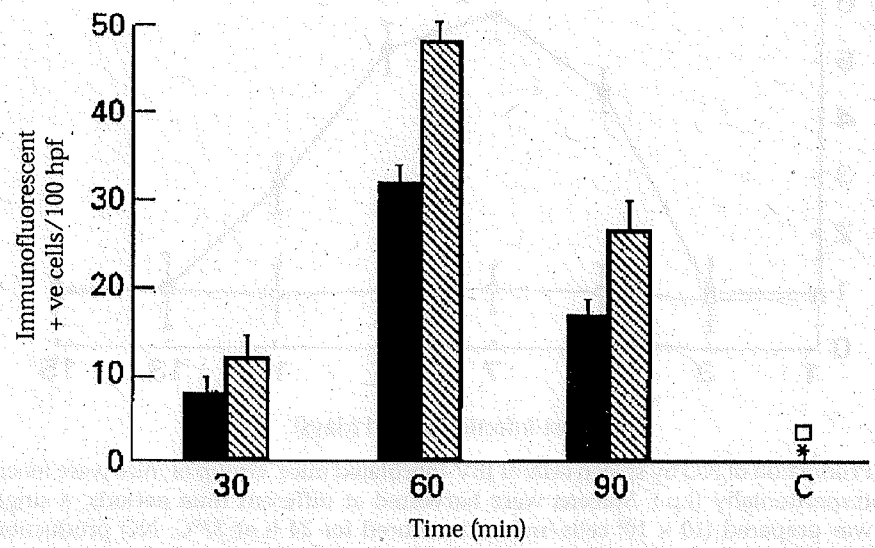


FIGURE 10: Demonstration of number of JEV positive immunofluorescent cells at different time periods after JEV (■) or costimulation with MDF and JEV (▨). Control cells (C) were stimulated either with MDF (□) or normal macrophage culture supernatant (*). IF positive cells were counted in 100 high power fields (hpf). Values are presented as mean ± SE of triplicate experiments.

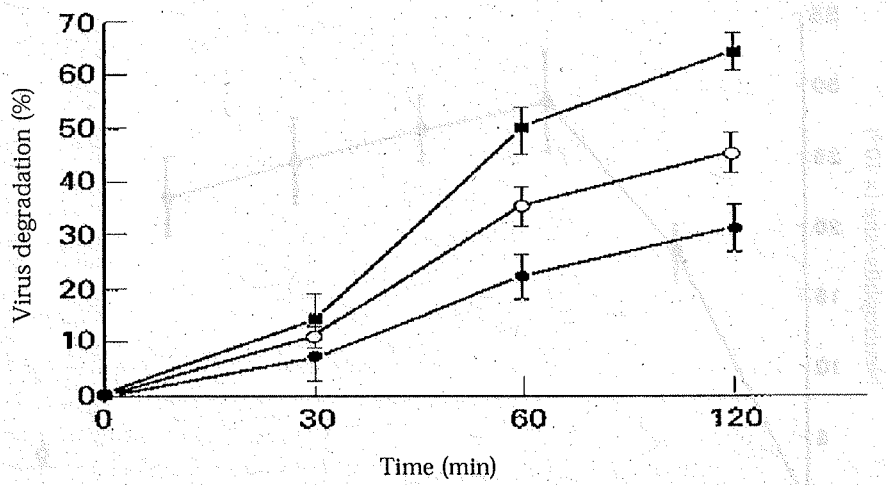


FIGURE 11: Virus degradation in neutrophils. (O) JEV only; (■) cells pretreated with MDF; (●) cells pretreated with staurosporine and MDF. Neutrophils were assayed in their ability to degrade viral protein at different time periods 3×10^7 cells/60 µl were incubated with (^{35}S) labeleld virus for 1 h at 37°C. % degradation was measured at different time periods as follows : % degradation = cpm in supernatant × 100/cpm in pellet + supernatant. Each value represents mean ± SE of three experiments.

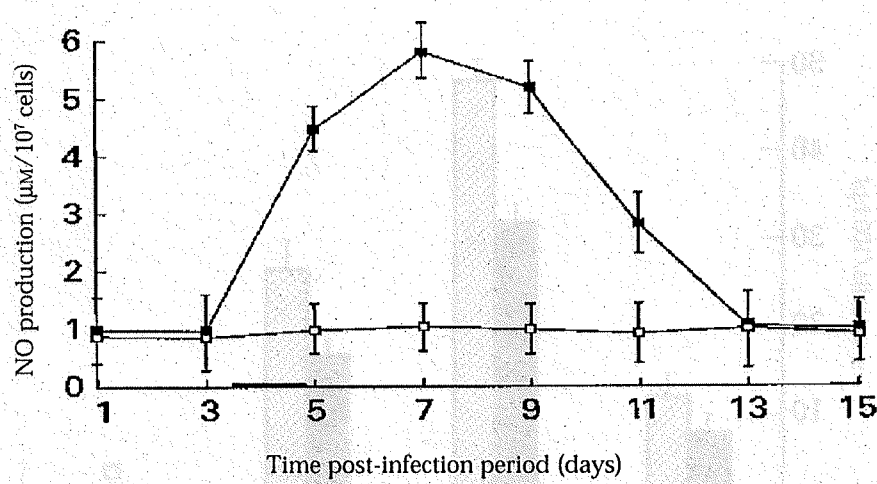


FIGURE 12: Production of NO by spleen cells of JEV-inoculated mice. Group of mice were inoculated with JEV intraperitonially (i.p.). Spleens were harvested at different time periods, a single cell suspension was prepared (10×10^6 cells/ml) and cultured for 24 h at 37°C. NO production was assayed in the cell free culture supernatants (■) as described in Materials and methods. Control (□) mice were inoculated with normal mouse brain suspension. Values are presented as A.M. ± SD from 10 cultures.

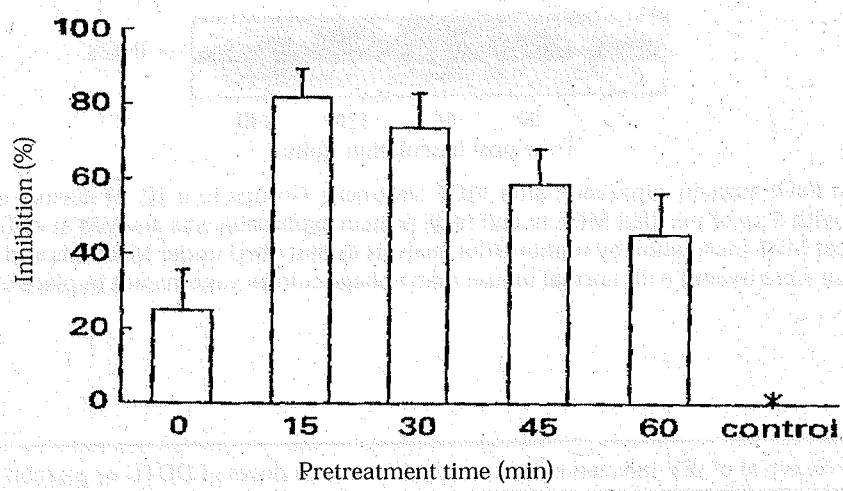


FIGURE 13: Inhibition of NO production by treatment with N^{G} monomethyl-L-arginine (L-NMMA). Normal mouse macrophage cultures were pretreated with 100 μM L-NMAA for indicated time periods followed by inoculation of 5 μg MDF for 60 min at 37°C. Control group of cells (*) were treated with MDF only. Cells treated with L-NMMA for different time periods were used for background values. NO production was assayed as described in Materials and methods. Results are presented after deduction of background values as A.M. ± SD for eight cultures.

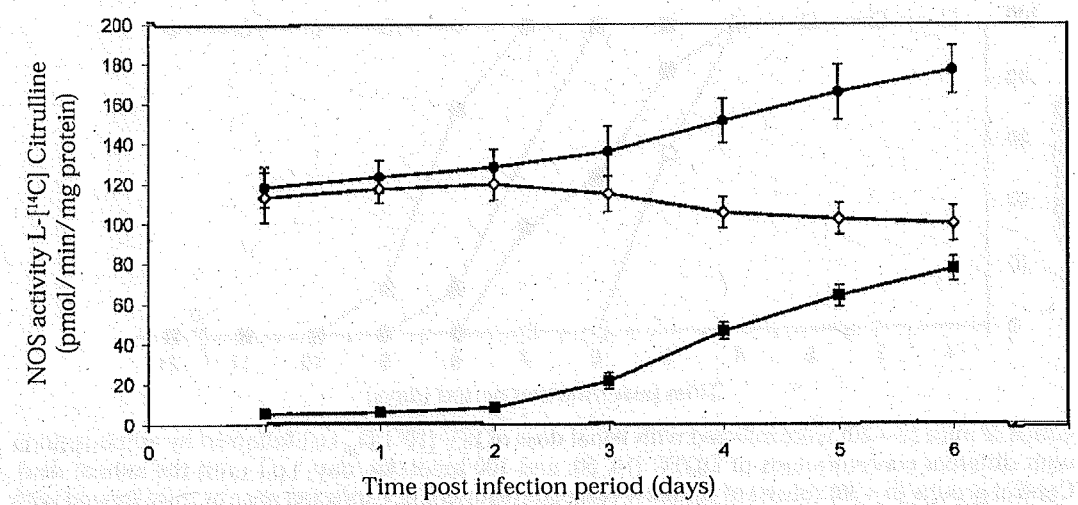


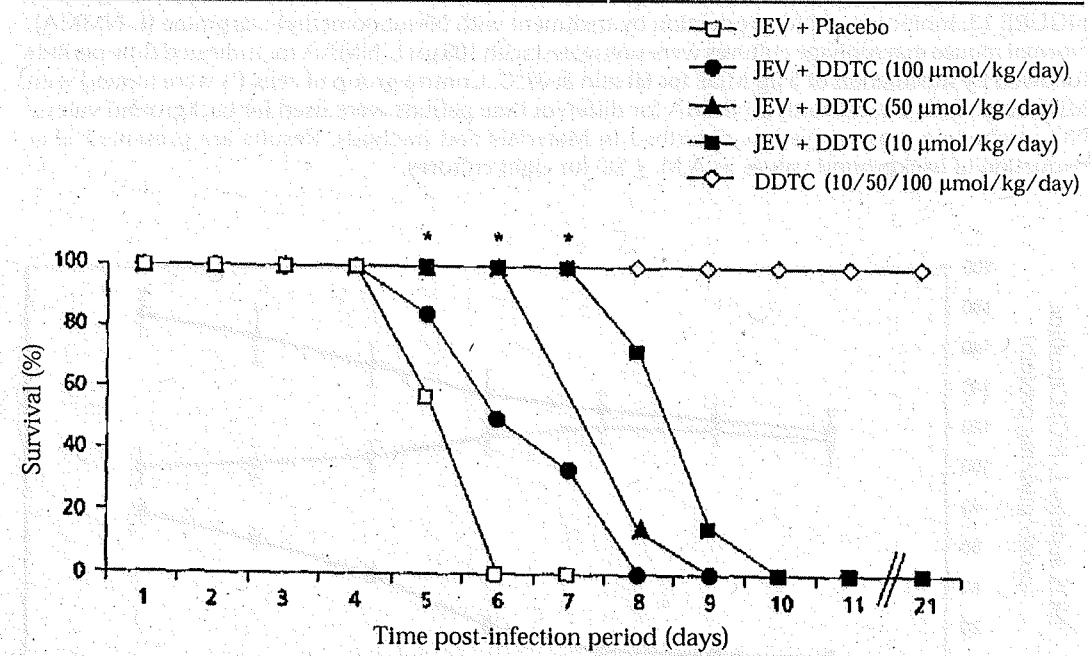
FIGURE 14: JEV-induced NOS activity in brain. Groups of mice (n = 10) were inoculated with 0.025 ml of 10^2 LD_{50} JEV (ic) or normal mouse brain suspension (for control). NOS activity was assayed in brain on different days p.i. as described under Materials and Methods. The NOS activity was expressed as A.M. ± SD of five experiments. (●) Total NOS. (◇) cNOS, (■) iNOS.

C 30 60 120 240

Time post inoculation (min)

FIGURE 15: iNOS protein expression after MDF treatment. Groups (n = 10) of normal mice were inoculated with 5 µg of purified MDF iv and NOS protein expression was assayed at different time intervals post MDF inoculation by immunoblot analysis as described under Materials and Methods. Control mice were treated with normal mouse macrophage culture supernatant in place of MDF.

FIGURE 16: Survival of JEV-infected mice treated with various doses of DDTC or placebo



Grops of mice (n = 20) were infected with lethal dose of JEV (10^2 LD_{50} i.c.) followed by adminisration with different concentrations of DDTC (10, 50, and 100 µmol/kg/day, i.p.) until the animal died. Control groups (n = 20) consist of similarly placebo-treatment JEV-infected mice or mice treated with different concentrations of DDTC alone. The survival rate of mice was monitored daily for 3 weeks.

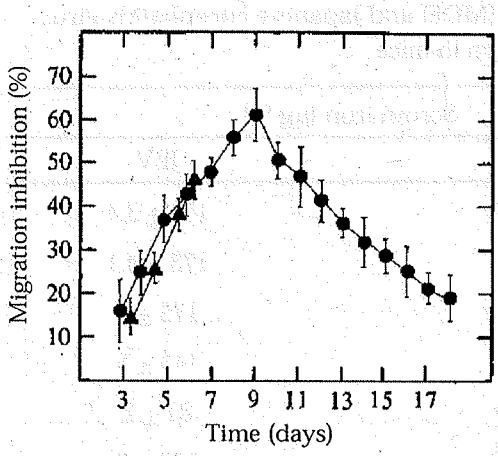


Fig. 1

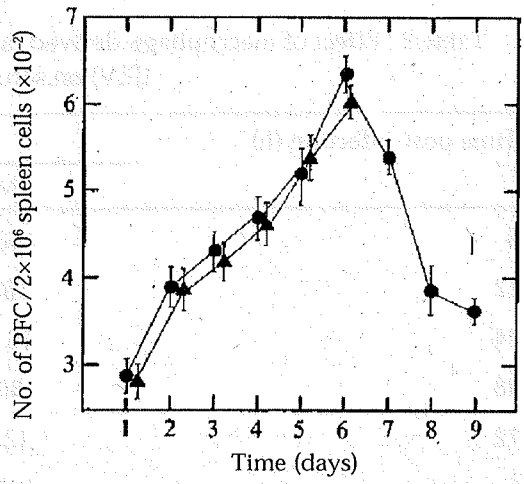


Fig. 2

FIGURE 17: Leukocyte migration inhibition of spleen cells of mice on different days after intraperitoneal (●) or intracerebral (▲) inoculation of JEV. Each observation represents the mean value with its standard error from multiple readings from five mice.

FIGURE 18: Antibody plaque-forming cells in the spleen of mice on different days after intraperitoneal (●) or intracerebral (▲) inoculation of JEV. Each observation represents the mean value with its standard error from multiple slides from five mice.

Table 1 : Peripheral blood leucocyte counts in MDF inoculated mice

Time post inoculation (h)	Total leucocyte counts (per min^3)	Neutrophil counts (%)
1	14980 ± 419	63 ± 5
2	13100 ± 575	59 ± 2
3	10228 ± 256	42 ± 1
Control	8600 ± 116	28 ± 2

The mice were injected with 5 μg of purified MDF i.v. Total leucocyte counts were done and smears were prepared from each mouse from tail vein at different intervals. The values are expressed as mean of 7-9 mice ± s.e.

Table 2 : Effect of macrophage-derived factor (MDF) and Japanese encephalitis virus (JEV) on serum iron in mice

Time post-infection (h)	Serum iron (μg %)	
	MDF	JEV
6	182 ± 11	170 ± 3.4
12	165 ± 5	173 ± 4.1
24	76 ± 2.3	175 ± 7
48	80 ± 3	143 ± 5
72	125 ± 9	87 ± 2
Control	180 ± 10	168 ± 3

Values are expressed as mean of five to seven serum sample ± s.e.m.

Table 3 : Degradation of viral RNA by neutrohils at different time intervals

Phagocytosis (min)	% Degradation	
	Test	Control
0	3 ± 0.9	2.9 ± 0.3
60	28 ± 9.1	3.1 ± 0.6
120	69 ± 17.0	3.3 ± 0.5

Degradation of viral RNA which was phagocytosed by neutrophils for different time periods. Results shown are TCA-soluble cpm as a percentage of the total associated cpm ± SE of triplicate experiments. The control values represent degradation of virus incubated in MEM alone at respective time periods. 0.069 ± 0.027

Ann Natl Acad Med Sci (India), **42(2)**: 135-146, 2006

Immunology of Lymphatic Filariasis: Connecting the dots

Dr. Balachandran Ravindran
Division of Immunology, Regional Medical Research Centre,
(*Indian Council of Medical Research*), Nandankanan Road, Bhubaneswar,751023,

SUMMARY

Immunological studies in filariasis, as in many other infectious diseases, have been undertaken using animal models and comparing them with human disease. A general disconnect in the observations made in these two apparently diverse models have resulted in generating a notion that animal models do not effectively reflect the situation in human filariasis. Notwithstanding the obvious differences between human and animal models of filariasis, it has been possible to identify common grounds that unify the underlying principles of immunology of filariasis. Insight into this has also resulted in clearer understanding of the vexed issue of natural history of human filariasis and has led to the proposal of a model that incorporates existing data from animal models as well as epidemiological and longitudinal observations made in human populations. The model explains the observations and proposes testable predictions.

Key Words: Immunology of Filariasis; Natural history of Filariasis, *W. bancrofti*, Infection and Disease, Btk mice, XID mice, IFN-g, Inflammation.

INTRODUCTION

Filariasis is a spectral disease. Based on parasitological examination of blood for Mf, serological testing for circulating filarial antigens (CFA) and clinical manifestations, the subjects in an endemic area can be classified as i) Parasite carriers: infected subjects who are often free of overt chronic disease manifestations; ii) Chronic pathology: patients who display one or more of the chronic disease manifestations with or often without patent infection; iii) acute disease: subjects who suffer from periodic episodes of acute symptoms characterized by adenolymphangitis and iv) Endemic normals. Typical clinical manifestations, the hallmark of human disease, are seldom observed in animal models of filariasis. If and when comparable clinical features are observed,

Correspondence : Dr. Balachandran Ravindran, Division of Immunology, Regional Medical Research Centre, (*Indian Council of Medical Research*), Nandankanan Road, Bhubaneswar,751023; e-mail : balaravi@sancharnet.in; ravindran8@gmail.com Dr. Pran Nath Chhuttani Oration 2004-2005 delivered at the Annual meeting of NAMS at Ahmedabad-2005.

as in *B. pahangi* infected cats, (1) the manifestations are transient, unlike chronic disease in humans which persist for several decades. Development of lymphadema persisting for longer duration has recently been demonstrated in *B. malayi* infected leaf monkeys (2).

A large body of work on human immune responses to all the developmental stages of filarial parasites has been reported from several geographical areas endemic for filariasis such as India, Brazil, Cook Islands, Haiti, Indonesia, PNG etc. (3-6) in both Bancroftian and Brugian Filariasis infected subjects. The immune response phenotypes differ significantly in endemic subjects between those infected with filarial parasites and those free of patent infection. Filarial specific T-cell proliferation, IFN-g, IL-5 and IL-10 production, levels of antibodies to filarial carbohydrates, levels of filarial IgG1, IgG2, IgG4 and presence of anti-sheath antibodies are significantly different between putatively immune subjects in comparison to the infected population. Taken together, these observations indicate that absence of current infection in the host is associated with enhanced Th1 responses and infected hosts display a significantly decreased filarial specific Th1 responses associated with enhanced T-regulatory cell activity resulting in enhanced production of IL-10 and TGF-b. Based on these results it was generally assumed that protective immunity is associated with enhanced Th1 responses in human filariasis. A mathematical model was also proposed on this basis. More detailed discussion on the cytokine balance in the clinical spectrum of human filariasis can be found in a recent review (7). Most of the above observations have been made in case controlled studies. There are a few cohort studies conducted in infected subjects and in endemic controls over a long period of time (8-11). These limited but valuable studies have shown that qualitatively the immune response phenotypes are sustained in both the cohorts over 13-18 years – these investigations have also revealed that immunological hypo-responsiveness associated with patent filarial infections observed in infected subjects persist even after loss of infection.

Rodents such as gerbils and Mastomys couch (multimamate rats) are the most susceptible animal models for human filarial parasites although the precise biological reasons for this are still not understood. However non-availability of crucial immunological reagents and limitations in amenability to genetic manipulations have precluded the use of these animal models to address finer aspects of protective immunity in experimental filariasis. Notwithstanding the limitation that mice are inherently not susceptible to complete development of the human filarial parasite B.malayi, they have been extensively used to study the nature of immune responses to infective larvae and microfilarial stages. Mice are most amenable to study immune responses due to the wide availability of reagents to dissect at the cellular, molecular as well as genetic levels to any antigen/pathogen. In recent years, normal as well as mouse strains with specific genetic deficiencies (gene knock-out mice for specific cytokines, cytokine receptors, immunoglobulins and specific populations of immune cells) have been

used widely to understand protective immunity in filariasis. Several scholarly reviews have been published summarizing these observations (12-14).

The only effective vaccination protocol for helminths, viz., infection with irradiated parasites has also been studied in mice with a view to understand the immune response phenotypes associated with protective immune immunity. Injection of filarial infective larvae or irradiated larvae induce very similar immune responses, viz., a Th2 dominant response that is associated with a down regulated production of Th1 mediating inflammatory cytokines (15-16) suggesting that Th1 responses have a limited role in protection induced by experimental vaccination in mice.

Although immunocompetanat mice are refractory to B.malayi infections, T cell deficient nude mice and IL-5 null mice are relatively more susceptible(12). Interestingly, Brutan's tyrosine kinase (Btk) deficient mice also known as XID mice are susceptible to development of filarial larvae into juvenile adult stage parasites and such mice also do not clear microfilariae in circulation as rapidly as wild type mice. Since a single mutation in one gene made significant differences to filarial susceptibility, extensive investigations were undertaken to study immune responses to filarial infections in XID mice (17-19). Susceptible XID mice exhibited a inflammatory and Th1 polarized immune response to filarial as well as non-filarial antigens, characterized by higher levels of production of TNF-a and IL-1b by their macrophages and higher levels of IFN-g by their T-lymphocytes associated with lower levels of IL-10 in comparison to wild type mice. These observations were juxtaposed with induction of Th2 dominant responses observed in normal wild type mice when injected with filarial larvae as described above (16) It thus emerges that a Th1 polarized immune response is associated with susceptibility to filarial parasites Btk mutant mice while down-regulated Th1 response is a feature observed in refractory immuno-competent mice. Based on this principle four years ago it was then proposed that an inflammatory response is needed for growth and development of filarial larvae while absence of such host responses would render the animals refractory to filarial parasite development (20). Experimental evidence for this proposal has been forthcoming in recent years: - a) Infective larvae of B.malayi (L3) induce primarily pro-inflammatory cytokines (TNF-a and IL-1b etc.,) when put in culture with normal human peripheral mononuclear cells (21) – a similar induction is not observed in PBMCs of microfilariae carriers when exposed to L3s S.Babu, personal communication indicating that non-induction of inflammatory cytokines when challenged with L3s concomitant immunity; could be manifestations as b) induction of Th1 cytokines by pre-injection of CpG nucleotides resulted in enhanced development of filarial larvae into adult worms in jirds (22); c) pre-injection of Carrageenan, a pan macrophage activator of inflammatory cytokines significantly enhanced filarial worm growth and development in both normal as well as nude mice (23) and d) administration of Cyclosporin-A and several other immunosuppressive drugs resulted in

decreased development of filarial worms in experimental animal models (24) ; the above observations strengthen the notion that inflammatory host responses could be needed for larvae development. Taken together these investigations suggest that enhanced Th1 response to filarial antigens is not associated with protective immunity, which appears to be in variance with the broad conclusions drawn in human filariasis as described above. However, a closer and critical appraisal suggests a common unifying basis of immune response in both mice and human if one considers that humans are naturally susceptible to filarial infection while normal mice are essentially resistant. Dissection of immune response phenotype in 'susceptible' and 'resistant' hosts suggests that growth and development of filarial larvae in susceptible hosts including humans are associated with an inflammatory response to filarial parasites (20). Using this central theme the natural history of human filariasis can now be understood and a model is being proposed below.

Very broadly, a "static immunological viewpoint" and a "dynamic model" have been put forward. The static immunological view-point proposes that individuals displaying filarial specific T-cell hyporesponsiveness (down-regulated filarial specific Th1 responses) are associated with development and maturation of filarial worms and such individuals harbor microfilaraemia, while those displaying filarial specific T-cell hyperresponsiveness (upregulated filarial specific Th1 responses) develop pathology and disease and are generally free of patent infection. This implies that differing immune responses predispose individuals either towards harboring infection or developing disease (25). The "dynamic model" proposes that there is a sequential progression from infection, microfilaraemia, and amicrofilaraemia to obstructive disease in all individuals who experience microfilaeraemia (26, 27) and/or that the lymphatic dwelling adult worms essentially mediate pathology and disease (28). Extending this model, it has been proposed that subclinical lymphangiectasia is caused by lymphatic dwelling adult worms and that loss/death of adult worms would result in an inflammatory reaction leading to development of pathology and consequently chronic disease, often assisted by co-factors such as secondary bacterial infections (29). A decade ago, it was also proposed that a breakdown of immunological tolerance associated with patent infection would result in recovery of immunological hyperactivity to filarial antigens and lead to development of pathology and chronic disease (30). While the "immunological view point" was proposed based on immunological read-outs, the dynamic model was proposed on the basis of mathematical derivation using epidemiological data and later by integrating clinical, surgical, ultrasonographic and histopathological data. High prevalence of microfilaraemia and more significantly filarial antigenemia (which detects presence of adult filarial worms in the host, a parameter that did not exist at the time when "immunological view point" was proposed) in elephantiasis and hydrocele patients in several geographical areas do not appear to validate this model

completely (25,31-33). On the other hand, the "dynamic model", proposed about a decade ago, suffers from more serious limitations – presence of patent infection or loss of patency leading to development of disease is central to this model. Since vast majority of patients with chronic disease display immunological hyper-reactivity to filarial antigens, epidemiological proof for the validity of the "dynamic model" is dependant on demonstration of a switch over from the state of immunological hypo-responsiveness (observed during patency) to that of hyper-reactivity and development of chronic disease over a period of time. Longitudinal studies conducted on the same cohort of subjects, the results of which have been reported in recent years, do not offer credence to such a scenario expected of the "dynamic model". Both the models thus continue appear to be limited in their scope (11).

AN ALTERNATIVE MODEL

The different components of an alternative model of progression of filarial infection and disease in naturally exposed human population are shown in Table 1. The model proposed here essentially extrapolates several immunological observations made in experimental animals to development of chronic disease in humans living in endemic areas. In susceptible animal hosts, such as gerbils, dogs, cats, monkeys and chimpanzees, infection with filarial larvae results in inflammatory immune responses followed by down-regulation of such responses, after onset of patency. The pre-patent period in infected animals is consistently associated with an immune response phenotype characterized by enhanced filarial specific T-cell proliferation and release of high levels of IFN-γ by the proliferating T-cells. These characteristic features are "switched-off" once patent infections (with microfilariae/adult worms) set in (20). Extending this sequence of events to infected human populations, the proposed model perceives two stages of parasite development. Stage I, during which the filarial larvae are still developing and are yet to reach maturity and thus the infected hosts do not have circulating filarial antigens. This stage is analogous to the pre-patent period observed in experimental animals. Individuals at Stage I display a hyper-responsive immune phenotype characterized by high levels of filarial specific IgG1, IgG2, IgE and presence of antibodies to Mf sheath. During Stage I, filarial specific lymphocytes proliferate vigorously *in vitro* releasing high levels of IFN-γ and also IL-5. However, production of anti-inflammatory cytokines such as IL-10 and TGF-β are low and levels of filarial specific IgG4 are minimal. The model thus places all subjects with the above features described in the literature (34-38) at Stage I.

Maturation of the developing larvae into adult stage parasites would result in a shift from Stage I to Stage II - a phase in which circulating filarial antigens are detectable; this stage is analogous to the patent phase observed in experimentally infected animals models. The immune response phenotype at Stage II is characterized by lower levels of filarial specific IgG1, IgG2, IgE and absence of antibodies to sheath; proliferation of filarial specific T cells and release of IFN-γ and IL-

5 are also significantly down regulated in this stage. This hypo-responsive phase is characterized by production of high levels of filarial specific IgG4 and release of higher levels of anti-inflammatory cytokines such as IL-10 and TGF-β. The model thus places at Stage II all infected subjects displaying immunological hypo-responsiveness described by several investigators (34-40). The duration of stay at Stage I could vary between individuals in a given endemic area – a few months in some to a few years in others. Several individuals may never move into the patent phase of Stage II. Host as well as parasite factors would contribute in shifting from Stage I to Stage II.

(1) A higher intensity of transmission (greater exposure to infective larvae) would contribute to successful maturation of larvae to adult stage parasites in a larger number of individuals in the area and at a shorter duration of time.

(2) Adult worms and/or their products could offer the required signal for down-regulation of hyper-responsive inflammatory host responses associated with Stage I.

(3) Host genetic factors and/or intra-uterine exposure to filarial antigens/ parasites would predispose the subjects to readily induce immunological hypo-responsiveness that is required for shifting from Stage I to Stage II; and

(4) Presence of intestinal worms in the host could augment filarial worms in down-regulating the inflammatory responses associated with Stage I and assist in establishing patent filarial infections. In general, subjects living in low endemic areas would behave more like experimental animals administered with trickle infections of filarial larvae. Susceptible subjects living in areas of high endemicity and satisfying one or more of the above mentioned predisposing factors would shift more readily from Stage I to Stage II, analogous to susceptible animals reaching patency when infected with a large inoculums of infective larvae.

Histologically, a lymph-node biopsy taken from individuals at Stage I would reveal dead/degenerating worms associated with a severe inflammatory reaction, while those collected from Stage II would have intact, live mature adult worms in dilated lymphatics without inflammatory reaction [41-42]. These are analogous to inflammation and formation of lymph thrombi during pre-patent phase and down regulation of such responses during patent phase in infected animals (43). All Mf carriers and those with cryptic infection (as shown by circulating filarial antigens) are those who have moved into Stage II, while endemic normals are those who have remained stationary at Stage I. A majority of patients with chronic filarial disease, particularly lymphedema/ elephantiasis are those who have remained at Stage I. However, infection pressure above a threshold could down-regulate the inflammatory responses associated with Stage I and shift some of these patients to Stage II, thus accounting for presence of Mf and/or CFA along with chronic symptoms. The relatively higher prevalence of CFA in

patients with hydrocele indicates that shift from Stage I to Stage II takes place more readily in them than in patients with lymphedema/elephantiasis (32,34). The model does not exclude pathogenesis of filarial disease mediated *per se* by lymphatic dwelling adult worms. Parasite-associated factors causing pathology could be operational at Stage II and contribute to the development of disease. Extrapolating from the observations in susceptible animal models of filariasis, the model presumes that a strong inflammatory hyper-responsive state (Stage I) is associated with the growth and development of infective larvae into mature adult worms and that successful persistence of developed worms in the host would depend on rapid down regulation of the inflammatory responses observed in Stage I to an immunologically tolerant Stage II (20,44). The model assumes that the life span of adult filarial worms in infected humans is in the range of 15-20 years, or more. Estimates of the life span of filarial worms are limited to calculations of "fecund life span" only, since they were based on the duration of microfilaraemic phase in Mf carriers (45). Longitudinal follow-up of Mf carriers for 13-16 years has indicated persistence of adult worms as shown by the presence of CFA several years after loss of circulating microfilariae. (10,46). The long life span of adult filarial worms is further indicated by several immunoepidemiological studies on the prevalence of CFA in age-stratified populations in endemic areas. Unlike intestinal worms, which follow a convex prevalence curve (47) filarial antigenemia increases in younger age groups (<20 years) and is maintained as a plateau in higher age groups (34,48,49). Persistence of adult filarial worms for several years (15-20 years or more) would thus maintain the host at the hypo-responsive Stage II. This hypo-responsive state would be irreversible and loss/ death of adult worms would not result in recovery of immunological hyper-reactivity and thus continue to sustain the host at Stage II (50,10). The loss of microfilariae and /or antigenemia does not result in recovery of immunological hypo-responsiveness. Microfilaraemic subjects continue to display decreased filarial specific T-cell proliferation and IFN-γ production and are free of antibodies to microfilarial sheath after loss of circulating Mf/ filarial antigens (50,10). The current model is partly similar to the "Immunological model" in which adult worm infestation is not considered a pre-requisite (unlike the "dynamic model") for development of disease. However, it is unique and clearly different from "immunological view-point" which is essentially bi-directional and considers infection and disease to be generally mutually exclusive. The current model is uni-directional and linear. Secondly, apart from a genetic pre-disposition, it considers infection load/transmission intensity as well as intra-uterine exposure to filarial antigens as crucial components for the consequence and progression of infection/ disease. The model thus accommodates disease development by inflammatory processes (proposed by immunological viewpoint) as well as by lymphatic dwelling parasites, (proposed by dynamic model) and it explains the presence of filarial infection in patients with chronic disease. It also accommodates the observations

made by all the long-term follow-up studies mentioned above (50,10,8,51). Three sets of immuno-epidemiological observations made in endemic areas provide more direct evidence for the major component of the current linear model, which proposes that inflammatory Th1 responses will be observed in the host during the pre-patent phase of infection i.e., before the onset of antigenemia/microfilaraemia. In endemic areas this phase can be expected to be observed under two circumstances – a) in early childhood or b) in transmigrants who have been moved from non-endemic areas to filarial endemic areas. This implies that Stage I features of the model would be observed in younger age groups and relatively more of Stage II features would be observed in the adult population in endemic areas. Existing epidemiological

Table 1: Progression of filarial infection and disease

	Stage-I		Stage-II
Parasite development	L3 → L4 → L5	→	.....L5 → Adults/Mf
Circulating Filarial Antigen (CFA)	Negative	→	Positive
Immune response phenotype	Hyper-responsive state	→	Hypo-responsive state
T-cell proliferation	High		Low
IgG1, IgG2, IgG3	High		Low
IgE	High		Low
IgG4	Low		High
Abs to Mf sheath	Present		Absent
IFN-?	High		Low
IL-5	High		Low
IL-10	Low		High
TGF-?	Low		High
Histopathology	Dead/degenerating worms associated with severe inflammatory reaction	→	Intact, live worms in dilated lymphatics without inflammatory reaction.
Distribution of filarial spectrum			
Mf carriers	0%	→	100%
Cryptic infection	0%	→	100%
Endemic Normals	100%	→	0%
Hydrocele[a]	60%	→	40%
Lymphodema[a] / elephantiasis	80%	→	20%

[a] The prevalence of Hydrocele and lymphoedema at Stage I or Stage II will be variable in different geographical regions depending on transmission intensity.

evidence offer credence to such a possibility (34,52). Transmigrants in Indonesia (who were moved from filarial non-endemic areas to endemic zones) displayed more of Stage I associated immune response phenotype in the early years of exposure (<3 years) and with increasing years of stay in endemic areas many of them moved towards the hypo-responsive Stage II (53). Finally, in Onchocerciasis, early human infection is associated with enhanced parasite specific cellular immune responses, which get down regulated in chronic infections (54). A more direct evidence that development of lymphedema is driven by repeated exposure to infective larvae and not so much by adult worms has been experimentally demonstrated in a primate model with B.malayi infections (2). This linear model of natural history of lymphatic filariasis has a vital bearing on the on-going global initiative for control of the disease. The model implies that infected subjects are prone for re-infection and effective blocking of transmission in human communities will be a critical requirement for successful control programme in disease endemic countries.

ACKNOWLEDGEMENTS

The Regional Medical Research Center is funded by The Indian Council of Medical Research, New Delhi. The work in the author's laboratory has been partly funded by the European Commission (IC-18- CT-970245) . The author thanks all his current as well as past laboratory colleagues and students who have been a constant source of inspiration.

REFERENCES

1. Denham DA and Fletcher C (1987). The cat infected with *Brugia pahangi* as a model of human filariasis. In: Filariasis, CIBA Foundation Symposium (127), John wiley & Sons Ltd, Chichester, UK, pp.225-235.
2. Murty PK, Khan MA, Rajani HB and Srivastava VM (2002) Pre-adult stage parasites and multiple timed Exposures to Infective larvae are involved in development of limb edema in *Brugia malayi* infected Indian leaf monkeys (Presbytis entellus) *Clinical Diagnosis Laboratory Immunology* **9**: 913-918.
3. Almeida ABD, Silva MCME, Braga C and Freedman DO (1998). Differences in the Frequency of Cytokine-Producing Cells in Antigenemic and Nonantigenemic Individuals with Bancroftian Filariasis. *Infection and Immunity* **66**:1377-1383.
4. Kazura JW (2001). Resistance to Infection with Lymphatic-dwelling Filarial Parasites. In: TB Nutman (ed), Lymphatic Filariasis, Tropical Medicine: Science and Practice, Imp. College Press, pp.83-102.
5. Yazdanbakhsh M, Paxton WA, Kruize YCM, Sartono E, Kurniawan A, Wout AVH et al. (1993). T Cell Responsiveness Correlates Differentially with Antibody Isotype Levels in Clinical and Asymptomatic Filariasis. *Journal of Infectious Disease* **167**: 925-931.
6. King CL (2002). Immune regulation and the spectrum of Filarial Disease In: TR Klei & TV Rajan (eds), The Filaria (5), World Class Parasites, Kluwer Academic Publishers, Boston, pp127-142.
7. Ravindran B, Satapathy AK, Sahoo PK and Mohanty MC (2003). Protective immunity in human lymphatic filariasis: problems

and prospects. *Medical Microbiology and Immunology* **192:** 41-46.

8. Simonsen PE and Meyrowitsch DW (1998). Bancroftian filariasis in Tanzania: Specific antibody responses in relation to long-term observations on microfilaremia. *American Journal of Tropical Medicine & Hygiene* **59**:667-672.
9. Sahoo PK, Geddam JJB, Satapathy AK, Mohanty MC, Das BK, Acharya AS at al. (2002). Bancroftian filariasis: a 13 - year follow-up study of asymptomatic microfilariae carriers and endemic normals in Orissa, India. *Parasitology* **124:** 191-201.
10. Satapathy AK, Sahoo PK, Geddam JJB, Mohanty MC and Ravindran B (2001). Human Bancroftian filariasis: loss of patent microfilaraemia is not associated with production of antibodies to microfilarial sheath. *Parasite Immunology* **21**: 163-167.
11. B. Ravindran (2003). Aping Jane Goodall: insights into human lymphatic filariasis. *Trends in Parasitology* **19(3)**: 105-109.
12. Hoerauf A (2002). Immune Effectors Important in Protective Resistance. World Class Parasites. In: T.R. Klei and T.V. Rajan (eds) The Filaria (5), Kluwer Academic Publishers, Boston, pp109-125.
13. Lawrence RA and Devaney E (2001). Lymphatic filariasis: parallels between the immunology of Infection in humans and mice. *Parasite Immunology* **23**:353-361.
14. Maizels RM, Allen JE and Yazdanbakhsh M (2001) Immunology of Lymphatic Filariasis: Current Controversies. In: TB Nutman (ed), Lymphatic Filariasis (1), Tropical Medicine, Science and Practice, pp. 217-243.
15. Devaney E and Osborne J (2000). The third-stage larva (L3) of Brugia: its role in immune modulation and protective immunity. *Microbes Infection* **2**:1363-1371.
16. Devaney E and Yazdanbakhsh M (2001). Prospects and challenges in lymphatic filariasis. *Parasite Immunology* **23**:323-325.
17. Mukhopadhyay S, Sahoo PK, George A, Bal V, Rath S and Ravindran B (1999). Delayed clearance of Filarial infection and enhanced Th1 immunity due to modulation of macrophage APC functions in XID mice. *Journal of Immunology* **163**: 1789-1792.
18. Mukhopadhyay S, George A, Bal V, Ravindran B and Rath S (1999). Bruton's tyrosine kinase deficiency in macrophages inhibits nitric oxide generation leading to enhancement of IL-12 induction. *Journal of Immunology* **163**: 1786-1792.
19. Mukhophadya S, Mohanty MC, Mangla A, George A, Bal V, Rath S et al. (2002). Macrophage Effector functions controlled by Bruton's Tyrosine Kinase are more crucial than the cytokine balance of T-cell responses for microfilarial clearance. *Journal of Immunology* **168**: 2914-2921.
20. Ravindran B, Satapathy AK, Sahoo PK and Geddam JJB (2000) Protective immunity in human bancroftian filariasis:inverse relationship between antibodies to microfilarial sheath and circulating filarial antigens. *Parasite Immunology* **22**: 633-637.
21. Babu S and Nutman T (2003). Proinflammatory cytokines dominate the early immune response to filarialparasite. *Journal of Immunology* **171**: 6723-6732.
22. Chirgurin SR, Nowling JM, Coleman SU and Klei TR (2003). Effect of immune stimulatory oligonucleotides of Brugia pahangi in Mongolian gerbils (Meriones unguiculatus). *Journal of Parasitology* **89**:483-489.
23. Rao UR, Vickery AC, Kwa BH, Nayak JK and Subramanyam D (1992). Effect of carrageenan on the resistant of congenitally athymic nude and normal Balb/C mice to infective larvae of Brugia malayi. *Parasitology Research* **78:** 235-240.
24. Zanner H and Schulthesis K (1987). Effect of Cyclosporine A and some derivatives in litomosoidea carinii infected Mastomys

natalenesis. *Journal of Helminthology* **61**: 282-290.

25. Ottesen EA (1992). Infection and disease in lymphatic filariasis: an immunological perspective. *Parasitology* **104**: S71-S79.
26. Bundy DA, Grenfell BT and Rajgopalan PK (1991). Immunoepidemiology of lymphatic filariasis: the relationship between infection and disease. *Immunoparasitol Today* (Gallager, R. and Ash, C., eds Elsevier A71-A75.
27. Srividya A, Pani SP, Rajgopalan PK, Bundy DA and Grenfell BT (1991). The dynamics of infection and disease in Bancroftian filariasis. *Transactions of the Royal Society of Tropical Medicine & Hygiene* **85**: 255-259.
28. Chan MS, Srividya A, Nomam RA, Pani SP, Ramaiah KD, Vanamail P *et al.* (1998). Epifil: A dynamic model of infection and disease in lymphatic filariasis. *American Journal of Tropical Medicine & Hygiene* **59**: 606-614.
29. Dreyer G, Noroes J, Figueredo-Silva J and Piessens WF (2000). Pathogenesis of Lymphatic Disease in Bancroftian Filariasis: A Clinical Perspective. *Parasitology Today* **16**: 544-548.
30. Maizels RM and RA Lawrence (1991). Immunological tolerance: The key features in human filariasis? *Parasitology Today* **7**: 271-276.
31. Kazura JW, Bockarie M, Alexander N, Perry R, Bockarie F, Dagoro H et al. (1997). Transmission intensity and its relationship to infection and disease due to *Wuchereria bancrofti* in Papua New Guinea. *Journal of Infectious Disease* **176**: 242-246.
32. Addiss DG, Dimoik KA, Eberhard ML and Lammie PJ (1995) Clinical, parasitologic, and immunologic observations of patients with hydrocele and elephantiasis in an area with Endemic lymphatic filariasis. *Journal of Infectious Disease* **171**: 755-758.
33. Gyapong JO (1998). The relation between infection and disease in *Wuchereria bancrofti* infection in Ghana. *Transactions of the Royal Society of Tropical Medicine & Hygiene* **92**: 390-392.
34. Ravindran B (2001). Are inflammation and immunological hyperactivity needed for filarial parasite development? *Trends in Parasitology* **17**: 70-73.
35. Maizels RM, Sartono E, Kurniawan A Partono F, Selkirk ME and Yazdanbakhsh M (1995). T-cell Activation and the balance of antibody isotypes in human lymphatic filariasis. *Parasitology Today* **11**: 50-56.
36. Freedman DO (1998). Immune Dynamics in the Pathogenesis of human lymphatic filariasis. *Parasitology Today* **14**: 229-233.
37. Dimock KA, Eberhard ML and Lammie PJ (1996). Th1-Like Antifilarial immune responses predominate in Antigen-Negative persons. *Infection and Immunity* **64**: 2962-2967.
38. Sartono E, Kruize VC, Kurniawan A, Maizels RM and Yazdanbakhsh M (1997). Depression of Antigen-Specific interleukin-5 and Interferon-? responses in human lymphatic filariasis as a function of clinical status and age. *Journal of Infectious Disease* **175**: 1276-1280.
39. Mahanty S, Mollis SN, Ravichandran M, Abrams JS, Kumaraswami V, Jayaraman K et al. (1996). High levels of spontaneous and parasite antigen-driven interleukin-10 production are associated with antigen-specific hyporesponsiveness in human lymphatic filariasis. *Journal of Infectious Disease* **173**: 769-773.
40. King CL Mahanty S, Kumaraswami V, Abrams JS, Regunathan J, Jayaraman K et al. (1993). Cytokine control of parasite-specific anergy in human lymphatic filariasis. Preferential induction of a regulatory T helper type 2-lymphocyte

subset. *Journal of Clinical Investigation* **92**: 1667- 1673.

41. Jungmann P, Figueredo-Silva J and Dreyer G (1991). Bancroftian lymphadenopathy: A histopathologic study of fifty-eight cases from northeastern Brazil. *American Journal of Tropical Medicine & Hygiene* **45**: 325-331.

42. Jungmann P, Figueredo-Silva J and Dreyer G (1992). Bancroftian lymphangitis in northeastern Brazil: A histopathological study of 17 cases. *Journal of Tropical Medicine & Hygiene* **95**:114-118.

43. Rao UR, Nasarre C, Coleman SU, Bakeer M, Dennis VA, Horohov DW et al. (1996). Cellular Immune Responses of Jirds to Extracts of life cycle stages and adult excretory products during the Early development of Brugia pahangi. *Experimental Parasitology* **82**:255-266.

44. Saeftel M, Volkmann L, Korten S, Brattig N, Al-Qaoud K, Fleischer B et al. (2001). Lack of Interferon γ confers impaired neutrophil granulocyte function and imparts prolonged survival of adult filarial worms in murine filariasis. *Microbes Infection 3*: 203-213.

45. Vanamail P, Ramaiah KD, Pani SP, Das PK,Grenfell BT and Bundy DA (1996). Estimation of the fecund life span of *Wuchereria bancrofti* in an endemic area. *Transactions of the Royal Society of Tropical Medicine & Hygiene* **90**: 119-121.

46. Simonsen PE and Meyrowitsch DW (1998). Bancroftian filariasis in Tanzania: specific antibody responses in relation to long-term observations on microfilaremia. *American Journal of Tropical Medicine & Hygiene* **59**: 667-672.

47. Anderson RM (1986). The population dynamics and epidemiology of intestinal nematode infections. *Transactions of the Royal Society of Tropical Medicine & Hygiene* **80**: 686-696.

48. Simonsen PE, Lamnge MM, Msangeni HA, Jakobsen PH and Bygbjerg IC (1996). Bancroftian filariasis: The patterns of filarial-specific immunologlobulin G1 (IgG1), IgG4, and circulating antigens in an endemic community of Northeastern Tanzania. *American Journal of Tropical Medicine & Hygiene* **55**: 69-75.

49. Day KP, Gregory WF and Miazels RM (1991). Age specific patterns of change in the dynamics of *Wuchereria bancrofti* infection in Papua New Guinea. *American Journal of Tropical Medicine & Hygiene* **44**: 518-527.

50. Steel C, Ottesen EA (2001). Evalution fo Immunogenic Responsiveness of persons leaving in an area of endemic Bancroftin filariasis: A 17 years follow-up. *Journal of Infectious Disease* **184**: 73-79.

51. Dissanayake S (2001). In *Wuchereria bancrofti* filariasis, asymptomatic microfilaraemia does not progress to amicrofilaraemic lymphatic disease. *International Journal of Epidemiology* **30**: 394-399.

52. Lammie PJ, Reiss MD, Dimock KA, Streit T, Roberts JM and Eberhard ML (1998). Longitudinal analysis of the development of filarial infection and antifilarial immunity in a cohort of Haitian children. *American Journal of Tropical Medicine & Hygiene* 59: 217-221.

53. Yazdanbakhsh M (1999). Common features of T cell reactivity in persistent helminth infections: lymphatic filariasis and schistosomiasis. *Immunology Letter* **65**: 109-115.

54. Cooper PJ, Mancero T, Espinel M, Sandoval C lovato R, Guderian RH et al. (2001). Early human infection with Onchocerca volvulus is associated with an enhanced parasite specific cellular immune response. *Journal of Infectious Disease* **183**:*1662*-1668.

Ann Natl Acad Med Sci (India), **42(2)**: 147-155, 2006

Hemophilia and allied disorders care in India : A story of dismay and success

Dr. Dipika Mohanty
Ex-Director, Institute of Immunohaematology (ICMR),
13TH Floor KEM Hospital M.S. Building, Parel,
Mumbai 400012

INTRODUCTION

Hemophilia and allied conditions collectively referred to as "hemophiloid disorders" are a group of disorders due to inherited deficiency of blood coagulation factors leading to life long bleeding disorders. The factors most frequently found deficient in hemophilias are factors VIII (FVIII) and IX (FIX), whose genes are located on the X-chromosome and when mutated, cause the X-linked recessive traits called hemophilia A and B. The reported incidence of hemophilia A is 1 in 10,000 births and that of hemophilia B is 1 in 60,000. Deficiencies of other coagulation factors, which are transmitted as autosomal recessive traits and affect both sexes; are much rarer (1 in 500,000 or less). There is paucity of epidemiological data on the incidence of hemophilia in developing countries. However, as per WHO data 4.8 billion out of 6.0 billion people in the world live in the developing countries belonging to Asia, Africa and South America (1). There is tremendous social and economic diversity within this group leading into significant differences in the incidence, prevalence and management of inherited disorders (2).

Hemophilias occur in mild, moderate and severe forms (corresponding to plasma factor levels of 6-30%, 1-5% and less than 1% respectively). Hemophilia A and B are clinically indistinguishable and are characterized by delayed, prolonged and repeated bleeding episodes. Although patients with mild hemophilia usually bleed only after trauma or surgery, those with severe hemophilia bleed spontaneously or after trivial trauma particularly into joints and muscles, on average 20 to 30 times per year but sometimes more frequently. Hemorrhages within joints and muscles, unless treated adequately with deficient factors results in painful, progressive joint damage and muscle atrophy; resulting in severe disability and limitations of daily activities. These physical disabilities are compounded by associated psychological problems. Hence, *Comprehensive care of Hemophilias* are very essential for successful management of these patients.

Correspondence : Dr. Dipika Mohanty, Sr. Consultant Haematologist, In-charge-Lab Services, Apollo Hospitals Dhaka, Bangladesh, e-mail : mohantydipika@yahoo.com. Sir Shriram Memorial Oration 2004-2005 delivered at the Annual Meeting of NAMS at Ahmedabad, 2005.

In this communication I will describe to you how we are able to establish the comprehensive hemophilia care in India: the problems, frustrations and ultimate success.

World Scenario in the end of 20th country : The modern management of hemophilia started in the 1970s, when the increased availability of plasma concentrates of coagulation factors and the widespread adoption of home replacement therapy led to the early control of bleeding and to the reduction of musculoskeletal damage typical of untreated or poorly treated patients. Furthermore prophylactic treatment was started in Sweden and other countries with very successful outcome of preventing the majority of bleeding episodes and also arthropathy. Therefore, on the whole during these years hemophilia care became one of the most gratifying examples of successful secondary prevention of a chronic disease. However, this optimistic perception changed dramatically in 1980s, when 60-70% of patients became infected with HIV virus. It was also found that they were also infected with hepatitis C virus (HCV) transmitted by factor concentrates manufactured from large pool of plasma.

The last 15 years witnessed the production of safer plasma concentrates of coagulation factors due to the efforts of the scientific communities. The availability of recombinant factors has been the result of progress in DNA technology. Analysis of patients DNA has permitted identification of the mutations in these disorders and have allowed secondary control of disease through carrier detection and antenatal diagnosis. New treatments have substantially improved the previously unfavorable prognosis of patients who develop alloantibodies (inhibitors) to F VIII and IX. Finally in the past few years the first experiments of somatic gene therapy started in persons with hemophilia which has promising results.

Indian Scenario:

Almost 100,000 patients with haemophilia exist in India, although there is no large epidemiological data available in our country. Inadequacy of health care facilities, lack of adequate knowledge of bleeding disorder among primary care physicians and poorly developed haematology services particularly with reference to good diagnostic facilities of bleeding disorders contribute to the fact that the vast majority of patients with hemophilia are undiagnosed Suffice to tell in short that it is an under diagnosed entity. The state expenditure on health is usually 1% of GDP. Considering the large population, the per capita expenditure on health is often $1 per annum. Diseases of greater public health importance i,e infectious diseases and malnutrition take major share from the health budget. The result is that "low-volume-high cost" disorders like hemophilia get little attention in the health planning. There are few laboratories that can provide accurate diagnostic services for bleeding disorders. The fact remains that the clinicians often have little interest in diagnosing diseases they cannot treat, leads to inadequate volume of work to sustain good laboratory services. It is also not enough to attract industry to market appropriate equipments, reagents and also the therapeutic materials i.e the factor

concentrates both recombinant and plasma derived. This again complicates in providing quality diagnostic services and management.

Establishment of Quality Laboratory: We established therefore good coagulation and hemostasis laboratory at I.I.H, Mumbai in the year 1993 which was recognized by WFH and ISTH in course of time. This is a tertiary centre and reference laboratory too. Regular quality control exercise is under taken here. Subsequently this centre was recognized as one of the International Training centre (IHTC) from the year 1999. The aim of the IHTC programme is to disseminate medical knowledge and experience in the diagnostic and management of haemophilia and other coagulation disorders in order to improve the quality of hemophilia care and services in developing countries. This overall activity is achieved by providing training to physicians, Surgeons and laboratory personnel's and also by holding regional workshops providing theoretical lectures and practical demonstrations on the care of haemophilia. These centres have been chosen by WFH for the excellence, for the appropriateness as role models within their region for the diversity of training which they can offer in various aspects of haemophilia care, for facilities they provide.

Carrier Detection and prenatal diagnosis in Hemophilia Families:

Since the treatment of hemophilia is very expensive in the developing country we decided to establish the "Prevention Programme" which is quite in-expensive compared to the huge cost of the management of PWH. It has a greater relevance in the developing country like India. These are achieved in the following ways:

A) Detection of hemophilia by pedigree analysis and phenotype assessment.

It is possible to detect hemophilia carriers by pedigree analysis and by performing some coagulation tests like factor assays both coagulant as well as antigenic.

However, both pedigree data and phenotype assessment are subjected to the limits of probabilistic evaluation, which in the best of the conditions carries no less than 3-20% of error rates. The lower values within the range have been obtained with rigorous testing procedures and sophisticated statistical analysis. Certainly this is an important parameter in many cases. The efficacy of the coagulation data in the classification of carriers and normal controls were assessed by us in our Indian population. (3) (Fig 1, 2)

In case of hemophilia A, the ratio of factor VIII : C and VWF was used as a discriminant and in case of hemophilia B, univariate analysis using factor IX: C was used as a discriminating parameter. In hemophilia A, with a ratio of 0.7 for F VIII: C and VWF Ag there was 92% agreement between the coagulation data and DNA analysis, where as in case of hemophilia B there was only 76% agreement between coagulation parameter and genetic analysis.

B) Carrier detection by DNA analysis:

After the cloning of factor VIII and IX genes, an accurate diagnosis of the carrier state and prenatal diagnosis of hemophilia A and B in the foetus is possible by DNA analysis.

There are two prevalent mutations in severe hemophilia A. About 36% of cases in our series were found to have inversion involving a gene within intron 22 of the F VIII gene, of which 2 further copies exist distal to the factor VIII locus on Xq 28. (4). Recently we also found inversion in intron 1 accounting for about 2% of cases. We have performed the carrier detection by linkage analysis with restriction fragment length polymorphism (RFLP). Usually polymorphic markers in and around factor VIII and IX genes are chosen. These markers are either biallelic or multiallelic and have successfully been used to track down the mutations through the hemophilic families. Following are the strategies for carrier detection in hemophilia A and hemophilia B families in our centre (Figs. 3&4). Our centre has, amongst the developing countries, experience of carrier detection in the largest number of cases so far and also prenatal diagnosis. The efficiency of three common intra and extragenic polymorphic sites of the factor VIII and IX genes has been examined by us in the Indian population (5). In the course of investigation we found a case of recombination between st 14 & the factor VIII gene. For our Indian population we found that for hemophilia A carrier detection Bcl 1, xba1 and taq 1 polymorphic sites for intron 18 and 22 and the extragenic locus st 14 respectively are most suitable amounting to 100% cumulative efficiency shown in Fig 5. For hemophilia B the polymorphic markers determination includes taq1, Dde1 and Hha1 for introns 4 & 1 and the 3' flanking region of the factor IX gene respectively. It indicated the low efficiency of the Taq1 restriction site (18%) in factor IX gene in our population as compared to 45% in caucasian.

Prenatal diagnosis: The strategy for prenatal diagnosis in our centre in the first trimester of pregnancy by CVS is shown in Fig 6. The prenatal diagnosis can be done either in the first trimester or second trimester of pregnancy in a carrier female. The first trimester diagnosis is based on RFLP method. The technique thus involves an index case of hemophilia in the family. In the second trimester the diagnosis is offered by determining the coagulation parameter like level of factor VIII:C activity and also VWF : Ag in the fetal blood sample obtained by cordocentesis under ultrasound guidance at 16 to 18 weeks of pregnancy. The difficulty arises when the index case in not available. Our experience has been published else where (5). The DNA diagnosis approximately costs $100 and is found to be cost effective. Chances of misdiagnosis is about 1-2%.

Genetic Counselling:

This is an important aspect of our procedure for carrier detection and antenatal diagnosis. It is mandatory that formal counselling should be done before any laboratory tests are even considered. Genetic counselling therefore remains an important aspect of hemophilia care helping obligate carriers / those with unknown status to make informed decision. Some of the important points that should be kept in mind while doing this procedure are:

1) The female who seeks antenatal diagnosis must have her carrier status confirmed by DNA analysis before CVS procedure.
2) The affected person in the family also should have a confirmed diagnosis and the factor level determined.

3) The coagulation factor level of the carrier female determined and if she is a symptomatic carrier care should be taken after the procedure if there is excessive bleeding.

4) All the relevant family members including the affected person should be available for investigations.

5) Improper blood sampling or inaccurate labeling may result in misdiagnosis. So, extreme caution is being applied to this procedure. Preferably the CVS sampling should be done in the same centre where the genetic tests are being offered and the person responsible should be present during the procedure.

6) The patients and their relatives should realize and give consent that occasionally the tests might end in inconclusive or inaccurate results based on the fact that not all females are informative for markers used or that there is recombination. However this occurs only in <0.5% of cases.

7) To eliminate maternal DNA contamination in CVS, one can have some different markers.

Now, with improved technology one can go for sequencing of the gene by automatic DNA sequencer to find out the mutation. It is also thought that impaired folding and /or altered conformation of the mutant factor VIII lead to both intra and extracellular instability, which in turn causes factor deficiency in plasma.

Development of Strategy for Economic use of clotting Plasma Products:

(a) Management during surgery: Attempt has been made by us to plan and manage with less amount of factor concentrate for patients with hemophilia who need an operation (6). A patient with an inherited bleeding disorder like hemophilia may need surgical intervention due to diverse common ailments just like his non-hemophilic counterpart. With the availability of factor concentrates in liberal amounts, the scenario of surgical management in patients with hemophilia in western countries has changed substantially except fro those with high inhibitor level. However, factor concentrates are costly and liberal use in major surgery for an adult patient may consume upto 50,000 to 80,000 I.U. of factor VIII in patients with severe hemophilia if carried out according to standards set by developed countries. These standards are arbitrary and were never established by double blind trials finding out the minimum. Under these circumstances there is a pressing need for planning the surgical operation in a patient with hemophilia in such a way that a limited amount of factor concentrates is used along with several other measures without risking excessive bleeding in the patient.

(b) Planning Operations: Any major or minor operative procedures must be well planned in a centre where experienced haematologists are available along with a good hemostasis laboratory, good blood bank services and surgeons having experience in managing such cases. Relevant imaging and other studies required to manage surgical procedure and postoperative period. The inhibitor

status determined preoperatively and if the patient has an inhibitor level of > 10BU/ml then the FEIBA or recombinant factor VII A may be made available.

Emergency Surgery:

If a known hemophiliac and the factor level is known then the patient should receive 100% factor correction before undergoing surgery. A problem arises when a patient with mild or moderate hemophilia who does not know about his disease comes for emergency surgery or even for elective surgery. The diagnosis can only be made after proper investigations. One of the most important things is to know about bleeding diathesis of the patient by collecting his past history about how he has tackled the hemostatic stress like previous operation, child birth, dental extractions, injuries etc and also the detail family history.

Our emphasis was on reducing the factor concentrate use and we are successful in doing so by following the steps described below:

(a) Use of sealant :

Cryoprecipitate which contains fibrinogen and tranexamic acid was taken in one syringe and thrombin in another syringe connected with plastic "Y" connector and then spread together on the wound surface. Immediately the reaction takes place, fibrin glue is generated and hemostasis obtained. This home made product is cheap and was able to stop bleeding from the surgical wounds where a large raw area of oozing remains following surgery. We have used this technique for circumcision with gratifying results and the patient needed only single dose of factor concentrate just before operation.

(b) Use of DDAVP:

DDAVP is known to increase factor VIII levels in patients with hemophilia A 3 to 5 times. So, milo & moderate hemophilia cases can be given the drug just 30 to 60 minutes before operation. It is usually administrated at a dose of 0.3mg/kg in 50ml of normal saline as an intravenous infusion. The medicine is contraindicated in cases of hypertension and coronary artery disease . But the problem is tachyphylaxis development and not all patients do responds to it. So, one has to find out the response in each case. The patients can be given initially daily for 3-4 days & then on alternate days for another 4-5 days keeping an eye on factor VIII level.

(c) Use of Drugs inhibiting Fibrinolysis:

We have found that concomitant use of fibrinolysis inhibitor drugs like EACA and tranexamic acid cut down the factor concentrate requirements. Infect in an in vitro study, we have been able to show that EACA may specifically improve the factor VIII economy in the patients with inhibitors. Tranexamic acid is a better inhibitor of fibronolysis than EACA (Epsilon Amino Caproic Acid). We use EACA more liberally except in urological surgeries and hematuria in patients with hemophilia. Antifibrinolytic drugs can be used locally over the operation field at a dose of 100mg/ml of EACA or 10mg/ml of Tranexamic acid. We have used EACA successfully for orthopedic, procedures like open reduction and plating in cases of fractured femur.

In our patients needing orthopedic surgical procedures we have been able to wean these patients of factor concentrates by 12 days and subsequently used 10IU/ Kg twice or thrice weekly during the beginning of active physiotherapy for first 2 wks. Hence it may be said that antifibrinolytic agents should be used in severe cases of hemophilia more liberally.

d) Problems with patients having an inhibitor:

It is a difficult decision to operate on patients with hemophilia A who have developed inhibitors. Of course the magnitude of the problem of this kind is low, we have successfully used FEIBA and EACA in high doses along with cryoprecipitate in some of the cases.

e) Economizing on the use of Factor concentrates in the post operative period:

Continuous use of factor concentrates has been shown to be one of the effective ways of maintaining constant level of haemostatic factors leading to substantial saving of factor concentrates. However, it is a must to have > 80% factor VIII level and > 60% factor IX level during the operation but subsequently keeping trough level at 30% with other measures will ensure adequate hemostasis. Adequate level of clotting factors can also be maintained by frequently giving relatively lower doses of the factor. The use of FFP and cryoprecipitate also cut down the factor concentrate amount.

Inhibitor to factor concentrate and management of such cases:

Hemophilia Patients with inhibitors pose a formidable challenge for patient management. This is particularly problematic in developing countries where porcine factor VIII, FEIBA, factor VIIa or immunoadsorption column are generally unavailable. We investigated both in vivo and in vitro, the effect of EACA on the inhibitory activity of the inhibitor to factor VIII. It was found that the in vitro EACA substantially inhibited the activity of the inhibitor and had no effect on other immunological reaction like red cell agglutination. The same was confirmed by antigen binding ELISA system also (7). Factor VIII inhibitors are IgG alloantibodies that arise during replacement therapy in 25 to 50% of patients with severe hemophilia A. The hydrolysis of factor VIII by anti-factor VIII antibodies has been found as a mechanism of inactivation of factor VIII. Of course not all antifactor VIII antibodies are found to be proteolytic or catalytic antibodies (8,9). It has an implication in the treatment of cases of hemophilia A having inhibitors.

We also have developed monoclonal antibody to factor VIII:C which we utilize for estimation of inhibitor to factor VIII by ELISA technique. Patent has been applied for the same.

Management of Chronic synovitis and Hemophilia Arthropathy:

Prevention of chronic synovitis is the key to management of hemophilic arthropathy. Hemophilic arthropathy is often seen in India due to inadequate management of the early bleed owing to the nonavailability of factor concentrate. About 30% of the total six hundred hemophiliac patients treated at our centre present with various grades of synovitis. Apart from the

factor infusion on demand, immobilization of the patient during an acute episode of bleeding forms an important aspect of management in chronic synovitis. The immobilization time may vary from couple of days to a few weeks, depending on individual patient response. Walking is not allowed till the patient is free of pain on weight bearing. Along with immobilization in appropriate splints, compression with elastocrepe bandage is applied.

Subsequent to immobilization, graded mobilization using appropriate exercise regimen is mandatory. Mobilization is generally started following infusion of factor concentrate like cryoppt. Mobilization and appropriate exercise regime is carried out by physiotherapists having experience in handling hemophilia cases.

Synoviorthesis:

It is a method of choice prescribed in the treatment of chronic synovitis, who present with repeated bleeds to the same joint. Two widely used methods are chemical synoviorthesis, radioactive synoviorthesis. We have carried out chemical synoviorthesis using rimpicin intraarticularly in some of our cases with gratifying results. (10).

Rehabilitation intervention is equally important to address the complications in chronic arthropathy which is usually done by: (a) management of the acute bleed in chronic synovitis. (b) Improving range of movements of joints. (c) Muscle strengthening. The details of these have been described elsewhere (10). Regular physiotherapy exercises are taught to the hemophiliacs by the physiotherapist. This procedure strengthens the muscles and reduces instability in the joints. Ultimately bleeds are found to be less.

Education:

Realizing the fact that where resources are scarce education remains the corner stone of hemophilia care, we prepared some Educative materials in Hindi, Gujarati & Marathi language for the patients and their family members.

Von Willebrand Disease:

Incidence, carrier detection and molecular basis; Although VWD is considerably more frequently encountered in clinical practice than hemophilia A, the common VWD variants are generally quite mild clinically. Type 3 von willebrand disease is severe and is inherited as autosomal recessive manner and has got very low or undetected level of VWF. Paucity of data regarding incidence, spectrum of clinical manifestations prompted us to undertake this study at our centre. In includes screening of 217 patients for VWD with a bleeding tendency. Out of these, 36 patients were diagnosed as VWD. On investigating the family members of these patients, 10 additional cases of VWD were diagnosed. The laboratory investigations include the screening coagulation test like PT, APTT, TT the factor VIII:C and VWD:Ag levels and VWF:Rco and multimeric pattern. The results are tabulated in tables 1-5.

For carrier detection polymorphisms in intron 40 of the VWF gene was studied. 300 normal controls were also screened for the various alleles in the VWF 1 and VWF2

VNTR polymorphic markers of the intron 40. Apart from the 8 alleles in VWF 1 and 6 alleles in VWF2 markers of VWF intron 40, we have found new alleles VNTR 9 consisting of 111bp in VWF 1 and VNTR 7 and VNTR 8 made up of 178 base pairs and 182 base pairs respectively in the VWF 2 markers. Details of the study are given in table 6. This data obtained subsequently was used in carrier detection in 2 severe type 3 VWD families.

REFERENCES

1. World Health organization. The would Health Report: Geneva: WHO, 1998.
2. United Nations Organizations. Trends and policies in the would economy. World Economic and Social Review, UNO 1997.
3. Mohanty, D (2001). Carrier detection and prenatal Diagnosis in Hemophilia Families in Developing Countries. In : Mohammad Tariq Sohail and Lily Heijnen. Comprehensive Hemophilia care in Developing Countries, Ferozsons Pvt Ltd, 2001, 138-147.
4. Shetty S, Ghosh K, Pathare A, Colah R, Badakere S, Mohanty D(1997). Factor VIII and IX gene polymorphism analysis in Indian population. *Am J Hematol* **54**:271-275.
5. Shetty S and Mohanty D(2001) Molecular Genetics of Hemophilia-Advances in carrier detection and antenatal diagnosis. *Paediatrics Today* 4: 252.
6. Ghosh K, Mohanty D (2001). Haematological Management during surgery. Suggestions for Economic use of clotting plasma products. In: Mohammad Tariq Sohail and Lily Heijnen. Comprehensive Hemophilia care in Developing Countries. Ferozsons Pvt Ltd, 47-57.
7. Ghosh k, Shetty S, Pathare AV; Mohanty D (2000). Epsilon Amino Carproic acid inhibits the activity of factor VIII inhibitors in patients with severe haemophilia in vivo and in vitro. *Acta Haemat* **103**:63-72
8. Lacroix-Desmazes S, Misra N, Bayry J, Mohanty D, Kaveri SV and Kazatchkinem MD (2002). Autoantibodies to factor VIII. *Autoimmunity Reviews* 105-110.
9. Lacroix-Desmazes S, Bagry J, Misra N, Horn MP, Villard S, Pashov A, Stieltjes N, d' oiron R, Saint- Remy J-M, Hoebeke J, Kazatchkine MD, Reinbolt J, Mohanty D, Kaveri SV(2002). The prevalence of proteolytic antibodies against Factor VIII in Hemophilia A. *New Engl Jl of Medicine* **346**: 662-667,2002.
10. Kale J, Ghosh K, Mohanty D, Jijina F (2001) Management of chronic synovitis and Haemophilic Arthropathy in Developing Countries. In: Mohammad Tariq sohail and Lily Heijnen. Comprehensive Haemophilia care in Developing Countries, Ferozsons pvt Ltd P86-101.

Ann Natl Acad Med Sci (India), 42(2): 157-168, 2006

Peptic Ulcer Disease: Managing the Paradigm Shift

Dr. M.P. Sharma
Head Deptt. Of Gastroenterology,
Rockland Hospital,
Qutub Institutional Area, New Delhi - 110016
Professor and ex Head
Department of Gastroenterology, All India Institute of Medical Sciences,
New Delhi, India

SUMMARY

Peptic ulcer is the most important organic gastrointestinal disease. The past several decades have seen dramatic advances in the diagnosis and therapy of acid related disorders. Two decades ago, almost all peptic ulcers were considered to be idiopathic, today, at least in the West, two distinct etiologies can be implicated in three fourths of patients of peptic ulcer. Helicobacter pylori is causally related to majority of cases of both duodenal and gastric ulcer both in the West and in developing countries. In developing countries almost 75% ulcers are associated with H. pylori. The second most common form of peptic ulcer is due to the use of non steroidal anti-inflammatory drugs (NSAIDs)

Management of peptic ulcers has undergone a radical change from the practice of antacids to H_2 receptor antagonists and now use of proton pump inhibitors (PPI). H. pylori eradication regimes is today the treatment of choice in peptic ulcers. By eradicating H. pylori in western countries recurrence of ulcer has been reduced tremendously in 5 years, however, in developing countries the recurrence of H. pylori infection is quite high so the ulcer recurrence is also high. The endoscopic techniques have been developed for treating complicated ulcers.

"Peptic ulcer is an epidemic during this century. It is the disease of the 20th century." This is what Sir Avery Jones had very rightly prophesised decades ago. Peptic ulcer is a disease which has its secrets unravelled over the century which has just gone by. From 1881 onwards, Billroth pioneered gastric surgery for peptic ulcer, Black swung the pendulum in 1972 by using H2 receptor blocking agents in the treatment of this disease and Marshall by implicating Helicobacter pylori as the ulcerogenic bacteria produced a revolution in the disease management. The presentation and course of the disease has not been uniform over all geographical regions and some differences exist in the disease manifestations between the tropics and other regions. This review will address key issues of peptic ulcer disease from epidemiology to management in the tropics.

Correspondence : Dr. M.P. Sharma, Head Deptt. of Gastroenterology, Rockland Hospital, Qutub Institutional Area, New Delhi-110016; Former Professor & Head, Department of Gastroenterology, All India Institute of Medical Sciences, New Delhi, India. Glaxo Oration 2004-2005 delivered at the Annual Meeting of NAMS at Ahmedabad, 2005.

Epidemiology

The epidemiology of peptic has been influenced by two major observations. First is the implication of H. pylori as a causative agent of peptic ulcer disease and second is the clear relationship between the ingestion of NSAIDs and gastroduodenal damage.

Peptic ulcers were rare in the 19th century and became highly prevalent in the 1950s and 1960s. Since, then peptic ulcer disease has declined in incidence, although the rate of decline has been more repaid in the developed countries as compared to developing countries. In the South East Asian countries, the incidence of ulcer disease rose as in the west, but the declined has started in the last decade and has been very gradual. Peptic ulcer disease affect males and females equally in the west while in India the men are affected more commonly than women. Both duodenal as well as gastric ulcer are equally common in the west but incidence times more commonly than women. Both duodenal as well as gastric ulcer are equally common in the west but incidence of duodenal ulcer is much more than the incidence of gastric ulcer in the tropics. The first epidemiological study on peptic ulcer in north India was conducted in 1963 (1). A population prevalence of 0.6% with a male to female ration of 1.7:1 was found in a population of 10096 urban dwellers. The ulcer was located in duodenal bulb in over two third of patients. An increased prevalence was noted in the high socio-economic groups, and dietary evaluation showed that 63% of patients were wheat eaters, thus casting doubt on the rice theory of southern India. Smoking habits were similarly in ulcer and control groups, but alcohol consumption was higher in the ulcer group. In a large country like India differences are bound to exist between regions. South India, where rice is the staple food, has a higher prevalence of peptic ulcer disease then the north (2).

Pathophysiology

The presence of gastric acid is obligatory for a peptic ulcer to form, but most patients have acid secretion within normal limits. Hypersecretion of gastric acid is rarely sufficient by itself to lead to ulceration. Currently, the most important factors in the pathogenesis of peptic ulcer disease are gastric acid secretion and Helicobacter pylori.

The following factors have a role in pathogenesis.

1. Acid secretion and duodenal ulcer

Duodenal ulcer patients a group have increased basal acid output (BAO is expressed as percentage of maximal acid output (MAO), only 10% to 20% of duodenal ulcer patients are beyond the normal range. Nocturnal gastric acid secretion is also increased in duodenal ulcer patients. Only about one third of duodenal ulcer patients have an abnormally large secretory capacity. Alternations in gastric emptying and altered duodenal pH have been implicated in the pathogenesis of duodenal ulceration, but their role has not been established. It has been seen that bicarbonate production in duodenal bulb is markedly diminished in patients with active duodenal ulcer as compared to normal individual. This leads to altered duodenal mucosal defence.

2. Acid secretion and gastric ulcer

The majority of gastric ulcers are associated with NSAID use and H.pylori infection. Resting and meal stimulated pyloric sphincter pressures are diminished in some patients with peptic disease. This permits greater duodenogastric reflux and bile and lecithin are potential gastric mucosal damaging agents. Mucosal blood flow is decreased in small group of gastric ulcer patients. NSAIDs decrease gastric mucosal blood flow in humans. It is possible that diminished blood flow may serve as a cofactor in ulceration.

3. H. pylori and ulcer disease

H. pylori infects the antral mucosa in 95-100% of patients with duodenal ulcer disease and in 70-80% of patients with gastric ulcer. The mechanism by which H.pylori leads to these disease states has not been established. Host factors as well as characteristics of bacteria interact to produce the disease state (Peura 1997, Crespo et al 2001, Nomura A et al 1994).

a) Virulence Factor

These include urease, adhesions, protease, lipase, catalase, superoxide dismutase and platelet activating factor. Strains of H.pylori posses a pathogenicity island which encodes for Cag A (cytotoxin associated gene protein) which encodes a product that promotes induction of cytokines. Approximately, 50% of H.pylori strains produce a vacuolating cytotoxin (vac A). Cag A expression was initially reported to represent an enhanced risk for the development of both gastric cancer and duodenal ulcer disease.

b) Mucosal immune responses

H.pylori induces host responses that promote inflammation and epithelial damage without conferring immunity against infection. Responses include increase interleukin-1 (IL-1), IL-6, IL-8, TNF-alpha.

c) Gastrin release

Hypergastrinemia in patients with H.pylori infection may result from a decrease in antral somatostatin content and somatostatin mRNA. There is a greater acid response to gastrin in patients with duodenal ulcer disease.

d) Mucosal Bicarbonate Secretion

Cure of H. pylori infection normalizes the decreased duodenal bicarbonate secretion in patients with duodenal ulcers.

e) Gastric metaplasia in duodenum

It has been postulated that H.pylori organisms from the stomach colonize areas of gastric metaplasia in the duodenal blub, leading to duodenitis and ulcer formation but this has not been substantiated.

4. Non steroidal antiinflammatory drugs and ulcer disease

NSAIDs are associated with approximately a five folds relative risk of developing a gastric ulcer. The incidence of new gastric ulcer in patients taking aspirin and NSAIDs is about 10-15% during the first 3 months of use. Those at risk are usually elderly patients with a history of ulcer disease, multiple NSAIDs and a high dose of these agents. Duodenal ulcers also occur as a result of NSAID use, but generally less frequently then gastric ulcers.

NSAIDs induce mucosal injury by direct topical injury and systemic effects mediated by prostaglandin depletion. Topical damage is because of 'Ion trapping' whereby the NSAIDs being weak organic acids their intracellular drugs concentration is higher then the outside. This ion trapping allows direct cellular injury, NSAIDs also directly attenuate the hydrophobic properties of systemic effects of NSAIDs are due to prostaglandin depletion. Inhibition of cyclooxygenase with a resultant decrease in PGE, PGE2 and PGI2 are though to be the most important mechanism of action. This leads to decreased mucin secretion, decreased in bicarbonate secretion, decreased surface active phospholipid secretion and decreased epithelial cell proliferation.

5. Smoking and Ulcer Disease

Peptic ulcer disease and smoking are strongly associated based on epidemiological and clinical studies. Cigarette smoking has also been associated with complications related to peptic ulcer disease. The mechanisms whereby smoking causes these effects are not understood. Chronic smoking increases maximal gastric acid secretion and it also acid secretion. Nicotine significantly reduces duodenal but not gastric mucosal blood flow. It also inhibits duodenal and pancreatic bicarbonate secretion.

6. Genetic factors

The concordance for peptic ulcer among identical twins is approximately 50% and is increased among non identical twins, although not to the same degree. The lifetime prevalence of developing ulcer in first degree relative of ulcer patients is about three fold greater then that in t he general population.

Clinical Course

Peptic ulcer disease is a chronic disease with frequent relapses and remission. Eradication of H.Pylori or the use of long term acid suppression diminishes the risks of complications and lowers the relapses rate. Epigastric pain is the predominant symptom in 60-80% of subjects. Typical pain of duodenal ulcer occurs 1-3 hours after meals and frequently awakens the patient at nigh. The discomfort is relieved by food or antacids and is sometimes described as a burning hunger pain or a vague discomfort. Ulcer symptoms are typically episodic with relapses lasting upto 2 week. Gastric ulcer is often asymptomatic, particularly in elderly patients taking NSAIDs. The pain of gastric ulcer is not helped by food and symptoms are also less likely to show periodicity. Vomiting in ulcer disease may signify gastric outlet obstruction as a consequence of chronic ulceration or pyloric obstruction. Weight loss may occur in patients with peptic ulcer, particularly gastric ulcer. Symptoms found to have discriminate value of ulcer are night pain and relief from pain with food, mild or antacids.

Complications of ulcer disease include haemorrhage, penetration and obstruction. Haemorrhage is the most common complication followed by perforation. Duodenal ulcers tend to perforate anteriorly while gastric ulcer tends to perforate along the anterior wall the lesser curvature of the stomach.

H. pylori and Peptic ulcer disease

Epidemiology

H. pylori is found in a substantial proportion of the population. It remains among the most universal of infection. Oral-oral and oral – fecal transmission accounts for most, if not nearly all, cases of infection. H.pyori infection has declined rapidly in all developing countries, which probably has contributed to declines in duodenal ulcer disease and gastric cancer. Several studies have reported H.pylori prevalence of at least 90% of persons with duodenal ulcer. However it now appears that the importance of H.pylori in ulcer disease has been overstated. The implication for epidemiologic studies is that a substantial minority of ulcers thought to be purely attributable to H.pylori must have other causes. The reported prevalence of H.pylori infection in healthy or asymptomatic persons in India varies from 31% to 84% and this depends on age, socio-economic class, housing and sanitation, rural versus urban dwelling and the method used for diagnosis. H. pylori is supposed to be an infection of the childhood in India, and the majority of the population in India is already exposed by the age of 20 yrs. In India, Gill et al showed that the prevalence of IgG and IgA antibodies to the organism was 22%, 56% and 87% and 48%, 58% and 83% in the 0-4, 5-9 and 10-19 years age groups, respectively; thereafter it remained constant up to the fifth decade with a fall in later decades.(3)

Prevalence in disease states

The strongest association of H. pylori has been with peptic ulcer disease. H pylori has been reported to be present in 64-90% of Indian patient with duodenal ulcer. The prevalence of H.pylori in other gastroduodenal disease in Indian patients is gastric ulcer; 50-65% gastric cancer; 38-62% and no ulcer dyspepsia; 42-74%.

Strain characterization

Indian strains of H.pylori seem to be distinct as compared to H. pylori strains in West. PCR tests with a focus on putative virulence genes indicated that 80-90% of strains in Calcutta carried the cag pathogenicity island and potentially toxigenic vacAs1 alleles of the vacuolating cytotoxin gene (vac A) independent of disease status. This is higher than in the West (where cag A and vacA s1 genotypes are disease associated) but lower than in East Asia. The ice A2 gene was weakly associated with disease in Calcutta strains, whereas in the West the alternative but unrelated ice A1 gene at the same focus is weakly disease associated. In a study from Delhi, it was seen that cag A status was not helpful in predicting non ulcer dyspepsia from peptic ulcer disease.

Diagnosis of H. pylori infection

The preferred schema for H.pylori infection is diagnosis, treatment and confirmation of cure. Choosing the appropriate H. pylori test depends on several factors, such as indications for endoscopy, previous H. pylori therapy current or recent medications, and accuracy as well as coast of available testing alternative. The diagnosis of H.pylori infection can be based on invasive test which are endoscopy related tests, non-invasive non endoscopic tests.(4,5,6) No

single test is considered as the gold standard. For a definitive diagnosis consensus of two or more tests is considered as positive result for the diagnosis of H.pylori. Although this also depends on whether this question is being asked in a clinical practice setting or research setting.

Endoscopy based tests

Rapid urease test

With the observation that H. pylori is a strong urease producer, urease has been used as a marker for H. pylori in rapid urease tests and urea breath test. Rapid urease test is the cheapest and most easily available of all available tests. It is based on the principle the H.pylori produces an enzyme urease which cleaves urea into ammonia and carbon dioxide which will turn the pH indicator solution red from yellow. A positive result is available from a few minutes to 24 hours. Commercially available kits are available. However, at most centres an in-house prepared solution is used. This has been validated at most centres in India. The sensitivity and specificity of this test are 95% and 90% respectively. For maximal speed, urease tests should be performed at room temperature with rewarmed media because of the enzyme's higher activity at increased temperature. All biopsy based methods suffer from the patchiness problem. For example one biopsy specimen can be colonized heavily with H. pylori whereas a second biopsy sample 1 cm away can reveal hardly any organisms.

Culture

H. pylori is a fastidious organism to culture, hence it is the most difficult test to perform. It is considered to be the gold standard for the diagnosis of H. pylori however it suffers from the disadvantage of poor sensitivity. The sensitivity at centres with special expertise in this technique has been reported to be 60-90%. Hence, it is not recommended as a routine diagnostic procedure. Culture is required if antibiotic sensitivity is needed or for isolating the strain prior to molecular biology studies. Microbiology laboratories are interested in culturing H. pylori a) for diagnostic purposes b) to establish antibiotics susceptibility of isolates c) to identify potential virulence factors and d) to investigate microbial host- cell interactions. The disadvantages of this technique are a) requirement of special conditions for specimen transportation b) the use of expensive and complicated media with special conditions for maintenance and c) need for special incubation conditions.

Histology

Due to poor yield of culture, histology is considered to be the gold standard for the diagnosis of H.pylori. Although histology may be considered as a gold standard the reliability of detecting H. pylori infection depends on the site, number and size of gastric biopsy specimens. Special stains like Warthin Starry and modified giemsa give better results then Haematoxylin- eosin.

Crushed smear sand imprint cytology

Crushed smear and imprint cytology are done with the aid of antral biopsy specimen. They are as accurate and convenient as histology. The sensitivity and specificity of histology touch smear RUT

and brush cytology of endoscopic antral biopsy from 49 patients of duodenal ulcer was evaluated in a study from Delhi and it was found that best method for diagnosis of H. pylori is a combination of the rapid urease test or brush cytology with histology. In this study it was also observed that brush cytology or touch smear are diagnostic test of choice if a single test is desired.

Polymerase chain reaction

The use of polymerase chain reaction (PCR) for successful detection and characterization of H. pylori from clinical and environmental specimens has been well documented and is being used widely. Using urea primer on antral biopsy specimens PCR has been used for the diagnosed of H. pylori. However, PCR cannot distinguish between viable and nonviable organisms. In daily clinical practice, PCR does not have to be performed to establish H. pylori infection.

Non invasive tests

Urea breath test

The urea breath test is the non-invasive method of choice to determine Helicobacter pylori status. The test is based on the organism's urease activity, which liberates carbon dioxide from urea and produces ammonia to buffer its acidic environment. Ingestion of labelled urea results in production of labelled carbon dioxide which then can be detected in breath. There are two labelled tests available: C-13 and C14 urea breath test. C13 is non radioactive is minimal at the doses used. It is a highly sensitive and specific test with a diagnostic accuracy of 93%. Moreover, it offers the advantage of being a global test i.e. there is no sampling error as seen in endoscopic tests. The most important advantage of UBT is that it obviates the need for antral biopsies to confirm eradication of H.pylori after completion therapy.

Serology

Serology testing is the commonest method on non-invasive diagnosis of H.pylori. Serology is the best method for epidemiological studies but cannot distinguish between past and present infection. Serology detects the presence of IgG and IgA antibodies against H.pylori antigens in the serum. ELISA using a commercial kit has a high sensitivity of 95-100%. These are excellent for use in primary health care setting. Serologic testing is more definitive and differentiating if the antigenic epitopes of H. pylori can be differentiated based on antigenic epitopes that specifically associated with gastric cancer, peptic ulcer and non ulcer dyspepsia.

Choice of test

The choice of a diagnostic test will depend on the clinical situation for which it is required. The selection test at different centres also reflects personal preferences, facilities and skill available and the purpose of the study.

Epidemiological studies

ELISA should be used in this situation.

Diagnosis in a clinical practice

If endoscopy is planned then any of the endoscopic tests like RUT or histology will be sufficient. To reduce the cost, one sample can be used for RUT and the other sample

preserved. If RUT is negative at 24 hours, the second sample should be sent for histology. The biopsies should be taken endoscopically from two sites: the gastric antrum and the corpus. If endoscopy is not planned then urea breath test is a good alternative.

Diagnosis after therapy

Test for H.pylori are done minimum of 4 weeks after completion of therapy. In cases of duodenal ulcer where symptomatic relief has been gained, C14 urea breath test is sufficient to demonstrate eradication of organism. For clinical trials a combination of two of the following tests can be used: RUT, histology or urea breath test.

Strategies for successful eradication

Treatment

Similar to any bacterial infection, the treatment of H. pylori infection is based on the use of antimicrobial agents. An adjuvant therapy is needed and until now the best adjuvant therapy has comprised drugs that increase the pH of the stomach (i.e. antisecretory drugs and especially proton pump inhibitors (PPI).

Indications for treatment

Eradication of H.pylori cures peptic ulcer disease and conversely relapses of peptic ulcer disease are associated with reappearance of H. pylori. This is the basic concept for which H. pylori eradication had been advocated. In 1994, National Institutes of Health consensus development conference had recommended eradication of H. pylori in all cases of duodenal ulcer infected with H. pylori. Subsequently, European H. pylori study group, American College of Gastroenterology and 1997 Asia Pacific working party had also recommended eradication of H. pylori in peptic ulcer disease. Eradication therapy was also recommended in all patients with low grade gastric MALT lymphoma with coexisting H. pylori. Eradication is not recommended in non ulcer dyspepsia with or without antral gastritis. In a bleeding peptic ulcer disease or a past history of ulcer with proven H. pylori infection eradicating therapy should be given.

First Line Therapy

The drugs effective against H. pylori are bismuth salts (colloidal bismuth sub citrate, bismuth subsalicylate), metronidazole, Tinidazole, secnidazole tetracycline, amoxicillin, Clarithromycin, azithromycin, omeprazole, lansoparazole, quinolones and ranitidine bismuth citrate.(7-12). However, a single drug is not effective and moreover it promotes drugs resistance. Hence, at present a three or four drug regimen is indicated. The regimen should include a proton pump inhibitor and two antibacterials in triple therapy while in quadruple therapy it should contain protein pump inhibitor, bismuth and two antibiotics. The time of therapy should be 14 days. As it has been seen that 7 days therapy does not produce optimal eradication rates. No single therapy can be recommended for all of India as there are wide variations in the resistance patterns in different parts of India. The following regimens may be considered:

- PPI (Lansoprazole 30mg BD, omeprazole 20mg BD) + amoxicillin 1 gm twice daily + clarithromycin 500mg twice daily for 14 days.

- PPI+amoxicillin 1 gmBD/clarithromycin 500mgBD+tinidazole 500mgBD for 14 days
- Colloidal Bismuth subcitrate 240mg BD+PPI+ amoxycillin/clarithromycin + tinidazole for 10-14 days

Factors Influencing outcome

Factors linked to treatment:

1. Dose of clarithromycin: Increasing the dose of clarithromycin to 1-1.5mg/day improves cure rates.
2. Duration of treatment: the optimal duration of treatment remains controversial. It has been shown that better cure rates are achieved for longer treatment duration: 14 days greater than 10 days, greater than 7 days.

Factors linked to strains:

1. Resistance of H.pylori to antimicrobial agents
2. Strain type

Factors to patients:

1. Geographical region
2. Patient compliance

Second Line therapies

The choice of the second line treatment largely depends on the treatment which was used initially. If a clarithromycin based regimen was used, a metronidazole based regimen should be used and vice-versa as acquired bacterial resistance to metronidazole and clarithromycin primarily results from previous treatment failure.

Quadruple therapy (i.e. PPI twice daily, colloidal bismuth subcitrate 120mg four times a day, tetracycline 500mg four times a day and metronidazole 500mg three times a day) has been recommended as the optimal therapy in several guidelines. A seven day treatment duration seems to be sufficient and increasing the duration does not increase the efficacy. In non responders, if the initial therapy has been metronidazole based then rescue therapy should be non metronidazole based. And if the first line of therapy had been non metronidazole based then either a quadruple drug regimen should be used or the length of therapy should be increased to a minimum of 14 days. Patient compliance also needs to be monitored rigorously in such cases. Testing for eradication needs to be done in patients with relapse of duodenal ulcer, complicated duodenal ulcer and patients with gastric ulcer when ulcer healing needs to be documented. Newer compounds currently being evaluated for eradication of Helicobacter pylori include macrolides other than clarithromycin, fluoroquinolones, rifamycin derivatives and others.

Macrolides

Azithromycin is able to reach high gastric concentrations persisting for several days and therefore may be administered at a dose of 500mg once daily for three days during a seven day triple eradication therapy. Eradication rates ranging from 28% - 93%59 have been reported for regimens employing this antibiotic. The absorption of azithromycin is markedly reduced when administered with food, which may account for the low eradication rates. In treatment regimens in which azithromycin was given to fasting patients,

cure rates were in the range of 86%-93%. Spiramycin is a well tolerated macrolide. It has shown eradication rates of 89%-91% when administered for 10 days with metronidazole and bismuth subnitrate or ranitidine bismuth subcitrate.

Fluroquinolones

Levofloxacin is being evaluated for its role in eradication of H.pylori. A therapeutic regimen comprising levofloxacin 500mg daily plus rabeprazole and either amoxicillin or tinidazole for 1 week has been found to promote eradication of H. pylori in 90%-92% of treated patients.

High dose dual therapy

PPI and amoxicillin dual therapy widely used in the early 1990's was abandoned because of the inconsistent results and an inferior eradication rate compared to the PPI based triple therapies. As compared to macrolides and metronidazole, amoxicillin never reaches high concentration in the gastric mucosa. Thus an alternative would be to given high doses of omeprazole (40mg 3 times a day) and amoxicillin (1 gm 3 times a day). 80% eradication rate has been reported in initial pilot studies.

Rifabutin

Rifabutin is a rifamycin derivative, which has been used in 'rescue' triple therapy for patients failing to respond to standard regimens for H.pylori eradication. Both quadruple and triple drug regimens employing rifabutin 150mg daily promoted eradication in 66.6% of cases, while the eradication rate was 86.6% ($p<0.025$) in the group employing rifabutin 300mg daily. The study indicates rifabutin is more effective than the so-called second line quadruple therapies, but it needs to be confirmed in futures studies.

Nitazoxamide

Nitazoxamide is a nitrothiazolamide with similar properties as nitroimidazoles but it has the advantage of being well tolerated and does not select resistant H. pylori strains. An eradication rate of 83% was obtained in a dose ranging trial of nitazoxamide with omeprazole.

Ketolides

Ketolides are macrolide derivatives developed to be active against macrolide resistant bacteria. Trials for their effectiveness against H.Pylori are yet to be conducted.

New drugs based on genomics

The complete genomic sequencing of two H.pylori strains may change the present approach to H.pylori eradication. Numerous genes are specific to H. pylori and are common to all strains. Post genomic methods allow an effective screening of these genes and once their vital role is confirmed by mutagenesis they can be screened against thousands of small molecules. This would lead to the development of active drugs, which specifically target certain functions of the bacterium.

Recurrence after eradication

Recurrence is defined as tests for H.pylori which were negative 4 weeks after eradication, becoming positive again. Recurrence can be due to recrudescence of

reinfection. Recrudescence is a pre-treatment strain of H.pylori which was suppressed by treatment and was undetectable 4 weeks after treatment, becoming detectable at a later stage. Reinfection is infection by another strain of H.pylori which infects after the original strain of H.pylori has been eradicated completely. Recrudescence is most likely to occur during the first 12 months after apparent eradication whereas reinfection my account for recurrence after this period. Data from India on reinfection are scarce. Very few Indian studies are available. In three studies reinfection rate was 16% per patient year follow up (range 11%-40%). In the fourth study H.pylori clearance (colonization status 4 weeks after therapy) was studied rather than eradication (colonization status 4 week after therapy) and it was found to be 59% at 3-6 months suggesting that it was due to recrudescence. The ulcer relapse rates were 17% during an average follow up of one year (13,14). This is in contrast to developed countries where the reinfection rate is 0-3% per patient year follow up. Relatively higher reinfection and ulcer relapse rates reported from India could be either due to genetic susceptibility or reexposure to H.pylori. There could be methodological flaws like improper assessment of H.pylori eradication rates as only one test like rapid urease test was used to document eradication in most studies.

Drug resistance in H.pylori infection

Drug resistance appears to be one of the main reasons for failure of therapy. Resistance is more frequent to metronidazole and clarithromycin. There are some reports of resistance to amoxycillin and tetracycline also (15).

Resistance to imidazoles

In Lucknow, metronidazole resistance was found in 66% of cases. In Mumbai, Mhaskar *et al* found resistance to both metronidazole and tinidazole in 100% of the cases. Another study from Mumbai reported resistance to metronidazole in 16% of the cases. In Hyderabad resistance to metronidazole was seen in 17% of the cases. Data collected from seven centres in India showed that 70% of the strains were resistant to metronidazole. Preliminary data shows that resistance to metronidazole from strains isolated in Delhi is about 100%. In Calcutta, 90% of the strains are resistant to metronidazole. Adding proton pump inhibitors to regimens containing metronidazole appears to overcome the problem of metronidazole resistance in vivo. The resistance can also be overcome by using a quadruple (bismuth containing) regime instead of triple regime.

Resistance to clarithromycin

Mhaskar *et al* found 91% of the strains to be resistant to clarithromycin. All strains from Mumbai which were resistant to metronidazole, tinidazole and clarithromycin were sensitive to quinolones. In 3-4% of the cases combined clarithromycin and metronidazole resistance occurs.

Antimicrobial resistance in vitro may not always translate into low eradication rates with triple or quadruple therapies. Various antibiotic combinations may have synergistic effect that may not be apparent when components are tested alone. A strain found to be metronidazole resistant in vitro might under in vivo conditions may prove sensitive through unknown mechanisms.

REFERENCES

1. Chuttani HK, Wig KL, Chablani TD, *et al* (1963). Epidemiology of peptic ulcer: prevalence of peptic ulcer in an urban community in Delhi. *Ind J Med Res* **55**:1121.
2. Tovey FI, Jayaraj AP (1990). Peptic ulcer in India. *Gut* **31**:123-4.
3. Gill HH, Desai HG. (1993). Editorial: Helicobacter pylori and gastroduodenal disorders in India-lessons from epidemiology. *J Clin Gastroenterol* **16**:6-9.
4. Saksena S, Dasarathy S, Verma K, *et al* (2000). Evaluation of endoscopy based diagnostic methods for the detection of Helicobacter pylori. *India J Gastroenterol* **19**:61-3.
5. Ahuja V, Bal CS, Sharma MP. (1998) Can the C-14 urea breath test replace follow-up endoscopic biopsies in patients treated for Helicobacter pylori infection? *Clinical Nuclear Medicine* **18**:330-2.
6. Sharma MP. (2000) Diagnosis of Helicobacter pylori infection: who should be tested with intention to treat? *Indian J Gastroenterol* **19**(suppl1):S36.
7. Gupta VK, Dhar A, Srinivasan S, *et al* (1997). Eradication of H.pylori in a developing country: comparison of lansoprazole versus omeprazole with norfloxacin in a dual therapy study. *Am J Gastroenterol* **92**:1140-2.
8. Ahuja V, Dhar A, Bal C, *et al* (1998). Lansoprazole and secnidazole with Clarithromycin, amoxicillin or pefloxacin in eradication of Helicobacter pylori in a developing country. *Alimentary Pharmacol Therap***12**:551-5.
9. Kumar D, Ahuja V, Dhar A, Sharma MP. (2001) Triple versus quadruple drug regime for eradication of H.Pylori in patients with duodenal ulcer-results of a prospective randomised trial. *Indian J Gastroenterol* **20**:191-4.
10. Ahuja V, Bhatia V, Dattagupta S, Raizada A, Sharma MP. (2005) Efficacy and tolerability of rifampicin-based rescue therapy for Helicobacter pylori eradication failure in peptic ulcer disease. *Dig Dis Sci.* **50**(4):630-3.
11. Bhatia V, Ahuja V, Das B, Bal C, Sharma MP. (2004) Use of imidazole-based eradication regimens for Helicobacter pylori should be abandoned in North India regardless of in vitro antibiotic sensitivity. *J Gastroenterol Hepatol* **19**(6):619-25.
12. Chaudhary A, Ahuja V, Bal CS, Das B, Pandey RM, Sharma MP. (2004) Rank order of success favors longer duration of imidazole-based therapy for Helicobacter pylori in duodenal ulcer disease: a randomized pilot study. *Helicobacter* **9**(2):124-9.
13. Ahuja V, Sharma MP. (2002) Recurrence of H. Pylori after successful eradication. *Gastroenterology* **123**: 2: 653-4.
14. Dhar A, Sharma MP. (1997) Lacunae in data on Helicobacter pylori from India. *Indian J Gastroenterol* **16**(suppl 1): S13-15.
15. Thyagarajan SP, Ray P, Das BK, Ayyagari A, Khan AA, Dharmalingam S, Rao UA,Rajasambandam P, Ramathilagam B, Bhasin D, Sharma MP, Naik SR, Habibullah CM. (2003) Geographical difference in antimicrobial resistance pattern of Helicobacter pylori clinical isolates from Indian patients: Multicentric study. *J Gastroenterol Hepatol* **18**(12): 1373-8.