

# The Indian Journal of Tuberculosis

---

---

Vol. 43

New Delhi, January 1996

No. 1

---

---

## Editorial

### TOBACCO USE

It is time we start thinking, discussing and planning in terms of tobacco use and its control and not just smoking and its control. Tobacco is used worldwide and in many forms, though smoking is the best recognised tobacco use form. Even smoking has many styles - cigarette, cigar, pipe, bidi, hubble-bubble (hooka), chilum, etc. - but cigarette smoking is the most popular way of smoking. In the vast rural India, however, bidi smoking appears to be the favourite. The other tobacco uses are taking of snuff (niswar), chewing zarda (chutki) and adding it to Pan (betel leaf), Pan Masala, and the like. Using tobacco in any form is injurious to health. In the face of massive evidence, relating mainly to smoking, there is little doubt now that tobacco is carcinogenic and affects powerfully the cardio-respiratory system. Second hand (passive) smoking kills too. Besides, nicotine in tobacco is powerfully addictive. Focussing on smoking control alone may be akin to securing the front door while the backdoor is left ajar, allowing free access to these preventable health hazards.

The use of tobacco originated in the U.S.A., long before it travelled to Europe and other continents. John Rolfe sent the first shipload of Virginia tobacco to England in 1613. From that time to the present day, the habit of smoking, which Christopher Columbus and his men learnt from the native Red Indians, has been spreading like wild fire the world over. Growing of tobacco and manufacturing its various products became one of the leading industries, first in the U.S.A. and later in other countries. And, as a result, tobacco barons came to acquire political, economic and social influence out of proportion to their number. Their malfeasant influence has become one of the main constraints now in the fight against tobacco use. Elsewhere in this and the preceding issues, we publish the resolutions of the Ninth World Conference on Tobacco and Health as well as the measures the Clinton Administration is proposing to curb teen-age smoking, which is on the rise in U.S.A., as well as the subtle propaganda of the tobacco industry to encourage tobacco use everywhere.

While data on the extent of smoking in India are few and far between, those in respect of the other use forms hardly exist. The general impression, however, is that in this country chewing and sniffing of tobacco could not be lagging far behind smoking. In the western world, smoking is the predominant habit which, perhaps, explains why the present stress is on anti-smoking efforts. In the U.S.A., one out of every 5 deaths is due to smoking

related illnesses; 20% of adults smoke and 80% of them started smoking before reaching the age of 21 years; around 3,000 more teenagers start smoking every day and the nearly 26 million smokers consume upto 10 billion cigarettes packs every year. Compared with this, the U.S.A. exports 194 billion cigarette yearly, mostly to the developing world where the smoking haoit is rising at 2.1% annually, using up the local product as well as the imported stuff. Faced with a declining rate of smoking among adults in the western world, as a result of the sustained anti-smoking efforts, the tobacco barons are fighting back with a vengeance, anyway and everywhere.

What can we do to expand the scope of our efforts, to become an anti-tobacco use drive, while we are not succeeding remarkably well in our anti-smoking campaign? We could (i) conduct surveys to measure the extent, forms and distribution of tobacco use, worldwide, (ii) study the sociology, economics and environment of tobacco use in different countries/communities, (iii) document scientifically the effect of tobacco use, other than smoking, on health of the people, (iv) detail the overt and covert practices adopted by the tobacco industry to keep the people “hooked on” to tobacco use, (v) evaluate the efforts being made to control smoking and devise suitable strategies for the wider objective, and (vi) pass appropriate legislation.

It may be timely to remind ourselves that laws can only have a limited reach, provided we have efficient law enforcement, and that in the long term it is the people who must change to help themselves to better health. Governments have limited power to change behaviour unless individuals decide not to start using tobacco or quit using it. Governments can certainly educate, cajole, tax tobacco products, set out smoking-free public areas/ facilities and restrict access to tobacco for those who are too young to decide what is good for them, but if governments go farther than that, they imperil themselves.

---

**D.R. NAGPAUL**



## BCG: DO WE HAVE AN ALTERNATIVE\*?

C.N. Paramasivan, Daniel Herbert and R. Prabhakar

Vaccination is generally used as a form of immunoprophylaxis, so that administration of the vaccine even a long time before exposure to the wild-type infectious organism should afford protection. Since effector T and B cells are short-lived, a prime requisite for a vaccine is to generate immunological memory<sup>1</sup>. In the case of organisms such as mycobacteria which are obligate intracellular pathogens and which elicit granulomatous tissue reactions, artificial immunisation with live bacteria is required to induce protection.<sup>2,3</sup> The only existing vaccine against tuberculosis is the BCG (*Bacille Calmette - Guerin*), an attenuated strain of *M. bovis*, and it is mandatory or officially recommended in 182 countries or territories. Under the Expanded Programme on Immunisation (EPI), started by the Government of India in 1978, BCG is recommended to be given to all infants 3-9 months after birth.<sup>4</sup>

### HISTORY OF BCG VACCINE

The history of BCG vaccine and the trials conducted to assess its effectiveness in humans have been reviewed by many workers.<sup>5-10</sup> BCG, the bile-tolerant, attenuated strain of *M. bovis*, was isolated by Calmette and Guerin.<sup>11</sup> Ox-bile was originally added to these cultures to prevent clumping of bacilli. This led to the fortuitous observation that growth in the presence of bile also resulted in attenuation or gradual loss of virulence. Such attenuated organisms multiply only to a limited extent in the animal or human body and can bring about an increase in the resistance of the host to a subsequent fully virulent infection by the same or other antigenically closely related organisms. Calmette further attenuated this strain by cultivation of the organism on a potato-glycerol-bile medium for 230 serial transfers between the years 1908 and 1918.

The bacilli resulting from this attenuation have never been cloned. The original strain of BCG has been lost and has been replaced by a variant while it was being transferred serially on artificial culture media at the Pasteur Institute<sup>12</sup>. It has since been maintained by many different laboratories, using many different methods. As a result, the BCG strains used today are not bacteriologically identical.<sup>13,14</sup> In 1966, a WHO Expert Committee on Biological Standardisation adopted a series of recommendations for the production of BCG vaccine.<sup>15</sup> These recommendations stated that the vaccine should be freeze-dried, and that the vaccine strain should be maintained by the seed-lot-system whereby no vaccine is produced from a seed more than 12 passages removed from the primary freeze-dried lot. Such a method of maintenance was soon adopted by most laboratories and this eliminated the possibility of more attenuated variants in later BCG vaccine lots.<sup>16</sup>

### BCG VACCINE PRODUCTION IN INDIA

In India, the BCG Vaccine Laboratory was started in Guindy, Madras in 1948 for the production of BCG vaccine for use in India and also for supply to some of the neighbouring countries. Since 1966, Danish strain 1331 is being used here for the preparation of both the liquid and the freeze-dried BCG vaccines, based on the seed-lot-system<sup>17</sup>.

For preparing the liquid and freeze-dried vaccines, the BCG Laboratory, Madras, uses the method followed at the State Serum Institute, Copenhagen, but using Sauton potato medium for maintaining the BCG strain. The prepared vaccine is tested for purity by Ziehl Neelsen smear for acid fast bacilli, and by culture on nutrient broth, thioglycolate medium and Sabouraud's Agar

\* Reprinted by permission from the ICMR Bulletin, 1995, 25, 33

Dy. Director, Research Officer & Director,  
Tuberculosis Research Centre, Madras

Correspondence : Dr. C.N. Paramasivan, Tuberculosis Research Centre, Chetput, Madras 600031

medium. Total bacterial count and the number of culturable particles in the preparation are estimated. Biological tests are carried out in guinea pigs to estimate the degree of virulence of the BCG vaccine, allergenicity and safety. In addition to the above tests, in the case of the freeze-dried vaccine, tests are carried out to estimate residual moisture and heat stability. Both types of vaccines are to be stored at refrigeration temperature, protected from light. Under these conditions of storage, the liquid vaccine can be used for 4 weeks from the date of manufacture while the freeze-dried vaccine can be used for 3 months.

BCG can be administered intracutaneously, orally, by scarification or by multiple punctures. The most widely used method of administration is by intracutaneous injection. The dose is usually 0.1 ml and the site of injection is the upper arm. In the newborn, the dose used is 0.05 ml. The Madras liquid. BCG vaccine administered by an intracutaneous injection of 0.1 ml of the vaccine contains 0.075 mg (moist weight) of BCG. The freeze-dried vaccine prepared there is reconstituted by the addition of sterile distilled water or sterile saline to contain 0.1 mg (moist weight) in 0.1 ml of vaccine which is given intracutaneously.

#### EFFICACY OF BCG VACCINE

BCG was used successfully in humans for the first time in 1921 by Weil-Halle, a colleague of Calmette and Guerin.<sup>18</sup> Scepticism concerning the safety and efficacy of BCG vaccine, and the Lubeck disaster in which 72 of 240 children vaccinated with BCG died as a result of being fed a batch of vaccine containing virulent tubercle bacilli, delayed the acceptance of BCG. A series of controlled trials was begun in the 1930s. Despite inconsistent results from the trials, WHO encouraged widespread dissemination of BCG vaccines, starting in the 1950s.<sup>7</sup> By the 1970s BCG became the most widely used vaccine in the world. About 3 billion doses have been given in the last four decades, and more than 70 per cent of the world children now receive BCG.<sup>5,19</sup>

Between the years 1935 and 1955, at least eight controlled trials were conducted to assess the efficacy of BCG vaccine against tuberculosis.

**Table: Protective efficacy of BCG vaccine against tuberculosis (trials between 1935 to 1955)**

Population group	Period of intake	Protective efficacy (%)
North American Indians	1935-1938	80
Chicago infants	1937-1948	75
Georgia school children	1947	0
Illinois children	1947-1948	0
Puerto Rico population	1949-1951	31
Georgia and Alabama population	1950	14
British children	1950-1952	78
South Indian rural population	1950-1955	31

The protective efficacy results obtained ranged from 0 to 80 per cent (Table).<sup>8</sup>

#### THE SOUTH INDIAN TRIAL

A new study was started in Chingleput, south India, in 1968 in an attempt to avoid the methodological errors that might have affected previous trials.<sup>10,20,21</sup> This south Indian BCG trial was organised by the Indian Council of Medical Research (ICMR) in collaboration with the WHO and Centres for Disease Control (CDC), US Public Health Services. The intake for the study started in 1968 and was completed in 1971, including about 2,60,000 participants out of a population of 3,60,000. The entire population of all ages was eligible and tuberculin reactors were not excluded, in contrast with previous trials. Two BCG strains, Copenhagen and Paris, were tested at two doses, 0.1 mg and 0.01 mg. Neither of the vaccines, whether in full or reduced dosage, gave any protection against the bacillary form of pulmonary tuberculosis, as assessed over a 7.5 year follow up period. No data were available from the study to evaluate protection in children. Very little disease was observed in the period immediately after infection.<sup>22</sup> Incidence peaks were absent in young children and in young adults but the incidence increased logarithmically with age.

The findings of the south Indian trial were disappointing. The ICMR convened an expert

committee meeting to scrutinise the trial methodology, wherein it was agreed that no errors in the conduct of the field operations or in the data processing could have been so serious as to invalidate the results.<sup>10</sup> In the first meeting of the ICMR/WHO Scientific Group<sup>23</sup> it was stated that the data obtained in this trial were unique and of great importance for tropical countries, and should be considered as the starting point for further intensive investigations into the epidemiological, bacteriological and immunological problems related to BCG vaccine and tuberculosis, as well as studies to test certain hypotheses, e.g. that the immune response of the population was unusual, that the vaccines were inadequate to confer immunity, that the south Indian variant of *M. tuberculosis* acted as an attenuated immunising agent, and that mycobacteria other than *M. tuberculosis* may have partially immunised the study population.

#### EXPLANATIONS FOR VARYING EFFICACY OF BCG

The explanations and hypotheses for the varying efficacy of BCG have been discussed in detail<sup>27</sup>. BCG's varying efficacy due to interactions with the immune responses to other mycobacterial infections still remains one of the most popular explanations. Palmer and associates<sup>24,25</sup> showed in animal experiments, and in studies of US navy personnel, that infections with certain non-tuberculous mycobacteria could impart some protection against infection with the tubercle bacillus and such naturally acquired protection could mask any protection due to BCG vaccination, partially or totally. This explanation was criticised by Hart<sup>26</sup> as being inadequate to explain all the differences between the various BCG vaccine trials. Comstock et al<sup>27</sup> also could not find any evidence for lowered protection by BCG in those with intermediate levels of tuberculin reactivity, and this was thought to be due to non-tuberculous mycobacterial infection, in the Puerto Rico trial.

In the 1980s, Rook, Stanford and associates<sup>28,30</sup> proposed that exposure to non-tuberculous mycobacteria (NTM) can result in two types of cell-mediated responses, the 'Listeria type' and the 'Koch type'. Which of these two types of responses is evoked depended, among

other factors, on the mycobacterial species inducing the response and the immunomodulating cells and the pathway brought into play. They further proposed that the 'Listeria type' of response enhances the protective effect of subsequent vaccination with BCG while the 'Koch type' response opposes the protective effect of BCG. Once Koch-like responsiveness is present, this blocks subsequent recognition of further species by Listeria-like responses. BCG vaccination of a person with a pre-existing Koch-like response will temporarily boost this response, but completely fail to reconvert to Listeria-like responsiveness or induce protection from pathogenic challenge. According to them, this is likely to have been the situation in the south Indian trial.<sup>33-36</sup>

Investigations carried out since then have been able to produce some evidence supporting the hypothesis that infection with NTM induces a protective response and does not interfere with the immunity produced by BCG. Attempts to demonstrate that prior infection with any of the mycobacteria induced a suppressive effect against BCG have failed.<sup>33,36</sup>

The study population in the south Indian BCG trial was characterised by a very high prevalence of nonspecific sensitivity.<sup>37</sup> Further, nearly 20 per cent of the NTM obtained from sputum samples of subjects in this area belonged to the *Mycobacterium avium-intracellulare-scrofulaceum* (MAIS) complex.<sup>38</sup> And a recent study on the isolation profiles of environmental mycobacteria present in soil, water and dust<sup>39</sup> samples, and sputum samples of symptomatics in this area has shown that isolates belonging to the MAIS complex are predominant in water, dust and sputum samples while organisms of the *M. fortuitum* complex are predominant in soil samples.<sup>39</sup>

The hypothesis that oral immunisation with *M. avium intracellulare* complex might induce tolerance which might interfere with the immune response to subsequent BCG immunisation was studied at the Tuberculosis Research Centre (TRC)<sup>40</sup> in guinea pigs challenged with *M. tuberculosis*, and it was found that there was no interference with the protective immunity induced by BCG. A later study using intradermal route

showed that while there was no interference with the immunity due to BCG by prior exposure to NTM on the early course of challenge infection, modulation could be taking place during the later course.<sup>41</sup>

The variation in the efficacy of BCG has also been attributed to the differences between the BCG preparations<sup>42,43</sup>. Another view is that BCG is more effective in stopping haematogenous spread of the bacteria as occurring in primary progressive disease and endogenous reactivation compared with exogenous reinfections.<sup>44</sup> Other explanations include the genetic or physiological differences between the trial populations.

More recently, another explanation for the varying efficacy of BCG has been proposed based on the observation that a subgroup of the population may actually be adversely affected by vaccination.<sup>45</sup> Several trials included many subjects with weak initial tuberculin sensitivity, due either to environmental mycobacterial infection or to infection with *M. tuberculosis*. While it is accepted that vaccine efficacy may be moderately reduced in the former subgroup, it has been postulated that the latter subgroup may be at risk of reactivation of tuberculosis soon after vaccination perhaps from focal reactions due to enhancement of their weak sensitivity. The low levels of efficacy in several trials, and the early adverse effect in the south Indian trial are broadly consistent with this hypothesis.

In the search for identifying the correlates of vaccine-induced protective immunity, more than 70,000 subjects in northern Malawi were skin tested with soluble antigens of the tubercle and leprosy bacilli, and then followed up for 5 years for tuberculosis and leprosy incidence. Incidence rate ratios were calculated to compare subjects with different levels of prior skin test sensitivity.<sup>46</sup> It was found that the delayed type hyper-sensitivity to mycobacterial antigens has different implications for tuberculosis and leprosy: low level hypersensitivity, probably attributable to environmental mycobacteria, was associated with protection, but persistent vaccine-associated hypersensitivity to mycobacterial antigens was not a correlate of vaccine-derived protection against mycobacterial diseases.

## BCG VACCINATION AND HIV INFECTION

With regard to BCG vaccination in HIV infected individuals, there are reports of BCG abscesses in HIV seropositives, and of disseminated infection due to BCG in at least one case given BCG.<sup>47</sup> However, in all these cases, the resulting infection could be successfully treated. Since the risks and known consequences of natural infection with tubercle bacilli are likely to be more serious than the risks associated with live attenuated vaccines, the WHO has recommended that all asymptomatic HIV infected children should receive the standard vaccines, both live and inactivated, and those with symptoms of AIDS Related Complex (ARC/AIDS) should receive all the vaccines but BCG. However, in developing countries like India, where extensive HIV testing is not possible, the WHO Expert Group has recommended that all infants should continue to receive immunisation against all the major preventable diseases.<sup>48</sup>

There is no evidence that BCG activates HIV infection.<sup>49</sup> Further, it has been observed that the incidence of disease due to *M. avium intracellulare* (MAI) in AIDS patients varies from region to region and it has been postulated that this difference is the result of a protective effect of neonatal BCG vaccination.<sup>50</sup> In the USA, 30 per cent of patients with AIDS develop MAI disease in contrast to only 10 per cent of AIDS patients in Sweden. This difference in incidence between the two countries could be due to BCG vaccination: most Swedish patients with AIDS would have received BCG in infancy while those in the USA would be unvaccinated. This is further supported by the fact that over 50 per cent of AIDS patients in Netherlands, where BCG vaccination is not given, developed disease due to MAI or *M. Scrofulaceum*. Also, in a limited follow up of HIV infected individuals at the TRC, Madras, it has been found that while a few HIV infected individuals developed disease due to *M. tuberculosis*, no case has been encountered so far with disease due to MAI (Tuberculosis Research Centre - unpublished observations). It has been suggested that MAI disease in AIDS is not due to direct infection but that it arises from long standing silent foci of MAI in the lymphatic

tissue of the patient.<sup>51</sup> It is possible that neonatal BCG vaccination prevents overt infection by MAI and may, therefore, prevent inapparent persisting infection of lymphoid tissue, thus removing the internal reservoir of these bacilli from which AIDS-related MAI disease may arise later in life.<sup>52</sup>

#### **BCG AS AN IMMUNOPOTENTIATING AGENT**

The widespread use of BCG has demonstrated its safety and its potent immunogenicity. This has also led to its suggested use as a carrier to vaccination against other diseases.<sup>53-54</sup> BCG and other mycobacteria are highly effective adjuvants. It is one of the few vaccines that can be given at birth, and with a single dose it induces long-lasting immune responses. Till now, nearly 3 billion vaccinations have been carried out using BCG with a long record of safe use. There is also a worldwide distribution network with experience in BCG vaccination. The adjuvant properties of BCG and its cell wall components have previously been made use of in experimental vaccines. Mixtures of BCG and schistosomal antigens have been used successfully to protect mice in a model of schistosomiasis.<sup>55</sup> Mixture of muramyl dipeptide, which is one of the mycobacterial cell wall components that contributes to the adjuvant properties, and killed simian immunodeficiency virus (SIV) has been shown to provide partial protection against SIV infection in monkeys.<sup>56</sup> Mixtures of BCG and killed *M. leprae* have been used in large scale trials to assess the efficacy of this leprosy vaccine candidate.<sup>57</sup>

#### **RECOMBINANT BCG AND BCG AS A MULTIPLE VACCINE VEHICLE**

Recently developed genetic engineering techniques for mycobacteria have provided the means for the introduction and expression of foreign genes in BCG.<sup>53,58</sup> Recombinant BCG vaccine vehicles can induce immune responses to foreign proteins produced by the bacillus, indicating that BCG can act simultaneously as an adjuvant and as a vehicle to produce and deliver specific antigens to the immune system. A BCG recombinant may provide a longer lasting

immunity to a pathogen than a simple mixture of BCG and the antigen because the antigen continues to be produced by BCG multiplying in the host.

There is no ready answer to the question whether there is an alternative to BCG vaccine for protection against tuberculosis. It is possible to improve the protective efficacy of the existing BCG vaccine against tuberculosis by using the tools of genetic engineering even though very little has been achieved in this direction to date. Such an approach requires a full understanding of the important factors in the virulence of *M. tuberculosis*, pathogenesis of tuberculosis, and protective response against tuberculosis. Genetic deletion or modification of mycobacterial virulence factors or the addition of appropriate mycobacterial antigens, important for protection, might improve the effectiveness of BCG as an antituberculosis vaccine.

#### **CONCLUSION**

Fine and Rodrigues<sup>7</sup> state that several factors, especially the differences in BCG strains and regional differences in mycobacterial ecology in addition to differences in trial methods, have all contributed to the observed variation in BCG's efficacy. They conclude that despite our inability to predict its precise effect, BCG is still judged worthwhile in many countries because there is a possibility that the vaccine might provide reasonable levels of protection against childhood forms of the disease in most populations.<sup>7</sup> Recent retrospective studies of BCG vaccine efficacy among newborns and children have reported a protective effect against all forms of tuberculosis ranging from 17 to 90 per cent. And protection against tuberculous meningitis and cavitory, miliary and bone and joint tuberculosis has been estimated to be 75 per cent or greater.<sup>59,61</sup> BCG vaccination, when effective, does not prevent infection but interferes with the haematogenous spread of tubercle bacilli, thus reducing the risk of severe primary disease and its complications.<sup>60</sup> A meta-analysis of 14 trials and 12 case-control studies showed that the protective effect of BCG against tuberculosis was 51 and 50 per cent respectively.<sup>62</sup> Combining data from 7 trials reporting on deaths from tuberculosis, the relative risk for death

among the vaccinated was 0.29 (71% protective effect). Five case-control studies reporting on tuberculous meningitis showed a 64 per cent protective effect, and 3 case-control studies reporting efficacy of BCG in preventing disseminated tuberculosis showed a 78 per cent protective effect. **The conclusion was that BCG reduces the risk of active tuberculosis on an average by 50 per cent, and the risk of tuberculosis death, meningitis and disseminated tuberculosis.** The fact that BCG provides variable though significant protection against leprosy increases its value in countries with high prevalence of leprosy.<sup>63</sup>

**BCG vaccination alone, at least with the present vaccine, cannot substantially influence the epidemiological situation but should still be continued for children because its use is justified.**<sup>64</sup> BCG vaccination of the newborns protects against the serious forms of tuberculosis, is safe and cheap, and, should be used in developing countries, including India, where tuberculosis is more prevalent. However, in such highly endemic areas, due to the frequent occurrence of exogenous reinfection and also due to the waning of protective effect over the years after vaccination, BCG vaccination of the newborns may not offer protection in the later years of life when revaccination, perhaps at the school going age, may have to be considered. In developed countries with low prevalence of tuberculosis, BCG should be given to high risk groups such as immigrants, their newborns, contacts of patients with tuberculosis and hospital staff.<sup>65</sup>

#### REFERENCES

1. Ada, G.L. The immunological principles of vaccination. *Lancet*, 1990. 335: 523.
2. Lagrange, P.H., Hurtrel, B. and Stach, J.L. Vaccines against mycobacteria and other intracellular multiplying bacteria. *Ann Inst Pasteur/Immunol*, 1985. 136D: 151.
3. Mackaness, G.B. Cellular resistance to infection. *J Exp Med*, 1962. 116, 381.
4. Sokhey, J., Bhargava, I. and Basu, R.N. *In: The Immunisation Programme in India: A Handbook for Medical Officers.* Government of India, Ministry of Health and Family Welfare, New Delhi, 1984.
5. Fine, P.E.M. BCG vaccination against tuberculosis and leprosy. *Br Med Bull*, 1988. 44, 691.
6. Fine, P.E.M. The BCG story: Lessons from the past and implications for the future. *Rev Infect Dis*, 1989. 11 (Suppl 2), 5353.
7. Fine, P.E.M. and Rodrigues, L.C. Mycobacterial diseases. *Lancet*, 1990. 335: 1016.
8. Luelmo, F. BCG vaccination. *Am Rev Respir Dis*, 1982. 125: 70.
9. Smith, D.W. BCG. *In: The Mycobacteria (Part B).* Eds. G.P. Kubica and L.G. Wayne, Marcel Dekker, Inc., New York and Basel, 1984, 1057.
10. Ten Dam, H.G. Research on BCG vaccination. *Adv Tuberc Res*, 1984. 21, 79.
11. Calmette, A. and Guerin, C. Sur quelques proprietes du bacille tuberculeux cultivate sur la bile. *CR Acad Sci*, 1908. 147, 1456.
12. Guerin, C. *In: BCG Vaccination Against Tuberculosis* Ed. S.R. Rosenthal. Little, Brown, Boston, Massachusetts. 1957, 48.
13. Frappier, A., Portelance, V., St Pierre, J. and Parisset, M. BCG strains: Characteristics and relative efficacy. *In: Status of Immunisation in Tuberculosis* Ed. E.G. Chamberlayne, Fogarty Int Cent Proc 14, Washington, 1972, 157.
14. Milstein, J.B. and Gibson, J.J. Quality control of BCG vaccines by the World Health Organisation: A review of factors that may influence vaccine effectiveness and safety. *WHO/EPI/GEN/89: 3*, 1989.
15. WHO Expert Committee on Biological Standardisation. Requirements for dried BCG Vaccine. *WHO Tech Rep Ser*, 1966, 392: 23.
16. Collins, K.M. Tuberculosis, *In: Bacterial Vaccines* Ed. R. Germanier. Academic Press, Inc., 1984, 373.
17. Suri, J.C. *In: Text-book on Tuberculosis* Ed. K.N. Rao, The Kothari Book Depot, Bombay, India, 1972, 495.

18. Calmette, A. La Vaccination Preventive Centre la Tuberculose par le BCG. Masson, Paris. 1927.
19. WHO EPI Update. August, 1989.
20. Tuberculosis Prevention Trial. Trial of BCG vaccines in south India for tuberculosis prevention: First Report. Bull WHO, 1979. 57: 819.
21. Tuberculosis Prevention Trial, Madras, Trial of BCG vaccines in south India for tuberculosis prevention. Indian J Med Res, 1980. 72 (Suppl): 1.
22. Baily, G.V.J. and Toman, K. Notes on the epidemiology of tuberculosis in the area of the Tuberculosis Prevention Trial in India. WHO/TRV/ScG/79.9, 1979.
23. ICMR/WHO Scientific Group: Vaccination against tuberculosis. WHO Tech Rep Ser, 1980, 651: 1.
24. Palmer, C.E. and Edwards, L.B. Identifying the tuberculous infection. J Am Med Assoc, 1968. 205, 167.
25. Palmer, C.E. and Long, M.W. Effects of infection with atypical mycobacteria on BCG vaccination and tuberculosis. Am Rev Respir Dis, 1966. 94, 553.
26. Hart, P.D. Efficacy and applicability of mass BCG vaccination in tuberculosis control. Br Med J, 1967. 1, 587.
27. Comstock, G.W. Livesay, V.T. and Woolpert, S.F. Evaluation of BCG vaccination among Puerto Rican children. Am J Pub Hlth, 1974. 64, 283.
28. Rook, G.A.W. The importance to the International Union against Tuberculosis of some recent advances in our understanding of cell mediated immunity to microorganisms. Bull Int Union Tuberc, 1983. 58, 60.
29. Rook, G.A.W., Bahr, G.M. and Stanford, J.L. The effect of two distinct forms of cell-mediated response to mycobacteria on the protective efficacy of BCG. Tubercle, 1981. 62, 63.
30. Stanford, J.L., Sheild, M.J. and Rook, G.A.W. How environmental mycobacteria may predetermine the protective efficacy of BCG. Tubercle, 1981. 62, 55.
31. Shield, M.J. The importance of immunologically effective contact with environmental mycobacteria. In: The Biology of Mycobacteria, (Vol.2) Eds. C. Ratledge and J.L. Stanford. Academic Press, London. 1983, 343.
32. Stanford, J.L. and Rook, G.A.W. Environmental mycobacteria and immunisation with BCG. In: Medical Microbiology, (Vol.2) Immunisation Against Bacterial Disease, Eds. C.S.F. Easmon and J. Jeljaszewicz, Academic Press, London and New York, 1983, 43.
33. Collins, P.M. Kinetics of the delayed hypersensitivity response in tuberculous guinea pigs and mice tested with several mycobacterial antigen preparations. Am Rev Respir Dis, 1983. 127, 599.
34. Edwards, M.L., Goodrich, J.M., Muller, D., Pollock, A., Ziegler, J.E. and Smith, D.W. Infection with *Mycobacterium avium intracellulare* and the protective effects of *Bacille Calmette-Guerin*. J Infect Dis, 1982. 145, 733.
35. Orme, I.M. and Collins, P.M. Efficacy of *Mycobacterium bovis* BCG vaccination in mice undergoing prior pulmonary infection with a typical mycobacteria. Infect Immun, 1984. 44, 28.
36. Smith, D., Reeser, P. and Musa, S. Does infection with environmental mycobacteria suppress the protective response to subsequent vaccination with BCG? Tubercle, 1985. 66, 17.
37. Narain, R., Vallishayee, R.S. and Venkatesh Reddy, A. Value of dual testing with PPD-S and PPD-B. Indian J Med Res, 1978. 68, 204.
38. Paramasivan, C.N., Govindan, D., Prabhakar, R., Somasundram, P.R., Subbammal, S. and Tripathy, S.P. Species level identification of non-tuberculous mycobacteria from south Indian BCG trial area during 1981. Tubercle, 1985. 66, 9.
39. Kamala, T., Paramasivan, C.N., Herbert, D., Venkatesan, P. and Prabhakar, R. Isolation and identification of environmental mycobacteria in the *Mycobacterium bovis* BCG trial area of south India. Appl Environ Microbiol, 1994. 60, 2180.
40. Narayanan, S., Paramasivan, C.N., Prabhakar, R. and Narayanan, P.R. Effect of oral exposure of *Mycobacterium avium intracellulare* on the protective immunity induced by BCG. J Biosci, 1986. 10, 453.
41. Herbert, D., Paramasivan, C.N. and Prabhakar, R. Protective response in guinea pigs exposed

- to *M. avium intracellulare* *M. scrofulaceum*, BCG and south Indian isolates of *M. tuberculosis*. Indian J Med Res, 1994. 99, 1.
42. Guld, J. BCG as an immunising agent. In: Status of Immunisation in Tuberculosis, Ed. B.C. Chamberlayne, Fogarty Int Cent Proc 14, Washington, 1972, 149.
  43. Willis, S. and Vandiviere, M. The heterogeneity of BCG. Am Rev Respir Dis, 1961. 84: 288.
  44. Wiegeshaus, E.H and Smith, D.W. Evaluation of the protective potency of new tuberculosis vaccines. Rev Infect Dis, 1989. 11: S484.
  45. Springett, V.H. and Sutherland, I. A re-examination of the variation in the efficacy of BCG vaccination against tuberculosis in clinical trials. Tuberc Lung Dis, 1994. 75: 227.
  46. Fine, P.E.M. Delayed-type hypersensitivity, mycobacterial vaccines and protective immunity, Lancet, 1994. 344: 1245.
  47. Von Reyn, C.F. Clements, C.J. and Mann, J.M. Human immunodeficiency virus infection and routine immunisation in childhood. Lancet, 1987. ii: 669.
  48. Indian Council of Medical Research. HIV infection-Current status and future research plans. ICMR Bull, 1991. 21: 125.
  49. Nunn, P.P. and McAdam, K.P.W.J. Mycobacterial infections and AIDS. Br Med J, 1988. 44: 801.
  50. Kallenius, G., Hoffner, S.E. and Svenson, S.B. Does vaccination with *Bacille Calmette-Guerin* protect against AIDS? Rev Infect Dis, 1989. 11: 349.
  51. Good, R.C. Opportunist pathogens in the genus *Mycobacterium*. Ann Rev Microbiol, 1985. 39: 347.
  52. Grange, J.M. Is the incidence of AIDS-associated *Mycobacterium avium intracellulare* disease affected by previous exposure to BCG, *M. tuberculosis* or environmental mycobacteria? Tuberc Lung Dis, 1994. 75: 234.
  53. Aldovini, A. and Young, R.A. Humoral and cell mediated immune responses to live recombinant BCG-ffIV vaccines. Nature, 1991. 251: 479.
  54. Young, D.B. and Cole, S.T. Leprosy, tuberculosis and the new genetics. J Bacteriol, 1993. 175: 1.
  55. Pearle, E.J., James, S.L., Hieny, S., Lanar, D.E. and Sher, A. Induction of protective immunity against *Schistosoma mansoni* by vaccination with *Schistosoma paramysin* (Sm 97), a nonsurface parasite antigen. Proc Natl Acad Sci USA, 1988. 85: 5678.
  56. Desrosiers, R.C. Wynad, M.S., Kodama, T., Ringler, D.J. Arthur, L.O., Sehgal, P.K., Letvin, N.L., King, N.W. and Daniel MD. Vaccine protection against simian immunodeficiency virus infection. Proc Natl Acad Sci USA, 1989. 86: 6353.
  57. Bloom, B.R. Learning from leprosy: A perspective on immunology and the third world. J Immunol, 1986. 137: 1.
  58. Jacob, W.R., Tuckman, R. and Bloom, B.R. Introduction of foreign DNA into mycobacteria using a shuttle plasmid. Nature, 1987. 327:532.
  59. Padungchan, S., Konjanarat, S., Kasiratta, S., Daramas, S. and ten Dam, H.G. The effectiveness of BCG vaccination of the newborn against childhood tuberculosis in Bangkok. Bull WHO, 1986. 64: 247.
  60. Snider, D.E. Jr., Rieder, J.L., Combs, D., Bloch, A.B., Hayden, C.H. and Smith M.H.D. Tuberculosis in children. Pediatr Infect Dis J, 1988. 7: 271.
  61. Tidjani, O., Amedome, A. and ten Dam. H.G. The protective effect of BCG vaccination of the newborn against childhood tuberculosis in an African community. Tubercle, 1986. 67: 269.
  62. Colditz, G.A., Brewer, T.F., Berkey, C.S., Wilson, M.E., Burdick, E., Fineberg, H.V. and Mosteller, F. Efficacy of BCG vaccine in the prevention of tuberculosis: Meta-analysis of the published literature. J Am Med Assoc, 1994. 271: 698.
  63. Ponninghaus, J.M., Fine, P.E.M., Sterne, J.A.C Wilson, R.J., Msosa, E., Gruer, P.J.K., Jenkins, P.A., Luxas, S.B., Liomba, N.G. and Bliss, L. Efficacy of BCG vaccine against leprosy and tuberculosis in northern Malawi. Lancet. 1992. 339: 636.
  64. Styblo. K. Overview and epidemiologic assessment of the current global tuberculosis situation with an emphasis on control in developing countries. Rev Infect Dis, 1989. 11 (suppl 2): S339.
  65. Citron. K.M. BCG vaccination against tuberculosis: International perspectives. Br Med J, 1993. 306: 222.
-



## ISOLATION OF PATHOGENIC NONTUBERCULOUS MYCOBACTERIA FROM PATIENTS WITH CHRONIC RESPIRATORY DISEASE

Mridula Bose<sup>1</sup>, Mohammad Isa<sup>2</sup> and Tapen Dam<sup>2</sup>

(Received on 22.3.95; Accepted on 25.7.95)

**Summary:** A two year study was conducted to determine the rate of isolation of non-tuberculous mycobacteria from chronic respiratory disease attending the V.P. Chest Clinic, University of Delhi. The aims of this study were (a) to find out the current rate of isolation of NTM and (b) to compare the results with results from earlier studies conducted in Delhi.

Though overall rate of isolation of NTM has not changed much over the last twenty years (0.86% in the present study as compared to 0.94% in 1973 and 0.83% in 1986), the frequency of isolation of potentially pathogenic NTM (Runyon Groups I and III) and commonly non-pathogenic (Runyon Group II and Group IV) seems to have been altered over the years. Isolation rate of Runyon Group I has increased from 14.36% in 1973 to 18.46% in 1992-93 and that of Runyon Group III from 14.89% to 38.84%.

In contrast, the rate of isolation of Runyon Group II has declined notably from 57.44% in 1973 to 27.69% in 1992-93 and that of rapid growers (Runyon Group IV) from 13.29% to 10.76%.

such variations. Also, comparison with studies conducted in the same geographic area, in a place not far away from Delhi, with a time interval of a decade has demonstrated a changed scenario<sup>6</sup>. Recent years have seen increased attention given to diseases due to NTM because patients suffering from Acquired Immuno-deficiency Syndrome (AIDS) contract infections from NTM, particularly *Mycobacterium avium-intracellulare* (MAI) as one of the more frequent opportunistic infections.<sup>7</sup> *Mycobacterium avium-intracellulare* complex (MAIC) reportedly causes severe infections in patients suffering from AIDS<sup>7,8</sup>. Although no case of AIDS with NTM infection has yet been reported from India, it may be useful to record prospective data regarding frequency of infections due to NTM including MAIC from various parts of India. Such data should provide important background information for future use.

During the last twenty years a few reports have appeared in the literature recording the rate of isolation of NTM from chronic respiratory infections from north India including Delhi.<sup>1,2,3,6,9</sup> The present study was conducted in patients attending the Patel Chest Institute during 1992 and 1993 to determine the present rate of isolation of NTM from these patients. In addition, we have compared the results of our study with those from earlier studies conducted in Delhi.<sup>1,2,9</sup>

### INTRODUCTION

Studies to determine rates of isolation of non-tuberculous mycobacteria (NTM) from different parts of India have recorded variable results<sup>1-5</sup>. Vast land mass, extremely diverse climatic conditions, variable density of population and atmospheric pollution in different parts of our country are probably the important reasons for

### MATERIAL AND METHODS

A total of 7,498 specimens including sputum, bronchial washings, laryngeal swabs, pleural fluids and FNAC material from patients with chronic respiratory infections attending V.P. Chest Institute Clinic were processed for presence

<sup>1</sup>Reader, <sup>2</sup>Doctoral students,

Department of Microbiology, V. P. Chest Institute, University of Delhi, Delhi

Correspondence Dr Mridula Bose, Reader, V.P. Chest Institute, University of Delhi, Delhi-110007

of acid fast bacilli by Petroff's method<sup>10</sup>. The processed specimens were cultured on a pair of Lowenstein Jensen (LJ) medium bottles and incubated at 37°C for 8 weeks. The slopes were examined every week for evidence of growth. Once growth appeared, it was examined by Ziehl-Neelsen (ZN) staining for acid-fast bacilli (AFB). The AFB positive cultures were screened for NTM by testing for PNB (Para nitrobenzoic acid) tolerance<sup>10</sup>. Repeat specimens were tested from those patients who yielded NTM to eliminate the possibility of laboratory cross contamination. Species identification of all NTM was done using fourteen biochemical tests described in CDC manual<sup>11</sup> namely, rate of growth, growth at 25°C, 37°C and 42°C, photochromogenicity, aryl sulphatase test (3 days and 7 days), niacin production test, nitrate reduction test, catalase production at 68°C, semiquantitative catalase test, tween hydrolysis (5 days and 7 days), 5% sodium chloride tolerance test, tellurite reduction test, urease test, pyrazinamidase test (4 days, 7 days) and TCH utilisation test. The identification tests were standardised and monitored by including CDC recommended mycobacterial cultures as positive and negative controls.

## RESULTS

Out of 7,498 specimens tested, 507, (6.76%) were culture positive for AFB. Among these, 65 cultures were finally identified as NTM (0.86%). Remaining cultures were identified as *M. tuberculosis* (5.89%). The NTM could further be categorised into various Runyon groups (Table 1). Identification for species level was possible for 59 (90.76%) of total NTM isolated (Table 2). Remaining six isolates could not be given any definite species status because of variable results after repeated testing. Such variability of biochemical properties and difficulties in identification have also been recorded by others<sup>19</sup>.

## DISCUSSION

In the present study, the rate of isolation of NTM from patients suffering from chronic respiratory disease was 0.86% of total samples tested and 12.82% of total cultures positive for AFB. The rate of isolation of NTM (% of total specimens studied-Table 2) has not changed much over the last twenty years. However, there is a variability in the percentage of NTM out of

**Table 1. Species level identification of non-tuberculous mycobacteria**

Runyon Group	Species	No. of strains	Percentage of total AFB isolated n=507	Percentage of NTM isolated n=65*
I	M. Kansasii	8	1.58	12.30
	M. Asiaticum	4	0.78	6.15
II	M. Scrofulaceum	5	0.98	7.69
	M. Zulgai	3	0.59	4.61
	M. Gordonae	6	1.18	9.23
	M. Flavescens	4	0.78	4.61
III	M. Avium-intracellulare Complex	18	3.55	27.69
	M. Gastri	2	0.39	3.07
	M. Terrae	2	0.39	3.07
IV	M. Fortuitum	4	0.78	6.15
	M. Vaccae	3	0.59	4.61

\*Six isolates could not be identified to species level.

total AFB positive cultures (Table 2). Shrinivas and Bhatia reported rate of 18.6% of total culture positives whereas Venkat Ram Shankar reported a rate of 5.87%. Shankar's work was based on a local clinic catering primarily to tuberculosis patients and the low rate of isolation of NTM in that study may be due to this reason. Our rate of 12.82% showed the more important, and interesting, overall increase in the rate of isolation of NTM belonging to Runyon Group I and Group III. Chandrasekhar<sup>1</sup> had no Runyon Group I NTM whereas in a study conducted around the same time Shrinivas and Bhatia<sup>2</sup> reported 14.36% of NTM isolates as belonging to Runyon Group I. Shankar, based on a more defined group, reported 4.16% of his isolates (NTM) as belonging to Runyon Group I. Our finding of 18.46% of NTM isolates as belonging to Runyon Group I, as compared to that of Shrinivas and Bhatia is considerably higher. Most of our Group I isolates turned out to be *M. Kansasii* (Table 1). Since majority of the cases reporting to this Institute are from Delhi, the possible reason for this high rate, like in Japan<sup>12</sup>, may be increased density of atmospheric pollution in Delhi which ranks among the world's most polluted cities. Another important observation of our study is a definite rise in the rate of isolation of NTM belonging to Group III: 33.84% as compared to 14.87% in 1968-73 (Table 2). Moreover, 18 out of 22 isolates belonging to Group III (Table 1), were strains belonging to MAIC, which are definitely pathogenic NTM and have been repeatedly isolated from patients who are suffering from pulmonary mycobacteriosis for more than one year. With the backdrop of lurking fear of AIDS assuming epidemic proportion in India, this may be an important observation since a recent report from Amritsar has cited maximum number of strains (4.6%) from Runyon Groups III<sup>6</sup>. A lower rate of isolation of NTM belonging to Runyon Group II, comprising non-pathogens, in our study (Table 2) may be due to suppression of temporary colonisation of the diseased lung by various NTM species because of widespread antituberculosis therapy<sup>6</sup>. Similarly, probably due to the same reason, only 10.76% of total NTM isolates belonged to the other potentially non pathogenic group (Runyon Group IV), compared with previous reports (Table 2).

To conclude, within the limited scope of this study, it may be said that overall rate of isolation of NTM has remained more or less the same over the last twenty years, the rate of isolation of pathogenic NTM (Runyon Groups I and III) seems to have gone up whereas the rate of usually non-pathogenic NTM has recorded a fall.

#### ACKNOWLEDGEMENT

We thank Mr. R.S. Negi and Mr. Daud Mia, for their excellent technical assistance.

#### REFERENCES

1. Chandra Sekhar, S.: Bacteriological & cultural studies on atypical mycobacteria isolated from patients with chronic non tuberculous respiratory diseases; Ind. J. Chest Dis; 1973, 15, 189.
2. Shrinivas and Bhatia, V.N.: A study of mycobacterial species isolated from patients with chronic respiratory disease; Am. Rev. Respir. Dis; 1973, 108, 378.
3. Chakraborty, A., Sharma, Meera and Dubey, M.L.: Isolation rates of different mycobacterial species from Chandigarh (north India); Indian J. Med. Res; March 1990, A(91), 111.
4. Trivedi, S.S., Desai, S.G. and Trivedi, S.B.: Non tuberculous lung mycobacteriosis in Gujarat; Ind. J. Tub.; 1986, 33, 175.
5. Paramasivan, C.N. Govindan D., and Prabhakar, R.: Species identification of non-tuberculous mycobacteria from south Indian B.C.G. trial area during 1981, Tubercle; 1985, 66, 9.
6. Aggarwal Meena, Jindal Neerja, Arora Rajeev, Aggarwal N.P. and Arora Satya: Non-tuberculous mycobacteria: The change in scenario at Amritsar; Ind. J. Tub.; 1993, 40, 25.
7. Greene, J.B., Sidhu, G.S., Lewins, et al.: *Mycobacterium avium-intracellulare*: a cause of disseminated life-threatening infection in homosexuals and drug abusers; Ann. Intern. Med; 1982, 97, 539.
8. Benson, C.A.: Disease due to the *Mycobacterium avium* complex in patients with AIDS: Epidemiology and clinical syndrome: Clinical Infectious Diseases; 1994, 18 (Suppl 3), S218.

Table 2. Different Runyon group percentages in various studies in and around Delhi

Year of study and reference	No. of specimens, (S <sup>1</sup> )	No. of cultures positive for AFB (C <sup>1</sup> )	Cultures positive for NTM (N <sup>1</sup> )		Distribution of NTM in different Runyon groups*			
			(% of S <sup>1</sup> )	(% of C <sup>1</sup> )	Group I (% of C <sup>1</sup> )	Group II (% of C <sup>1</sup> )	Group III (% of C <sup>1</sup> )	Group IV (% of C <sup>1</sup> )
(1968-73) <sup>2</sup>	19888	1010 (5.07% of S <sup>1</sup> )	188 (0.94)	(18.6%) (2.6)	27 (14.36)	108 (57.44)	28 (14.89)	25 (12.29)
(1973) <sup>1</sup>	not given	230	20 (N.D.)	(8.69)	Nil	10 (4.34)	5 (25.00)	5 (25.00)
(1986) <sup>8</sup>	5741	818 (14.2% of S <sup>1</sup> )	48 (0.83)	(5.87) (0.24)	2 (4.16)	17 (2.07)	3 (0.36)	19 (2.32)
(1992-93) Present Study	7498	507 (6.76% of S <sup>1</sup> )	65 (0.86)	(12.82) (2.30)	12 (18.46)	18 (27.69)	22 (33.84)	7 (10.76)

\* NTM cultures not identified for species are excluded.

9. Venkat Ram Shankar S., Prevalence of atypical mycobacteria in patients Undergoing treatment in a tuberculosis clinic; Thesis submitted for M.D. (Tuberculosis and Respiratory Diseases) to Delhi Univ., 1987.
10. Albert Balows: Manual of Clinical Microbiology; 5th Ed., Am. Soc. Microbiology, Washington 1991.
11. Patricia, T.K. and Cubica, G.P.: Public Health Mycobacteriology; A guide for the level III laboratory, U.S. Department of Health and Human Services, Centres for Disease Control, 1995.
12. Tsukamura, M., Sekine K., Yokota A., MKuze A., Shibato M. and Sato K. Studies on the epidemiology of non-tuberculous mycobacteriosis in Japan; Am. Rev. Respir. Dis; 1988,137,1284.



## ROLE OF FINE NEEDLE ASPIRATION BIOPSY IN CYTOLOGICAL DIAGNOSIS OF VERTEBRAL TUBERCULOSIS

Asitava Mondal<sup>1</sup>, Susheela Mitra<sup>2</sup>, D. Mitra<sup>3</sup>, S. Banerjee<sup>4</sup>,  
Dilip Kumar Misra<sup>5</sup> and Bidhan Pal<sup>6</sup>

(Received on 5-4-95; Accepted on 9-10-95)

**Summary:** Fine needle aspiration biopsy monitored with computerized tomography was used for the diagnosis of vertebral tuberculosis in 42 patients. The diagnosis was confirmed by culture or Ziehl-Neelsen staining of the smear in 38 of the 42 patients. Cytological examination of aspirated material revealed collections of epithelioid cells, scattered multinucleated Langhans giant cells and necrosis. In the remaining four patients, sputum for acid-fast bacilli was positive and the patients were being treated for pulmonary tuberculosis. All the patients responded to conservative therapy, There were no complications related to the needle biopsy and an operative biopsy was, thus, avoided. Fine needle aspiration biopsy is a safe and quick diagnostic procedure with high accuracy in the hands of trained cytopathologists. It should be practised in all diagnostic centres of our country, even for suspected vertebral tuberculosis

with destruction of bone with or without soft tissue involvement, adequate material can be obtained by fine needle aspiration biopsy and the results of this procedure have been encouraging.<sup>5-7</sup> Few reports of the use of this technique for non-neoplastic lesions of bone have been reported.<sup>8,9</sup> Cytological findings of tuberculosis after fine needle aspiration of a fifth lumbar vertebra was reported in 1986.<sup>10</sup> The present article reports on 42 patients in whom a diagnosis of vertebral tuberculosis was made on the basis of cytological findings after fine needle aspiration biopsy. The diagnosis was confirmed by culture or Ziehl-Neelsen staining of (he aspirated material in 38 patients.

### MATERIAL AND METHODS

From the beginning of January, 1985 till the end of October, 1994, fine needle aspiration biopsy of 132 osteolytic lesions of vertebrae was performed and the diagnosis of tuberculosis was made in 42 patients. A definite diagnosis of malignant tumour, either primary or metastatic was made in the remaining 90 patients on the basis of cytological examination. There were 28 male and 14 female patients who ranged in age from 12 years to 88 years. All the 42 patients who were diagnosed to be having tuberculosis had pain in the back when they were first seen; in some the pain radiated to the hips or the lower extremities. Detailed clinical examination revealed that all the patients toad local tenderness and a limited range of movements of the back. Except for an elevated erythrocyte sedimentation

### INTRODUCTION

Clinical examination, roentgenographic study and operative biopsy with histopathological examination have been considered essential for the accurate diagnosis of osseous lesions.<sup>1,2,3</sup> Recently, however, it has been shown that histological examination of cells recovered by fine-needle aspiration biopsy too can be of great value in the diagnosis of various malignant bone tumours.<sup>4</sup> As most osseous lesions are associated

- 
1. Head of Department of Cytopathology, S.V.S. Marwari Hospital and Cancer Centre, Calcutta 2. Lecturer in Surgery  
3. Professor in Surgery, S.S.K.M. Hospital, Calcutta 4. Ex-Professor and Head of the Department of Orthopaedic Surgery  
E.G. Kar Medical College and Hospital, Calcutta 5. Head of the Department of Radiology, The Calcutta Diagnostic Centre,  
Calcutta

Correspondence : Dr. Asitava Mondal, D-I, Cluster-1: Purbachal, Salt Lake City, Calcutta - 700091

rate in 28 patients, routine laboratory studies of the blood were unremarkable. Roentgenograms of the chest showed evidence of tuberculosis of the lung with or without hilar lymphadenopathy and pleural involvement in 21 patients. For the remaining patients, the roentgenogram of the chest was normal. Spinal roentgenograms showed destruction of the vertebral bodies and intervertebral discs: thoracic vertebral were involved in 14 patients and lumbar vertebral in 28 patients. (Figure 1). Computerized tomography scans revealed additional destruction of the vertebral bodies with or without protrusion of necrotic material into the surrounding soft tissues. (Figure 2).

Computerized tomography was used to guide the fine-needle in all the patients. A 20 or 22-gauge needle, 12 to 15 centimetres long was used (Figure 3). Some of the aspirated material was spread on glass slides, air-dried and stained by May-Grunwald-Giemsa method. When examination of the slide led to a presumptive diagnosis of an inflammatory reaction, with a strong suspicion of tuberculosis, the remainder of



**FIGURE 1.** Lateral roentgenogram showing almost complete destruction of body of the second lumbar vertebra



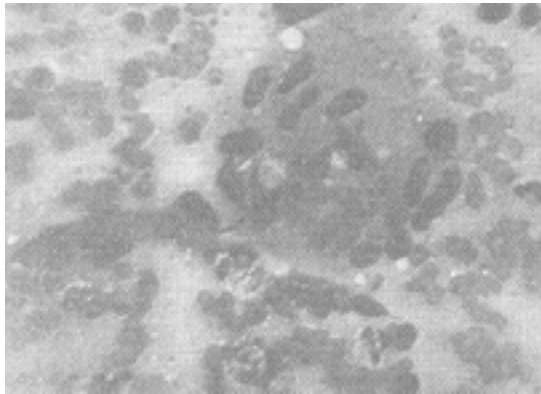
**FIGURE 2.** Computerized axial tomography scan showing extensive destruction of body of second lumbar vertebra, with extrusion of necrotic material into the surrounding soft tissues



**FIGURE 3.** Computerized axial tomography scan showing the placement of a fine biopsy needle in the osteolytic part of the second lumbar vertebra

the aspirated material was sent for Ziehl Neelsen staining or culture, and anti-tuberculosis therapy was started as indicated. *Mycobacterium tuberculosis* grew on culture of material from the aspirates of 38 of the 42 patients, but acid-fast bacilli could not be isolated in the remaining four, presumably because of previous antituberculosis therapy. Previous sputum examination from these 4 patients had shown acid-fast bacilli.

*Cytological Findings:* Cytological examination of the air-dried smears stained by May-Grunwald and Giemsa method showed typical



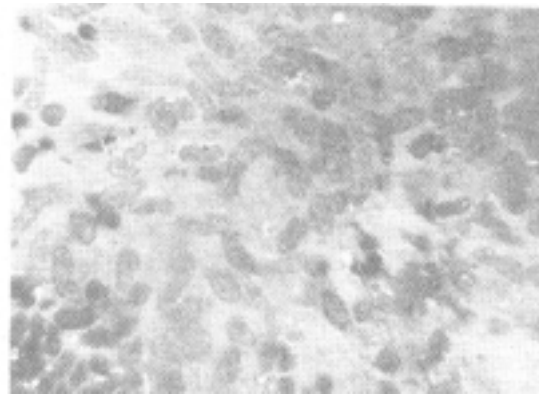
**FIGURE 4.** Photomicrograph showing clusters of epithelioid cells with pale cytoplasm and slipper shaped elongated nuclei. The cytoplasm is arranged in syncytial fashion. A few lymphocytes are also seen (Stain-May Grunwald and Giemsa: magnification - 400)

collections of epithelioid cells. (Figure 4). These cells were oval or elongated with pale cytoplasm and slipper-shaped nuclei. Scattered multinucleated and Langhans giant cells and inflammatory cells were present (Figure 5). Variable necrosis was present in all the cases. No organism was identified in the Gomori methenamine silver stained smears. In fifteen patients, mycobacteria (assumed to be *Mycobacterium tuberculosis*) were identified on Ziehl-Neelsen stained smears.

All the patients responded dramatically to conservative therapy.

## DISCUSSION

*Mycobacterium tuberculosis* involves the skeletal system in 1 per cent of the patients in Western countries.<sup>11-2</sup> It is the most common form of extrapulmonary tuberculosis in the Western world<sup>11</sup>. Skeletal tuberculosis is often seen in India too. In developing countries, most cases of tuberculosis of the vertebrae reach an advanced stage by the time the patient is first seen by an orthopaedic or thoracic surgeon. At that point, most patients have gross destruction of vertebrae, with or without involvement of surrounding soft tissues in the region of the psoas muscles. In many patients, these lesions cannot be distinguished from malignant tumours with routine



**FIGURE 5.** Photomicrograph showing a Langhans giant cell, necrotic tissue and inflammatory cells. (Stain-May Grunwald and Giemsa : magnification - 400)

roentgenographic studies<sup>25-11</sup>. Fine needle aspiration biopsy can help a clinician to reach a definite diagnosis, avoid an operation and begin immediate treatment. This procedure was acceptable to all our patients, was safe and did not require anaesthesia or hospitalization. In the present series, adequate material was obtained from all the patients and there were no complications.

***If smears madefrom material aspirated by FNAB show the presence of granuloma, especially when necrotic material is present, the patients should be treated for tuberculosis even when AFB are not found.***

Twenty-one of the 42 patients had concomitant tuberculosis of the lungs. This finding is in accord with reports that almost 50 per cent of patients who have skeletal tuberculosis do not have a pulmonary focus.<sup>11-13</sup> A common site of involvement is the metaphysis of vertebra, adjacent to the disc with destruction of the end-plates and intervening intervertebral disc. There may be other changes, such as destruction of anterior vertebral cortex with sparing of the discs or gross destruction of vertebral body with extrusion of

pultaceous material into the surrounding tissues, and vertebral collapse.

If smears made from aspirated material show the presence of granuloma, the patients should be treated for tuberculosis, especially when necrotic material is present, even when organisms are not found on Ziehl-Neelsen staining, particularly in countries like India where tuberculosis is widespread.<sup>2,3</sup> In the present series *Mycobacterium tuberculosis* grew on culture from the aspirates of 38 patients, but not from the remaining 4, possibly because of previous therapy for tuberculosis of lung. Follow-up of these 4 patients showed marked improvement with antituberculosis therapy. Since the roentgenographic appearance of vertebral tuberculosis can be similar to that of pyogenic infection, histiocytosis X, fungal infection and neoplasm, morphological confirmation is needed before appropriate treatment can be started. Fine needle aspiration biopsy can provide diagnostic material for morphological and microbiological confirmation of tuberculosis and thus can help the patient to avoid core biopsies<sup>14</sup> or open biopsies<sup>1</sup> done under general anesthesia.

#### REFERENCES

1. Koss L.G, Woyke S and Olszewski W: Aspiration biopsy: Cytologic Interpretation and Histologic Bases: New York: Igaku-Shoin 1984: pp-422-429.
2. Nathanson L and Cohen W: A statistical and roentgen analysis of two hundred cases of bone and joint tuberculosis. Radiology: 1941: 36: 550.
3. Versfeld G.A., and Solomon A: A diagnostic approach to tuberculosis of bones and joints. J. Bone and Joint Surg: 1982: 64-B(4): 446.
4. Hewes R.C., Vigorita V.J. and Freiburger R.H.: Percutaneous bone biopsy: the importance of aspirated osseous blood. Radiology: 1983: 148: 69.
5. deSantos L.A., Lukeman J.M., Wallace S, Murray J.A. and Ayala A.G.: Percutaneous needle biopsy of bone in the cancer patient. Am J. Roentgenol: 1978: 130: 641.
6. El Khoury G.Y., Terepka R.H., Mickelson M.R., Rainville K.L. and Zaleski M.S.: Fine needle aspiration biopsy of bone. J. Bone and Joint Surg: 1983: 65A: 522.
7. Mondal A, and Misra D.K: Fine needle aspiration cytology (FNAC) in the diagnosis of osteolytic lesions of bone: A study of 376 cases. The Antiseptic: 1995: 92: 17.
8. Schajowicz F and Derqui J.C.: Puncture biopsy in lesions of locomotor system, Review of results of 4050 cases, including 941 vertebral punctures. Cancer: 1968: 21:531.
9. Stormby N and Akerman M: Cytodiagnosis of bone lesions by means of fine needle aspiration biopsy. Acta Cytol: 1973: 17: 166.
10. Silverman J.F., Larkin E.W., Carney M, Weaver M.D. and Norris H.T.: Fine needle aspiration biopsy cytology of tuberculosis of the lumbar vertebra (Pott's Disease). Acta Cytol: 1986: 30: 538.
11. Davidson P.T. and Horowitz I: Skeletal tuberculosis. A review with patient presentations and discussion.. Am J. Med; 1970: 48: 77.
12. Cropper G.R., Acker J.D. and Robertson J.H.: Computed tomography in Pott's disease. Neurosurgery: 1982: 10: 506.
13. Waldvogel F.A., Medoff G and Swartz M.N: Osteomyelitis : a review of clinical features, therapeutic considerations and unusual aspects. New England J Med: 1970: 282: 198.
14. Gladstein M.D, and Grantham S.A: Closed skeletal biopsy. Clin. Orthop: 1974: 103: 75.



## TUBERCULOSIS MANAGEMENT IN PRIVATE PRACTICE AND ITS IMPLICATIONS\*

M.W. Uplekar, S.K. Juvekar, S.D. Parande, D.B. Dalai,  
S.S. Khanvilkar, A.S. Vadair and S.G. Rangan

*Summary:* This study of 81 rural and 96 urban private medical practitioners, which included 67 allopaths and 110 non-allopaths, was conducted to understand how patients of lung tuberculosis are diagnosed and treated in their clinics as well as their interactions with and perceptions regarding the public health services available for tuberculosis control. A majority of private doctors gave little importance to sputum examination and considered X-ray of the chest as the single most important diagnostic test for lung tuberculosis. They were neither aware of nor employed inexpensive standard regimens for treating their patients. While all private doctors used short course chemotherapy in the treatment of lung tuberculosis, few recommended under the NTP. Private doctors were aware of but sceptical about tuberculosis treatment available at public health facilities.

and treatment facilities, a large number of chest symptomatics and patients of tuberculosis prefer to go to private clinics. All kinds of private medical practitioners, from quacks to consultants, diagnose and treat a significant number of tuberculosis patients all over the country<sup>1</sup>. While this could greatly influence the outcome of tuberculosis control efforts, few studies have examined the private doctors' practices of managing lung tuberculosis<sup>2</sup>. The present study was taken up to fill this gap in the knowledge. The main objectives of the study were:

1. To understand the diagnostic and treatment practices about tuberculosis among private medical practitioners and,
2. To determine the type and extent of interaction between the private doctors and public health functionaries working in tuberculosis control.

### INTRODUCTION

While reliable statistics are unavailable, private medical practitioners in India diagnose and treat as many, if not more, patients of tuberculosis as do public health services under the National Tuberculosis Programme (NTP). It has also been acknowledged that the success of measures for tuberculosis control in the country will depend, in large part, on effective involvement of private doctors. Yet, very few studies have looked into the practices of private doctors in diagnosing and treating patients of tuberculosis.

In spite of the public health service's efforts to control tuberculosis by providing diagnostic

### METHODS

The study was conducted in both rural and urban areas of Pune district in Maharashtra. In all, 177 private medical practitioners, were included in the study.

To understand the management practices of private medical practitioners, a semi-structured questionnaire was administered and obtained duly filled in the presence of research investigators. The limitation of this approach was that while it provided a good insight into the knowledge of the doctors, it could only be an indirect evidence of their actual practices of managing tuberculosis. To meet the second objective, of determining the present public-private interaction in tuberculosis control, a joint group discussion was conducted

\* Paper presented at 49th National Conference on Tuberculosis and Chest Diseases, Pondicherry, 6-9 October, 1994

for private doctors and public health functionaries in a rural Primary Health Centre.

The responses in the questionnaires were compiled, coded and analyzed using SPSS, a computer package for statistical analysis. The reported treatment prescriptions of doctors were analyzed using another computer software EPI-INFO and the group discussion was tape-recorded, transcribed and edited.

## RESULTS

There were 67 allopaths and 110 non-allopaths, 81 practicing in rural areas and 96 in Pune city. The non-allopaths included were qualified doctors in Homeopathy and Ayurveda as well as Registered Medical Practitioners.

All the private medical practitioners reported that they did manage patients of tuberculosis in their clinics. Only 4 practitioners were uncertain about the number of patients of lung tuberculosis under their treatment at the time of the study (Table 1).

**Table 1. Tuberculosis patients in private clinics**

Number	Private doctors (%)	
	Allopaths n=67	Non-allopaths n=110
None	18	34
<5	60	40
6-10	12	15
11-20	3	5
Can't say	3	2
No response	4	3

## Diagnosis

Private medical practitioners relied upon X-ray as the most important diagnostic test for tuberculosis. About 40% of doctors reported that they would depend on X-ray while only about 10% of doctors advised sputum examination along with an X-ray chest (Table 2).

**Table 2. Diagnostic practices of private medical practitioners**

Investigation	Private Doctors(%)	
	Allopaths n=67	Non-allopaths n=110
X-ray only	36	39
X-ray+sputum	59	54
Others*	5	7

\* Includes investigations like sputum culture, Mantoux test & blood counts

## Treatment

Sixty-four doctors did not respond to a question on writing out a prescription for a 50 kg adult suffering from sputum positive pulmonary tuberculosis. Among the 113 who responded, Streptomycin was the most common drug used by as many as 90 practitioners. Other drugs employed included Isoniazid, Rifampicin, Ethambutol, Pyrazinamide, and Thioacetozone. Four doctors mentioned injection Kanamycin and one doctor mentioned Ampicillin among drugs used commonly in treating tuberculosis. Considering the drugs used and the duration for which the drugs were employed, 90 different treatment regimens were prescribed by 113 doctors. The drug regimens varied with regard to one or more of the aspects of a drug regimen, including the drugs used in the initial intensive phase and in the continuation phase, dosages employed and durations recommended Table 3 groups the regimens prescribed by them in the treatment of tuberculosis according to the acceptability of the drugs and durations of the regimens. Most drug regimens for tuberculosis reported by private medical practitioners did not conform to those recommended nationally or internationally. Among these, as many as twenty two of the 113 practitioners were not prescribing appropriate regimens and another 34 were prescribing

***Most drug regimens reported by private medical practitioners did not conform to those recommended nationally or internationally***

**Table 3. Classification of 113 practitioners according to quality and duration of prescriptions**

Regimen	Duration		Table
	appropriate	inappropriate	
NTP	13	8	21
Non-NTP but Acceptable	44	26	70
Unacceptable	22	-	22
Total	79	34	113

appropriate regimens for incorrect durations (including 6 who continued drugs too long). Only 57 i.e nearly half of the practitioners were prescribing regimens which could be described as acceptable (13 conforming to NTP and 44 appropriate but not recommended by NTP).

#### Case-holding by private doctors

All except two doctors, one rural and the other urban, reported that less than 50 per cent of their patients of tuberculosis completed treatment. Yet, it is noteworthy that none of the private practitioners had any mechanism for defaulter retrieval, nor could they suggest any that might be suitably employed in private practice.

#### Private-Public Interactions

The joint group discussion of private medical practitioners and public health functionaries in tuberculosis control including the medical officers of Primary Health Centres (PHC), multi-purpose workers, pharmacists and laboratory technicians, conducted to understand mutual perception revealed several important aspects. These were:

1. While public health functionaries were well aware of services provided under the National Tuberculosis Programme, private medical practitioners were largely unaware of the diagnostic and treatment practices followed under the programme. Those aware of these practices were unconvinced about their appropriateness.
2. A mutual distrust was obvious. Public health functionaries attributed the flow of patients to private medical practitioners to

**Table 4. Inappropriate drug regimens**

Regimens	Private doctors (%)	
	Allopaths n=67	Non-allopaths n=110
Using Pyrazinamide throughout	12	7
Using single drug in continuation phase	2	7
Not using INH some time during treatment period	9	15

their liberal use of expensive and highly effective drugs of short course chemotherapy. Private medical practitioners ascribed the poor opinion patients have about PHCs to unresponsive attitudes of the health functionaries, lack of secrecy of diagnosis, irregular supplies, poor quality of drugs And unsuitable clinic timings. Some of these views were shared by public health functionaries as well.

3. Despite the differences in approaches to and perception about tuberculosis control, the private medical practitioners and public health functionaries maintained good and friendly relations with each other.
4. Some areas for joint efforts were identified by both the sides, the private and the public. The private medical practitioners suggested holding joint camps in the areas of their practice wherein, they felt, many new patients could be detected who could then be treated in public or private clinics according to the patients' choice and convenience. Some practitioners, it was pointed out, may well be willing to maintain records if drugs were supplied to them by public health services. The public functionaries offered to visit private clinics for collecting informations to be included in the PHC registers and tracing of defaulters.

#### DISCUSSION

Not with standing the fact that utilization of peripheral public health services in both urban and rural areas is poor, most national disease control programmes are solely run by and

through the public health services. Few attempts have been made to involve private doctors in implementing disease control programmes. The main reason for this appears to be the mutual distrust which clearly came out of the joint group discussion that was conducted in the present study.

The private medical practitioners' estimates of tuberculosis patients under their treatment, if true, indicate that a significant proportion of patients is being treated by them in rural and urban areas alike and this reinforces the need to find ways of involving private practitioners in tuberculosis efforts.

Over-dependence of doctors on chest X-ray diagnosis of lung tuberculosis has major implications. This may lead to over-diagnosis, over-medication and wastage of expensive drugs. This aspect needs to be investigated further. Use of sputum examination for diagnosis and for deciding treatment regimen by only a small proportion of doctors highlights not only their ignorance of the value of this simple test in clinical practice but also their indifference to the public health implications of the sputum status of tuberculosis patients.

An important finding of this study is the private medical practitioners' inappropriate practice of combining drugs available for tuberculosis treatment at their will, but without any scientific rationale. This is evident in 113 doctors prescribing 90 different combinations, considering the drugs used and their durations in the intensive and continuation (not shown in Table) phases of the treatment. This showed lack of knowledge and awareness among private medical practitioners, urban as well as rural, about important advances in the treatment of tuberculosis and the absence of communication between those involved in the implementation of disease control activities and the practising private medical practitioners. Most doctors use more drugs than required for treating a case of tuberculosis. Use of a single drug in continuation phase by 9 doctors and non-inclusion of INH in the regimen by 23 doctors could have serious implications with regards to development of secondary drug resistance which is said to be increasing in alarming proportions.

Although non-allopaths do not receive any

formal training in the management of tuberculosis, they routinely treat patients in their clinics. No significant differences were found between allopaths and non-allopaths in their diagnostic or treatment protocols. This clearly points out the need for providing continuous education to all the private doctors.

In the virtual absence of any collaborative effort between private medical practitioners and public health services, several specific areas of mutual co-operation could be identified, like some of those which came up during the group discussion conducted in this study. The important prerequisites before any such venture is undertaken would be a reasonably well functioning programme and rapid retraining of private medical practitioners in tuberculosis.

Some specific steps could range from involving representatives of private medical practitioners in planning tuberculosis control activities to providing them free or subsidized facilities for diagnosis, drug treatment and defaulter tracing in return for assurance of compliance with nationally recommended practices and timely submission of prescribed reports and records. It is reasonable to expect that addressing the needs of private practitioners could give an impetus to private medical practitioners to improve their tuberculosis management practices which in turn could further augment the efforts and the yield of tuberculosis control activities by the public health services.

#### ACKNOWLEDGEMENTS

This study is a result of the ongoing study of "Social and Operational Constraints in Tuberculosis Control in Maharashtra", supported by the International Development Research Centre, Canada. The authors acknowledge the help offered by Ms. Sushma Jhaveri and Dr. Rajam Iyer for helping in analysis.

#### REFERENCES

1. Uplekar, M.W. and Shepard, D.S. : Treatment of tuberculosis by private general practitioners in India, *Tubercle*; 1991, 72, 284.
2. Uplekar, M.W. and S. Rangan : Private doctors and tuberculosis control in India, *Tubercle and Lung Disease*, 1993, 74, 332.

## COMPARTMENTALISATION OF LYMPHOCYTES IN TUBERCULOUS PLEURAL EFFUSION : A PRELIMINARY OBSERVATION

A.G. Ghoshal<sup>1</sup>, K. Mukherjee<sup>2</sup>, A.K. Saha<sup>3</sup> and T.K. Chowdhury<sup>4</sup>

(Original received on 28.2.95; Revised version received on 14.9.95; Accepted on 21.11.95)

*Summary:* The relative distribution of total lymphocytes, T and B lymphocytes, and their subsets in peripheral blood & pleural fluid were measured in 35 patients with moderate to massive tuberculous pleural effusion. Compared to 10 controls, they showed significant peripheral depletion of T lymphocytes. Both deficiencies had high correlation with diminished response to PPD (1TU) in these cases.

### INTRODUCTION

Aberration of immunoregulation has been known to occur in tuberculous pleural effusion<sup>1</sup>. This has been used to explain why tuberculin skin test may be negative in a considerable number of patients with this form of tuberculosis<sup>2,3</sup>. Tuberculin negative tuberculous pleural effusion is a diagnostic dilemma though, reportedly, these patients become tuberculin positive within a few weeks.

In this prospective study, we examined the response to tuberculin test in untreated patients of moderate to massive tuberculous pleural effusion, and whether the distributions of T & B lymphocytes in peripheral blood and pleural fluid in them had any correlation with the tuberculin response. We also examined the helper CD4 and suppressor CD8 subsets among the T lymphocytes and the blast cell proportions, in the different compartments, as a measure of immunogenic activity of lymphocytes. All the parameters were also studied in peripheral blood in a control group.

### MATERIAL & METHODS

Thirty five patients with the clinical and radiological diagnosis of moderate to massive pleural effusion were selected between July, 1993 and March, 1994. All the patients (aged 15 to 40 years - mean age 27 years; 22 males and 13 females) had a complete physical examination and blood, urine and pleural fluid study. The latter included smear test of pleural fluid for AFB and pleural biopsy. In all the cases, tuberculin test was done with 1TU of PPD-RT23. Other tests like sputum smear for AFB and malignant cell were done as found necessary. Empyema and malignancy were excluded by appropriate tests at the time of intake.

Total lymphocyte counts in peripheral blood and pleural fluid were done in all the 35 cases and in peripheral blood of 10 healthy tuberculin positive age-matched volunteers (Controls). Separation and estimation of T and B lymphocytes (nylon wool columns) were done in each. The CD4 and CD8 cells were isolated from the T Cell population with the help of Dynabeads M-450 CD4 and Dynabeads M-450 CD8 respectively. Immunogenic activity of the lymphocytes, indicated by blastogenic transformation was measured by proportion of total lymphoblasts, T-blasts and B-blasts (by inverted phase contrast microscope). Any cell with diameter greater than 7  $\mu$ m was scored as transformed blast cell.

### RESULTS

The relative distribution of lymphocytes and their subsets is shown in Table 1.

1. Reader, Department of Chest Medicine, Calcutta National Medical College, Calcutta

2,3. Reader/Lecturer, Departments of Pathology and Medicine, N.R.S. Medical College, Calcutta

4. Lecturer, Department of Life Sciences, North Bengal University, Siliguri, West Bengal

Correspondence: Dr. A.G. Ghoshal, 40/17, S.C. Chatterji Street, P.O. Belur Math, 711202

**TABLE 1. Relative distribution of lymphocytes and their subsets**

	Controls		Cases			
	Blood		Blood		Pl. Fluid	
	Range	Mean	Range	Mean	Range	Mean
Total Lymphocytes ( $\times 10^9/L$ )	2.6-4.8	3.2 $\pm$ 0.2	1.0-3.03	2.0 $\pm$ 0.3	0.09-5.1	1.3 $\pm$ 0.2
Lymphocytes	62-76%	68% $\pm$ 2	40-70%	51% $\pm$ 3	37-58%	53%
T	14-20%	16% $\pm$ 2	26-60%	39% $\pm$ 5	40-53%	44%
B						
T Lymphocytes						
CD4+	52-61%	55.05% $\pm$ 0.11	35.50-44%	41.25 $\pm$ 0.82	32-48%	41% $\pm$ 0.58%
CD8	14-18%	16.05% $\pm$ 0.147	18.25-21%	18.75 $\pm$ 0.43	16.50-18%	17.75 $\pm$ 0.82
Blast Cells						
T	-	-	23-40%	29%	18-31%	23%
B			13-27%	19%	19-35%	25%

The peripheral total lymphocyte count in the blood of cases ranged from 1.0-3.03  $\times 10^9/L$  with mean value of 2.6  $\pm$  0.3. The pleural fluid lymphocyte count among them was 0.09 to 5.1  $\times 10^9/L$  with mean value of 1.3  $\pm$  0.2. The proportion of T lymphocyte population in the peripheral blood of the patients varied from 40-70% (mean 51  $\pm$  3) and of B lymphocytes 26-60% (mean 39  $\pm$  5) of the total lymphocytes. In pleural fluid, proportion of T lymphocytes was 37-58% (mean 53%) and of B lymphocytes 40-53% (mean 44%) of the total lymphocyte population.

About the subsets, CD4 (helper cell) among the T lymphocytes had a mean proportion of 41.25%  $\pm$  0.82 of the total T lymphocytes in the peripheral blood and 41.00%  $\pm$  0.58 in the pleural fluid. CDS (suppressor cell) subset of T lymphocytes showed a mean proportion of 18.75%  $\pm$  0.43 in the peripheral blood and 17.75%  $\pm$  0.82 in pleural fluid.

Blastogenic transformation was taken as the measurement of activity of the lymphocytes. Blast cell transformation was noted in 29% of the T cells and 19% of the B cells in peripheral blood and 23% T blast with 25% B blast in pleural fluid.

Respective values among tuberculin positive healthy volunteers (controls) showed peripheral total lymphocyte count of 2.6 to 4.8  $\times 10^9/L$  with

a mean value of 3.2  $\pm$  0.2  $\times 10^9/L$  with T lymphocyte proportion of 68%  $\pm$  2 and B lymphocyte at 16%  $\pm$  2 of the total lymphocyte population. Among the subsets of T lymphocytes, CD4 helper cells were 55.05%  $\pm$  0.111 with CDS cells 16.05%  $\pm$  0.147 of the total T lymphocytes in the peripheral blood.

Mantoux test indurations among cases were of size 15 mm (transverse diameter) in only 5 patients,  $\geq$ 10 mm. in 15 patients, 8 mm in 6 patients and no induration in the rest (9 patients).

## DISCUSSION

Delayed hypersensitivity mediated by T lymphocytes sensitized to tuberculo-proteins is believed to be primarily responsible for tuberculous pleural effusion.<sup>4,5</sup> Tuberculin skin test is also a T cell mediated phenomenon: the infiltration of CD4 cells at the site outnumbering CDS cells by about 2:1.<sup>6</sup> Several explanations have been advanced to explain the findings of negative tuberculin skin test in tuberculous pleural effusions. The first hypothesis is that effusion occurs before body has chance to develop tuberculin positivity; secondly, circulating adherent cells suppress the specifically sensitized T lymphocytes in peripheral blood;<sup>7</sup> thirdly, sensitized T lymphocytes are preferentially sequestered into the pleural space thereby causing peripheral depletion.<sup>8</sup>

Our study suggests a **major redistribution**

**occurring in the lymphocyte population in untreated cases of moderate to massive tuberculous pleural effusion. Compared with controls, the cases showed total lymphocytopenia in peripheral blood and also major depletion of the peripheral T lymphocytes. Both changes were statistically significant ( $P < 0.01$ ). The relative proportion of CD4: CDS T cells in the cases was also altered, with increased CDS cells found in peripheral blood, but this did not attain statistical significance. The first two parameters had a direct correlation with the diminished response to PPD test in these cases. Individual data relating to peripheral blood total lymphocytes, T lymphocytes, and tuberculin reactivity in all the patients are given in Appendix. The correlation coefficient in respect of the first two parameters was 0.89, between the second and third 0.88 and between the first and third 0.91. Thus, all three parameters were found to be highly correlated with each other.**

#### REFERENCES

1. Seaton A, Seaton B, Leitch A.G. *in* Crofton and Douglas's Respiratory Diseases. 4th Ed. Oxford University Press, Delhi, 1989; 1089.
2. Murray J.F., and Nadel J.A. Textbook of Respiratory Medicine. 2nd Ed. Saunders, Philadelphia, 1994. II 2172
3. Chopra R.K. Adenosine Deaminase and T Lymphocyte levels in patients with pleural effusion. *Ind. J. Tub.* 1988, 35,22
4. Ellner J.J., Barnes P.P., Wallis R.S, and Modlin R.L. The immunology of Tuberculous Pleurisy. *Semin. Respir. Infec.* 1988, 3, 335
5. Alien J.C., and Apicella M.A., Experimental pleural effusion as a manifestation of delayed hypersensitivity to tuberculin PPD. *J. Imm.* 1968. 101, 481.
6. I. Roitt, J Brostoff, D. Mole *in* Immunology (3rd ed.). Mosby, London 1993.
7. Ellner J.J. Pleural fluid and peripheral blood lymphocyte function in Tuberculosis. *Ann. Int. Med.* 1978; 89, 932
8. Rossi G.A., Balbi B, and Manca F. Tuberculous pleural effusion: Evidence for selective presence of PPD-specific T-lymphocytes at site of inflammation in the early phase of the infection. *Am. Rev. Resp Dis* 1987, 136, 575

*APPENDIX***Peripheral total lymphocytes, T lymphocytes and tuberculin indurations in the 35 patients**

Case No	Peripheral total lymphocyte (X10 <sup>9</sup> /L)	Peripheral T lymphocyte (%)	Size of induration (mm)
1. SP.	2.6	54	12
2. N.S.	2.8	57	13
3. D.P.	3.0	70	15
4. B.N.	3.0	68	15
5. D.T.	1.4	44	8
6. P.M.	1.4	44	8
7. G.M.	1.0	40	Nil
8. P.D.	1.1	41	Nil
9. N.B.	2.5	52	11
10 N.K.	2.6	54	12
11 A.K.	2.6	54	12
12 P.D.	2.4	52	11
13 N.C.	3.0	70	15
14 K.P.	1.6	44	8
15 S.B.	1.4	44	8
16 R.S.	1.1	40	Nil
17 M.G.	2.8	56	12
18 A.S.	2.1	53	11
19 D.S.	2.6	56	12
20 S.B.	1.0	41	Nil
21 G.C.	1.4	44	8
22 A.M.	2.0	53	11
23 A.G.	2.1	66	15
24 C.S.	1.0	42	Nil
25 S.G.	1.0	40	Nil
26 M.D.	3.0	68	15
27 N.C.	2.8	56	13
28 D.P.	1.0	41	Nil
29 S.T.	1.4	43	8
30 A.S.	2.0	54	11
31 A.G.	1.1	41	Nil
32 T.S.	2.8	57	13
33 G.D.	2.6	54	12
34 S.S.	1.0	41	Nil
35 R.S.	2.6	52	11



## PULMONARY ALVEOLAR PROTEINOSIS WITH PULMONARY TUBERCULOSIS

Rekha Chaudhuri<sup>1</sup>, Pralhad Prabhudesai<sup>1</sup>, Pradeep Vaideeswan<sup>1</sup>, and A.A. Mahashur<sup>2</sup>

(Received on 25.2.95, Accepted on 12.4.95)

**Summary:** Pulmonary alveolar proteinosis (PAP) is an uncommon condition. We present a case report of PAP, followed by tuberculosis showing remarkable clinical and radiological improvement with anti-tuberculosis therapy alone.

relatively asymptomatic and sought medical advice as he was refused a visa due to an abnormal chest radiograph.

On examination, there was no tachypnoea, fever, cyanosis or finger clubbing. He had fine inspiratory end crackles, bilaterally, in the interscapular region. Other systems were normal.

### INTRODUCTION

Pulmonary alveolar proteinosis was first described by Rosen and colleagues in 1958<sup>1</sup>. This is a pathological diagnosis based on filling of pulmonary alveoli by periodic acid-Schiff (PAS) stain positive proteinaceous material rich in lipids.

Males preponderate (4:1) in PAP and the patients usually present in the fourth to sixth decades of life<sup>2</sup>. Although most cases have no well-defined causative agent, more than 50% are exposed to dusts and chemicals<sup>3</sup>, including silica, fibre glass and aluminium dusts. Fungal and Nocardia infections are also common in patients of PAP but mycobacterial infections are only rarely reported. Such infections may occur prior to PAP, acting as a stimulus for the type II pneumocytes<sup>4</sup> or may occur as a complication of macrophage dysfunction.

### CASE REPORT

A 26 year old male, non-smoker, exposed to dust, as a civil engineer, at construction sites, presented at our chest clinic in April 1992 with a six month history of grade I dyspnoea on exertion and an occasional dry cough. He was

Routine haematological and biochemical investigations were normal. The chest radiograph revealed bilateral acinar opacities in the mid and lower zones, with no other abnormality (Fig. 1). The initial pulmonary function tests were normal, with a DLCO (Diffusion Lung Capacity for Carbon-monoxide) of 22.63 (80% of the predicted value). His basal ABG (Arterial Blood Gases Analysis) showed a PO<sub>2</sub> of 90 mm Hg, which dropped to 81 mm after 10 minutes of exercise (80 watts) and rose to 633 mm Hg with 100% oxygen, suggestive of a diffusion defect. LDH (Lactic De-hydrogenase), was 7 IU. RA, LE and serum calcium were normal. Sputum for acid fast bacilli and the Mantoux test (8x8 mm) were negative. His CT scan showed bilateral, patchy, soft tissue shadows, with no cavitation, calcification or lymphadenopathy (Fig.2).

A broncho-alveolar lavage was performed which contained 9.75 x 10<sup>6</sup> cells. The cells/cmm were 177, with plenty of eosinophilic proteinaceous material, suggestive of PAP.

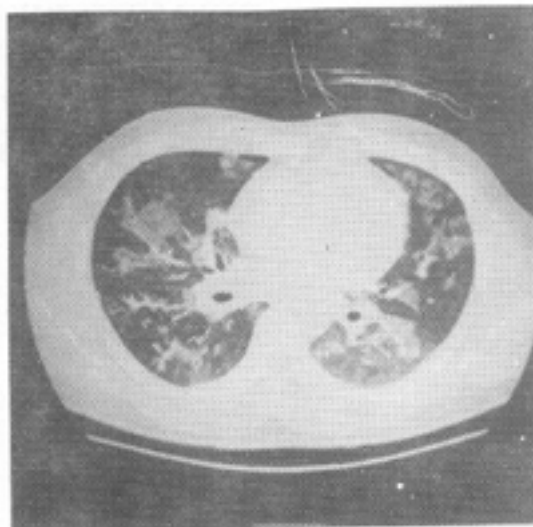
In June 1992, an open lung biopsy was done which showed large areas of alveoli filled with a granular pink material with occasional small clefts. The alveoli at the periphery showed

<sup>1</sup> Lecturer <sup>2</sup> Professor and Head  
Departments of Chest Medicine, Pathology and Environmental Pollution Research Centre,  
King Edward VII Memorial Hospital, Bombay

Correspondence: Dr. Rekha Chaudhuri, C/o Dr. A.A. Mahashur, Dept. of Chest Medicine and Environmental Pollution Research Centre, K.E.M. Hospital, Parel, Bombay 400012



*Fig 1. Chest radiograph suggestive of PAP*



*Fig 2. Thorax CT Scan suggestive of PAP*

macrophages and lymphocytes. Special stain studies confirmed that the proteinaceous material was PAS stain positive and diastase resistant. There were no tubercles or acid-fast bacilli in tissue sections, and cultures of the lung tissue were sterile. Thus, the diagnosis of pulmonary alveolar proteinosis was confirmed.

As the patient was relatively asymptomatic, a therapeutic lavage was not performed.

In July 1992, he developed cough with mucoid expectoration and his dyspnoea increased to grade II. The chest radiograph showed an increase in the opacities and the  $PO_2$  was reduced from 90 to 60 mm Hg. The pulmonary function tests showed a mild restriction.

Sputum was sent for examination and it was found to be positive for acid-fast bacilli. Nocardiosis was ruled out by culture and serology. He was, therefore, diagnosed as pulmonary tuberculosis and given anti-tuberculosis therapy with Isoniazid, Rifampicin and Pyrazinamide. He improved symptomatically and repeat chest radiograph after 2 months of treatment showed a dramatic clearing of the shadows,

Chemotherapy was continued for 6 months (2HRZ/4HR). At the end of one year, the patient was asymptomatic and the X-ray chest was clear.

## DISCUSSION

PAP is associated with pulmonary infections but accompanying tuberculosis is rare<sup>2</sup>. There have been eight such cases described in the literature, four of whom were discovered at autopsy<sup>4</sup>. Tuberculosis was the primary diagnosis followed by that of PAP in seven of the eight cases. In the solitary case where PAP was discovered first<sup>5</sup>, the anti-tuberculosis therapy was ineffective, till a lavage was performed for PAP. In this respect, our patient is unusual as he had a definitive diagnosis of PAP, following which he developed tuberculosis, and on chemotherapy his symptoms and chest radiograph resolved completely. The PAP may have shown a spontaneous resolution, while tuberculosis was being treated effectively.

There is a definite relationship between PAP and tuberculosis<sup>4</sup>: This could be the defective alveolar macrophages, found in PAP, getting choked with lipid and PAS positive material. On electron microscopy, the macrophages show vacuolations and giant secondary lysosomes containing inclusion bodies. These cells show decreased chemotactic ability and impaired fungicidal capacity<sup>6</sup>.

*In vitro* studies have shown that pulmonary

washings from a PAP patient could support the growth of mycobacteria in the absence of other nutrients<sup>7</sup>.

This combination of a rich culture medium with superimposed macrophage deficiency becomes a perfect setting for tubercle bacillus to grow<sup>4</sup>.

Thus, in a patient of pulmonary alveolar proteinosis the possibility of complicating tuberculous disease should be considered, especially in countries with high prevalence of tuberculosis.

#### ACKNOWLEDGEMENT

We thank Dr. P.M. Pai, the Dean, for permission to publish this case report.

#### REFERENCES

1. Rosen S.H., Castleman B., and Liebow A.A.. Pulmonary alveolar proteinosis. *N.Engl. J. Med.* 1958; 258: 1123
  2. Prakash U.B.S., Barham S.S., Carpenter H.A., Dines D.E., Marsh H.M.. Pulmonary alveolar phospholipoproteinosis. Experience with 34 cases and a review. *Mayo Clin Proc* 1987; 62: 499.
  3. Davidson J.M. and Macleod W.M.: Pulmonary alveolar proteinosis. *Br J Dis Chest* 1969; 63: 13.
  4. Reyes J.M. and Putong P.B. Association of pulmonary alveolar lipoproteinosis with mycobacterial infection. *Am Clin Pathol* 1980; 74: 478.
  5. Lathan S.R., Williams J.D., McLean R.C. et al. Pulmonary alveolar proteinosis. *Chest* 1971; 59: 452.
  6. Golde D.W., Territo M., Finley T.N. et al. Defective lung macrophages in pulmonary alveolar proteinosis. *Ann Intern Med* 1976; 85: 304.
  7. Ramirez R.J. Pulmonary alveolar proteinosis: Treatment in a case complicated by Tuberculosis. *Am Rev Resp Dis* 1967; 95: 491.
-



## CHRONIC MASSIVE RECURRENT PLEURAL EFFUSION ASSOCIATED WITH ASYMPTOMATIC PANCREATIC DISEASE

Lalita Fernandes<sup>1</sup> and Anthony Mesquita<sup>2</sup>

(Received on 4.5.95; Accepted on 17.9.95)

**Summary:** Three patients are described in whom massive recurrent pleural effusion developed secondary to asymptomatic pancreatic disease.

On examination, there was mild pallor, no lymphadenopathy, signs of massive pleural effusion on left side, no hepatosplenomegaly, mass or ascitis.

### INTRODUCTION

Massive pancreatic pleural effusions are uncommon and often go unrecognised. They result from internal pancreatic fistula and usually present as an exudative effusion of unknown cause. The effusion frequently occurs without clinical evidence of pancreatitis but may be associated with a pseudocyst of pancreas

Chronic massive pancreatic pleural effusion is usually recurrent and is characterised by high level of amylase in the pleural fluid. Two of the three patients presented had pseudocysts while one had acute haemorrhagic pancreatitis. Mortality and morbidity can be greatly reduced when a definite and early diagnosis is established and appropriate therapy is rendered.

### Case Reports

*Case 1:* A 30 year old male was referred to our hospital with history of recurring left haemorrhagic pleural effusion. He was treated with anti-TB therapy for 2 months without clinical response. His chief complaints on admission were: left sided dull chest pain radiating to the back, cough with minimal mucoid expectoration, loss of weight by 10 kg, over last 3 months. There was no history of injury and no abdominal discomfort or pain. He was a non-smoker but consumed 1 bottle of beer/day for the preceding eight years.

X-ray chest revealed massive pleural effusion (L) with mediastinal shift to the opposite side (Fig. 1). Serum and pleural fluid amylase levels were raised (Table). An ultrasound of abdomen revealed a hypoechoic mass in the tail of pancreas measuring 5 x 8 cms (Fig. 2) and extending upto splenic hilum. Liver, spleen, gall bladder and kidneys were normal. There was no ascitis nor pericardial effusion. Pleural biopsy was normal. Mantoux test to 1 TU was 6mms. Liver function tests were normal. Hb-9.5 g%, TC-6,000/cmm, N-54%, L-30%, E-14%, ESR- 46mm/1st hr, Fasting blood sugar level 82 mg%. Bronchoscopy was normal and washings were negative for malignant cells and AFB. Patient was treated conservatively. Repeated thoracentesis was done, draining 8 litres of serosanguinous fluid. He was subsequently referred to the surgical unit for the management of the pseudocyst.

*Case 2:* A 32 year old male patient, heavy alcoholic and a known case of pancreatitis with a pseudocyst was hospitalised for progressive breathlessness. On admission, his respiratory rate was 30/min, pulse 90/min and B.P. 120/80 mmHg. There were signs of massive left-sided pleural effusion. The patient, however, did not have any abdominal complaints. His Hb was 8 g% and rest of haematocrit picture was within normal limits. Fasting blood sugar level was 84 mg% and LFT's within normal limits. His abdominal ultrasound showed a cyst in the

1. Senior Resident, 2. Prof, and Head, Dept. of TB and Respiratory Diseases, Goa Medical College, Goa.

Correspondence: Dr. Lalita Fernandes, Sodiem Maina, Siolim, Bardez, Goa 403 517

**Table: Results of laboratory investigations**

Case No.	Sr. amylase	PL amylase	PL protein	Sr. protein	Ultrasound
1 140		300	3.5	6.8	Pseudocyst
2 576		480	3.3	6.5	Pseudocyst
3 528		600	3.4	6.2	Not done

#### Amylase levels - Somogyi Units

pancreas measuring 2.4 x 5 cms while liver, gall bladder, spleen and kidneys were normal. He was treated conservatively and discharged. Three months later, he was readmitted with massive left sided effusion. Abdominal ultrasound showed a slight increase in the pseudocyst. Even at this stage, he did not have any abdominal complaints. He was again treated conservatively and after 4 months of follow up showed complete resolution of both effusion and the pseudocyst.

*Case 3:* A 40 year old male alcoholic presented with history of low grade fever for 3 months. He had cough with minimal mucoid expectoration, progressive breathlessness and left sided chest pain for 8 days and dull aching pain in the upper abdomen since then. There was no history of diarrhoea, vomiting or suggestive symptoms of peptic ulcer. There was no other relevant history. He had been smoking 40 bidis/day for the last 20 years.

On examination, the patient had average build, pulse 100/min, B.P. 100/70 and respiratory rate 30/min. There was marked pallor, no lymphadenopathy, and signs of right sided massive pleural effusion. There was epigastric tenderness, but no evidence of a mass or fluid in the abdomen. Liver and spleen were not palpable. Hb was 8g%, counts were normal, LFT's within normal limits. Other systems were normal.

X-ray chest showed right-sided massive pleural effusion. Hence, thoracocentesis was done and 3.5 l of straw coloured fluid was aspirated in three sittings. Pleural fluid was exudative. Pleural fluid aspirate as well as pleural biopsy were negative, both for malignancy as well as tuberculosis. Mantoux test was negative to 1 TU. Pleural fluid amylase was 600 S. units and serum amylase was 528 S units. On the 3rd day, repeat

chest X-ray showed refilling of fluid and patient developed signs of shock. Physical examination revealed tenderness over the epigastrium and ascites. The ascitic tap revealed haemorrhagic fluid, and a simultaneous pleural tap was also haemorrhagic. Both aspirates were exudates. The patient expired the same evening in spite of all measures taken.

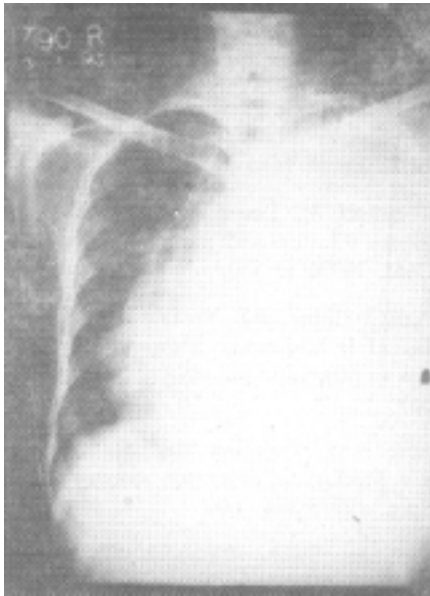
A clinical diagnosis of acute haemorrhagic pancreatitis with shock was made. An ultrasound could not be carried out as the machine was out of order. A post mortem liver biopsy was normal.

#### DISCUSSION

Pleuro-pulmonary complications secondary to pancreatitis are well known but rare. The frequency of pleural effusion in patients with acute pancreatitis varies between 3 to 17% but they are often small and transient.<sup>2</sup> The effusions are usually left sided, asymptomatic and resolve with control of pancreatitis. No specific treatment is required. Typically, they are non-haemorrhagic and show elevated amylase level.

In contrast, large blood stained exudative pleural effusions may develop upto several years after an episode of acute pancreatitis.<sup>3,4</sup> They are left sided but may be right sided or even bilateral. They tend to recur after thoracocentesis, as was seen in all the three cases. Most patients do not have abdominal symptoms but some may have tenderness or a swelling. None of our patients had history of flatulence, dyspepsia or loose motions. Only one patient had dull aching epigastric pain and tenderness.

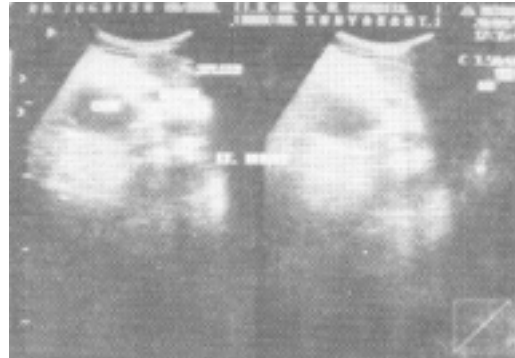
The serum amylase levels are usually normal or mildly elevated. When serum amylase is increased, it is thought to be due to back diffusion



**Fig. 1. X-ray chest (PA view) showing massive left sided pleural effusion and mediastinal shift**

from pleural space rather than acute pancreatitis.<sup>5</sup> The pleural fluid amylase levels are usually more than 1000 IU/l with reported values as high as 475,000 Somogyi units/dl<sup>6</sup>.

The exudative amylase rich pleural fluid may occur due to transfer of pancreatic secretions through transdiaphragmatic lymphatics.<sup>6</sup> Alternatively, direct contact of pancreatic enzymes with the diaphragm may lead to rupture or perforation. In some cases, a pancreatico-pleural fistula can be demonstrated either by endoscopic retrograde Cholangiopancreatography or by computed tomography<sup>7</sup> or by the injection of contrast medium in the pleural cavity.<sup>8</sup> Fistula is commonly associated with pancreatic pseudocyst and obstruction of the main pancreatic duct. The pseudocyst can also rupture into the pleural cavity and, on ultrasonography, no pseudocyst may be seen in the pancreas<sup>9</sup>. Pancreatic secretions probably leak into the retroperitoneal space and track upwards beside the aorta and oesophagus through the diaphragmatic hiatus into the mediastinum<sup>5</sup>. Occasionally, secretions are contained within the mediastinum presenting as a mediastinal pseudocyst<sup>1,10</sup> or it may rupture into the pericardium<sup>9</sup>, but usually there is penetration into the pleural cavity.



**Fig. 2. Ultrasonography showing hypoechoic area in pancreatic tail extending to splenic hilum**

A major pitfall in the diagnosis and management of chronic massive effusion is failure to recognise that intra-abdominal disease is responsible for the pleural effusion.<sup>11</sup>

Patients are often diagnosed to be suffering from tuberculous effusion and are put on prolonged chemotherapy with no avail.

Therefore, the clue to the diagnosis — the high level of amylase in pleural fluid — along with elevated serum amylase level must be looked for. Some degree of elevation of amylase level can also occur in thoracic neoplasms, both primary and secondary. Also, very high levels are seen in oesophageal rupture but the clinical presentation and natural history make it easy to differentiate these conditions<sup>1</sup>.

Therapy is somewhat controversial because of 48% effectiveness, and conservative management should be attempted initially<sup>4</sup>. Reduction of pancreatic secretions by naso-gastric suction and starting parenteral nutrition may allow some fistulae to close spontaneously. Surgery is often recommended after a fistula has been demonstrated, and should certainly be undertaken if effusion recurs after 2 weeks of conservative management.

Resolution of recurrent pleural effusion by drainage of a pancreatic pseudocyst through a percutaneous catheter is also described<sup>12</sup>. Complete drainage by introduction of intercostal tube is necessary in order to avoid complications like pneumothorax, empyema and broncho-pleural fistula.

#### REFERENCES

1. Naresh A. Dewan, Wesley W Kinney, Walter J O'Donoheu. Chronic massive pancreatic pleural effusion. *Chest* 1984; 85(4): 497.
2. Mckenna J, Chandrashekar A, Skorton D, Craig R M, Caugell D.W. The pleuropulmonary complications of pancreatitis, *Chest* 1977; 71: 197.
3. C.B. Cooper, P.A. Bardsley, S.S. Rao and M.C. Collins. Pleural effusions and pancreatico-pleural fistulae associated with asymptomatic pancreatic disease. *Br. J. Dis Chest* 1988; 82: 315.
4. Cameron J.L., Kelffer R.S., Anderson W.J., Zuidema G. Internal pancreatic fistulae: Pancreatic ascitis and pancreatic pleural effusion. *Ann. Surg.* 1976; 184: 587.
5. Cameron J.L. Chronic pancreatic ascitis and pancreatic pleural effusion. *Gastroenterol.* 1978; 74: 134.
6. Kaye M.D. Pleuropulmonary complications of pancreatitis. *Thorax*, 1968; 23: 297.
7. Louie S, McGahan J.P., Frey C, Cross C.E.. Pancreatic pleuropericardial effusions: Fistulous tracts demonstrated by computed tomography. *Arch. Intern Med* 1985; 145: 1231.
8. Tombroff M, Loicq A, Dekoster J.P. et al. Pleural effusions with pancreatico-pleural fistula, *BMJ*, 1973; 1: 330.
9. A R J Girbes, P.E. Postmus, W. Jansen, E.J. Jagt, J H Kleibeuker. Massive pleural effusion due to pancreatic pseudocyst. *Thorax* 1990; 45: 563.
10. Jaffe B.M., Ferguson T.B., Holtz S., Sheilds J.B. Mediastinal pancreatic pseudocysts. *Am. J. Surg.* 1972; 124: 600.
11. Willliama S.C.J., Bhupalan A, Zureikat N, Jhuluvath PJ, Santis G, Theodorou N, Westaby D. Pleural effusions associated with pancreatico-pleural fistula. *Thorax*, 1993;48(8) 867.
12. Faling L.J., Cerzof S.G., Daly B.P.T., Pugatch R.D., Snider GL. Treatment of chronic pancreatic pleural effusion by percutaneous catheter drainage of abdominal pseudocysts. *Am J Med.* 1984; 76: 329



## DIAGNOSIS OF INTRACRANIAL TUBERCULOMA

Ravindra Kumar Garg\*

### INTRODUCTION

Tuberculomas of the brain account for 20 to 30 per cent of intracranial tumors in India<sup>1</sup>. In pediatric age group, upto 41% of intracranial space occupying lesions (ICSOLs) have been found to be tuberculous in nature<sup>2,3</sup>. Tuberculomas develop in the brain when the initial "Rich focus" does not rupture into the meninges but expands locally within the brain parenchyma. The tuberculoma may also originate in the meninges, and may be found in the superficial cortex. The meningeal form may resemble a meningioma.

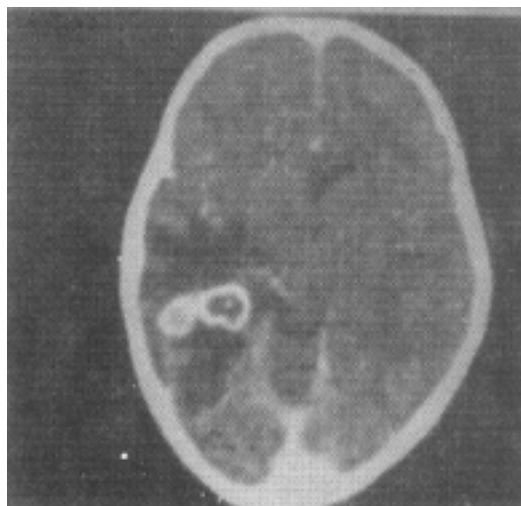
Patients with intracranial tuberculoma most often present with seizures (60 to 100%), symptoms and signs of raised intracranial pressure (56-93%), and focal neurological deficits (33-68%). In a magnetic resonance imaging (MRI) based study, Gulati et al<sup>4</sup> found tuberculoma as the commonest cause of chronic seizures, in 64 out of 158 patients. In the brain, there may be multiple caseating granulomas, although most of the patients (66-73%) have single or confluent large granulomas with necrotic centre. Tuberculomas may also be multiple or miliary<sup>5</sup> (Figs. 1, 2).

Although tuberculoma appears avascular when studied angiographically, its appearance on computerised tomographic (CT) scan and MRI varies. It is consistent with the evolving granulomatous nature of the disease. During the initial phase of the disease, oedema and necrosis may appear as a low attenuating area on CT scan. Once the granuloma has begun to organize, there may be high attenuation, contrast enhancement and calcification, as well as ring enhancement and a variable degree of surrounding oedema. The enhancement may be homogenous or there may be a central radiolucent area corresponding to the central zone of necrosis<sup>6,7</sup>.

MRI is considered to be more sensitive than CT in detecting tuberculomas of the cerebral parenchyma. Tuberculomas are isointense with grey matter on T1-weighted MR images. On T2-weighted images, lesions show central hyperintensity. In some cases, a hypointense ring is present within the wall of the tuberculoma on T2 weighted images. Most tuberculomas are further outlined by a collar of high signal, resulting from oedema, on T2-weighted images. Tuberculomas, typically, "enhance" after the intravenous administration of gadopentetate dimeglumine in a solid or ring pattern<sup>8-11</sup>.

### DIFFERENTIAL DIAGNOSIS

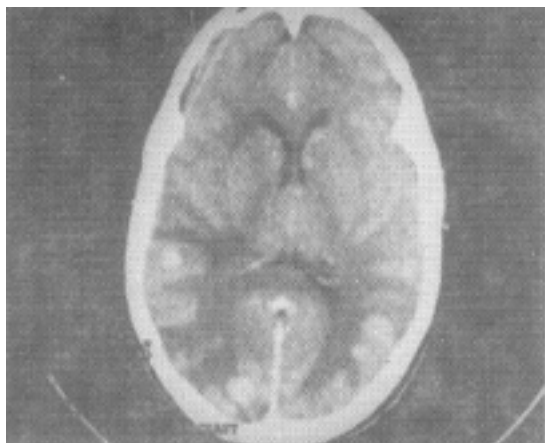
The CT/MRI diagnosis of tuberculoma is largely presumptive in view of its nonspecific appearance. Cysticercus granuloma, pyogenic abscess, metastases, fungal granuloma, and at times, glioma may be indistinguishable from tuberculoma.



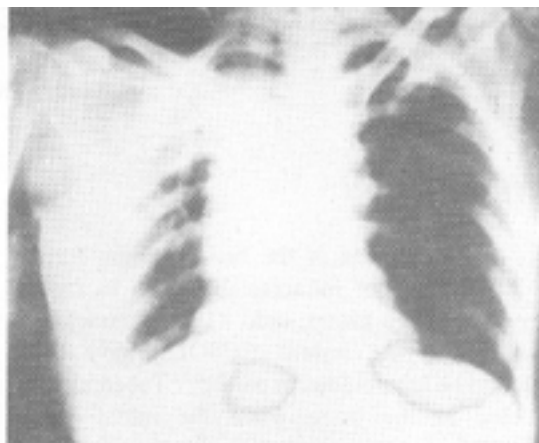
**Fig. 1.** Contrast enhanced computerised tomographic scan showing intracranial tuberculoma

\* Department of Neurology, King George's Medical College, Lucknow

Correspondence: Dr. R.K. Garg, Department of Neurology, King George's Medical College, Lucknow - 226003.



**Fig. 2. (A) A contrast enhanced tomographic scan showing multiple intracranial tuberculomas**



**(B) X-ray chest of same patient showing evidence of pulmonary tuberculosis (sputum was positive for AFB)**

#### **Differentiation from neurocysticercosis**

Clinical picture and CT scan in both the diseases are very similar. Several diagnostic points have been suggested from time to time but could not prove useful. In tuberculoma, a central speck of calcification, 'target sign', had been considered pathognomonic<sup>12</sup>. Similar punctate calcification may also be seen in cysticercus granuloma: McCormick et al<sup>13</sup> noted it in 55% of their patients.

In patients with partial seizures the cranial CT scans usually show small ring enhancing lesions. Initially, these lesions were considered tuberculomas and were prescribed antituberculosis treatment<sup>14</sup>. Later, Rajshekhar et al<sup>15</sup> from Vellore unequivocally demonstrated that majority of these lesions were cysticercus in nature; only a few were tuberculomas. In their study, presence of signs of raised intracranial pressure, focal neurological deficits, along with certain CT features of the lesion (>20 mm size, irregular margin, and midline shift) were suggestive of intracranial parenchymatous tuberculoma. However, none of these features are specific enough to start the antituberculosis therapy.

#### **Differentiation from other localised brain lesions**

Brain abscesses are usually characterised on CT scan by a central cystic lesion contained

within a well defined enhancing ring lesion with a substantial amount of surrounding oedema. A tuberculosis abscess may also be clinically and radiologically indistinguishable from pyogenic abscess. The protracted course and presence of calcium inside the intracranial lesion make the diagnosis of tuberculoma likely. A syphilitic gumma may be a solitary circumscribed lesion in the brain, but this lesion would be unusual without evidence of syphilis elsewhere<sup>16</sup>. *Nocardia*, an aerobic 'Gram positive' bacillus that behaves more like a fungus than bacterium, occurs mostly in immunocompromised persons, and produces poorly capsulated, frequently multiloculated, liquefied abscesses in the brain. There is evidence of pulmonary disease in 60 per cent of cases<sup>17</sup>. Actinomycosis, which invades the nervous system in 1 to 3 per cent of patients with systemic infection, produces a well encapsulated pus filled cavity containing characteristic sulphur granules. Evidence of cervicofacial, thoracic or abdominal disease is invariably present<sup>16,18</sup>.

Protozoal disease may produce focal brain lesions, especially those due to amoebiasis and toxoplasmosis. Acquired toxoplasmosis is predominantly a disease of immunocompromised persons, and usually causes encephalitis, circumscribed microglial nodules, or haemorrhagic and necrotic lesions in brain parenchyma<sup>19</sup>. Certain fungal diseases that may produce intracranial granulomas need to be considered in differential

diagnosis. *Cryptococcus neoformans*, which usually causes a chronic meningitis, may result in solitary granuloma. *Candida albicans* may produce multiple parenchymal brain abscesses or granulomas in an immunocompromised host. It closely resembles tuberculoma, although the *Candida* granuloma tends to be located predominantly in white matter rather than in the cortex and is usually associated with spinal fluid pleocytosis and poor prognosis. Evidence of candidiasis elsewhere in body should be present. Aspergillosis, which causes bronchopulmonary infection in immunocompromised patients can also result in solitary or multiple brain abscesses which progress to form granuloma that may calcify. Another fungal disease which produces intracerebral granuloma is mucormycosis especially in those with uncontrolled diabetes. Hydatid cysts of brain appear lucent on radiographic studies and may transform into a gelatinous mass very rarely<sup>18,20</sup>.

Primary brain tumours or the more common localized intracranial lesions are likely to be mistaken for tuberculoma, especially oligodendrogliomas which are more likely to calcify and produce a hyperdense lesion demonstrable on plain CT scan. Tumors metastatic to nervous system are often multiple, and a few appear hyperdense on CT scan like secondaries from lung cancer, melanoma, choriocarcinoma and renal cell carcinoma. Absence of substantial oedema and mass effect on CT scan, the presence of calcification in the lesion and the slow evolution of the lesion exclude this possibility. Primary central nervous system lymphoma is an uncommon lesion of brain. It has a rapid course otherwise indistinguishable, on clinical and radiological grounds, from tuberculoma<sup>18</sup>.

### BIOPSY

Accurate diagnosis of tuberculoma is not possible till brain lesion in question is subjected to histopathological examination. The small single enhancing lesions (in patients of epilepsy), which were earlier considered as tuberculoma, were found to be cysticercus granuloma in the majority, on biopsy<sup>15</sup>. In a study by Jaya Kumar et al (1993)<sup>21</sup> correct preoperative diagnosis of tuberculoma had been made in only 39 of the 52 (75%) patients. It was mistaken for glioma

in 7 and medulloblastoma in two. In a study by Traub et al<sup>22</sup> only in 3 patients out of 11 who presented with mass lesion tuberculoma could be confirmed after brain biopsy. They also reported that brain biopsy was a risky procedure, and might even lead to death.

### SEROLOGICAL EVIDENCE OF TUBERCULOSIS

Serological tests for the diagnosis of tuberculosis, based on the recognition of serum IgG antibodies of selected mycobacterial antigens and the use of enzyme-linked immunosorbent (ELISA) technique have been developed. When diagnosis is in doubt, serological evidence of tuberculosis may prove useful in the absence of histopathological confirmation. Antimicrobial antibodies are absent in healthy individuals. A positive test by ELISA technique can be used as supportive evidence in the diagnosis of intracranial tuberculoma<sup>23</sup>. For example, in a patient having multiple nodular enhancing lesions of brain along with subcutaneous nodules, ELISA was negative for neurocysticercosis while it was positive for tuberculosis. Biopsy of subcutaneous nodule also showed tuberculous granuloma<sup>24</sup>. However, a major limiting factor with serological tests remains the high cost.

### TUBERCULOSIS ELSEWHERE

If facilities for serological studies are not available, a reliable diagnosis can be made if there is evidence of tuberculosis elsewhere<sup>25</sup>. A chest X-ray should be done in every patient. In the study by Jaya Kumar et al<sup>21</sup>, pulmonary tuberculosis was evident in 14 out of 52 patients and seven others had history of close contact with other tuberculous patients in the family. In all these cases brain biopsy confirmed tuberculous nature of intracranial mass lesions. One of our patients had multiple small nodular lesions scattered throughout the brain. X-ray chest showed unequivocal evidence of pulmonary tuberculosis. Patient responded well to antituberculosis therapy (Fig. 2).

### ASSOCIATION WITH TUBERCULOUS MENINGITIS

It is not uncommon to find co-existing

tuberculomas in the presence of tuberculous meningitis. These lesions appear as discrete nodules or grape like clusters of ring enhancing lesions adjacent to the basal cisterns. In the presence of clinical, cerebrospinal fluid and CT criteria diagnostic of tuberculous meningitis, diagnosis of tuberculomas can be made with confidence. Demonstration of tubercle bacilli on culture or guinea pig inoculation is positive only in small proportion of patients and so it can not be relied upon<sup>23,26,27</sup>.

#### NEWER METHODS OF RAPID DIAGNOSIS

Gene amplification by the polymerase chain reaction (PCR) to identify mycobacterial DNA has been used with great sensitivity and specificity. If this technique is available, it offers great promise for rapid diagnosis<sup>27</sup>. In reference laboratories with sufficient instrumentation, high performance chromatographic techniques are capable of rapidly identifying mycobacteria in caseous material, by the presence of characteristic mycobacterial lipids<sup>23</sup>.

#### RESPONSE TO ANTITUBERCULOSIS TREATMENT

When the diagnosis of tuberculoma is considered probable, a trial of antituberculosis therapy may be instituted even without histopathological confirmation<sup>25</sup>. Improvement in clinical and radiological features may provide valuable evidence for the diagnosis of these lesions. However, the response to antituberculosis treatment may not be rewarding every time as these lesions are known to increase in size on treatment adding to the problem of diagnosis and management<sup>28,30</sup>.

#### CONCLUSION

Despite recent advances in imaging techniques, the diagnosis of intracranial tuberculoma remains a challenge. However, a diligent search for certain indicators of tuberculous nature of intracranial lesion should be made. Presence of these markers helps in making fairly accurate diagnosis of intracranial tuberculomas and antituberculosis treatment may be started with confidence.

#### REFERENCES

1. Ramamurthi, B. and Varadarajan, M.G. Diagnosis of tuberculomas of the brain: Clinical and radiological correlation. *J. Neurosurg.* 1961, 18, 1.
2. Dastur, A.M. and Desai, A.D. A comparative study of brain tuberculoma and glioma based upon 107 case records each. *Brain* 1965, 88, 375.
3. Dastur, O.K. and Dave, U.P. Ultrastructural basis of vasculopathy in and around brain tuberculomas possible significance of altered basement membrane. *Am. J. Pathol.* 1977, 89, 35.
4. Gulati, P., Jena, A., Tripathi, R.P. and Gupta, A.K. Magnetic resonance imaging in childhood epilepsy. *Indian Pediatr.* 1991, 28, 761.
5. Gree, G.T., Bazan III, C. and Jinkings, J.R. Miliary tuberculosis involving the brain. MR findings. *AJR* 1992, 159, 1075.
6. Jinkins, J.R. Computed tomography of intracranial tuberculosis. *Neuroradiology* 1991, 33, 126.
7. Draout, S., Abdenabi, B., Ghanem, M. and Bourjat, P. Computed tomography of cerebral tuberculoma. *J. Comput. Assist. Tomogr.* 1987, 11, 594.
8. Gupta, R.K., Jena, A., Sharma, A. et al. MR imaging of intracranial tuberculomas. *J. Comput. Assist. Tomogr.* 1988, 12, 280.
9. Gupta, R.K., Jena, A., Singh, A.K. et al. Role of Magnetic Resonance (MR) in the diagnosis and management of intracranial tuberculomas. *Clin. Radiol.* 1990, 41, 120.
10. Desai, B.B., Shah, V.C., Tavri, O.J. and Rao, P. MRI: More specific than CT in cranial tuberculomas. *Neuroradiology* 1991, 33 (suppl) 216.
11. Gupta, R.K. Pandey, R., Khan.E.M., Mittal, P., Gujral, R.B., Chhabra, O.K. MRI Signal intensity correlation with histopathology and localized proton spectroscopy. *Magn. Reson. Imaging.* 1993, 11, 443.
12. Van Dyke, A. CT of intracranial tuberculomas with specific reference to the 'target sign'. *Neuroradiology* 1988, 30, 329.
13. McCormick, G.F., Zee, C.S. and Heiden, J.

- Cysticercosis Cerebri. Review of 127 cases. Arch. Neurol. 1982, 39, 534.
14. Bhargava, S. and Tandon, P.N. Intracranial tuberculomas: A CT study. Br. J. Radiol. 1980, 53, 935.
  15. Rajshekhar, V., Haran, R.P., Prakash, S. and Chandy, M. J. Differentiating solitary small cysticercous granulomas and tuberculomas in patients with epilepsy. J. Neurosurg. 1993, 78, 402.
  16. Sze, G. and Zimmerman, R.D. The magnetic resonance imaging infections and inflammatory diseases. Radiol. Clin. North. Am. 1988, 26, 839.
  17. Adair, J.C., Beck, A.C., Apfelbaum, R.I. and Baringer, R. Nocardial cerebral abscess in acquired immunodeficiency syndrome. Arch. Neurol. 1987, 44, 540.
  18. Ramsey, R.G. Neuroradiology, 3rd Ed., W.B. Saunders Company, Philadelphia, 1994.
  19. Navia, B.A, Petito, C.K, Gold, J.W., et al. Cerebral toxoplasmosis complicating the acquired immunodeficiency syndrome: clinical and neuropathological findings in 27 patients. Ann. Neurol. 1986, 19, 224.
  20. Lyon, R.W. and Andriole, V.T. Fungal infections of CNS. Neurol. Clin. 1986, 4, 159.
  21. Jaya Kumar, P.N., Kolluri, P.V., Iyer, V. et al. Intracranial tuberculoma. A CT study of 52 histologically verified cases. Indian. J. Radiol. Imag. 1993, 3, 193.
  22. Traub, M., Colchester, A.C.F., Kingsley, D.P.E. and Swash, M. Tuberculosis of central nervous system. Q. J. Med. 1984, 53, 81.
  23. Daniel, T.M. New approaches to the radial diagnosis of tuberculous meningitis. J. Infect., Dis. 1987, 155, 599.
  24. Puri, V., Gupta, R.K. and Malhotra, V. Intracranial tuberculoma with cutaneous miliary tuberculosis. Indian pediatr. 1991, 28, 1197.
  25. Vengsarkar, U.S., Pisipaty, R.P., Parekh, B., Panchal, V.G. and Shetty, M.N. Intracranial tuberculoma and the CT Scan. J. Neurosurg. 1986. 64, 568.
  26. Dadachanji, M. Tuberculous Meningitis, Indian J. Radiol. Imag. 1993, 3, 199.
  27. Ahuja, G.K., Mohan, K.K., Prasad, K. and Behari, M. Diagnostic criteria for tuberculous meningitis and their validation. Tubercle & Lung Dis. 1994, 75, 149.
  28. Tandon, P.N. and Bhargava, S. Effect of medical treatment on intracranial tuberculoma - A CT study. Tubercle 1985, 66, 85.
  29. Chambers, S.T., Hendrickse, W.A., Record, C. and Rudge, P. Paradoxical expansion of intracranial tuberculomas during chemotherapy. Lancet 1984, 2, 181.
  30. Teoh, R., Hymphries, M.J., O'Mahony, G. Symptomatic intracranial tuberculoma developing during treatment of tuberculosis: A report of 10 patients and review of literature. Q.J. Med. 1987. 63, 449.
-



## **FUNCTIONS & RESPONSIBILITIES OF STATE TUBERCULOSIS CENTRES\* (Tuberculosis Demonstration & Training Centres)**

### **INTRODUCTION**

The purpose of this document is to recommend the organisational pattern and outline the main functions and responsibilities, of the State Tuberculosis Centres (STC), which are required to achieve the objectives of National Tuberculosis Programme (NTP).

The central and state governments have already accepted the concept and the various provisions underlining the NTP. Accordingly, the establishment of District Tuberculosis programme (DTP), the basic functional unit under NTP, has been going on in all states. It was envisaged that by the end of fourth plan all the districts would have been covered under DTP. As of today, only 80% of the districts have been implemented. It was also one of the objectives to fill up the existing gaps in the organisational "supra-structure" of the programme, in order to achieve a full fledged NTP, by the end of 1971. Establishment of a fully functioning State Tuberculosis Centre was a prerequisite in that direction. A large number of personnel had to be trained to run a public health programme of such magnitude and the states themselves should have taken timely and adequate steps to cope of with that requirement. So far, this responsibility had been shouldered solely by National Tuberculosis Institute (NTI), Bangalore. In the 70 training courses conducted so far, 1317 Medical Officers (DTO), 1274 Treatment Organisers (TB Health Visitors), 985 Laboratory Technicians, 833 X-ray Technicians and 837 Statistical Assistants, making a total of 5246 DTP key personnel have been trained. In spite of training this large number of personnel, only 15% of DTPs have the full complement of trained key staff. It is essential that personnel who have to

organise DTPs are trained according to a uniform pattern in order to have a high technical working standard.

### **Justification for decentralisation**

- (1) There are a number of important responsibilities and functions of the NTP, which the states must shoulder themselves because "health" is primarily a state subject under the constitution.
- (2) Most of the district tuberculosis programmes require considerable administrative and technical assistance which can only be provided by STCs for reasons of operational feasibility, familiarity with local conditions and administrative convenience.
- (3) Any public health programme has a tendency to slacken and gradually disintegrate without proper and continuous supervision. This can be effectively undertaken only by the states.
- (4) The other factors which demand decentralisation of this activity are;
  - (a) a steady decline in the number of trainees sponsored by the respective state governments for training at NTI,
  - (b) problem of communication and understanding faced by the trainees as the medium of instruction is English,
  - (c) administrative constraints like difficulty to meet the travel and dearness allowance by the state governments and inability to enhance the stipend for the trainees,

---

\* Prepared by National Tuberculosis Institute, Bangalore, Directorate General of Health Services, Ministry of Health & Family Welfare, New Delhi

*Correspondence:* Director, National Tuberculosis Institute, 8, Bellary Road, Bangalore - 560003

- (d) rapid depletion of the trained personnel in the form of retirements, transfers and bifurcation of districts,
  - (e) underutilisation/diversion of the trained man power,
  - (f) effective coverage of training the medical and paramedical personnel in orientation in NTP can be achieved only if the states come forward to train their own personnel, and
- (5) At present only 70% of the DTP reports are received at NTI and there is considerable delay in receipt of the reports and giving of feedback to the states. The delay can be avoided, if the states take up monitoring.

Against this background, it is felt that it is time now for the NTI to take up the responsibility of training the trainers (STC personnel) and also provide necessary technical guidance for the states to conduct training courses and monitor the programme. STCs will get the manuals printed and impart training preferably in local languages.

In almost all states there already exist Tuberculosis Demonstration and Training Centres (TDTC). These centres were established at the time when the aim was to practically demonstrate that it was possible to treat tuberculosis patients on ambulatory basis from TB clinics, as effectively as in a TB hospital or sanatorium. Many of the functions and responsibilities as stipulated in this document are not within the ambit of TDTCs. However, by **upgrading and strengthening the existing TDTCs**, wherever necessary, they can be made to play their role effectively and take the right place in the supra-structure of the NTP. The additional funds required for this will have to be borne by the state governments and the Centre as per the prevailing pattern of assistance.

In general, there will be one STC in each state. However, in some very large states more than one STC may be justified. While in some very small states, or in union territories, there may be no STCs, they can avail the facility of neighbouring big states for training and the District Tuberculosis Centre (DTC) of the headquarters could look after monitoring and supervision.

### **Building for STC**

Existing building of Tuberculosis Demonstration Centre should serve the purpose. Any additional accommodation required, will have to be provided by modification/extension of the existing building. As such, no defined plan for STC building is suggested.

### **STAFFING PATTERN OF STC**

Director is the overall head of the STC and all the sections work under his guidance. Director is assisted by a number of specialised personnel who discharge the various functions, such as training, supervision, assessment etc. The following are his responsibilities:

- (a) Organising training programmes,
- (b) Supervision of DTPs,
- (c) Monitoring of DTPs,
- (d) Assessment of DTPs,
- (e) Co-ordinating and assisting DTPs through provision of necessary feedback.

The different sections, corresponding categories of staff and their key functions, mentioned in brief, are given in a tabular form on next page:

### **SECTIONS AND FUNCTIONS OF STC**

The STC shall comprise the following functional sections even though it would be one composite unit from the administrative point of view. Within the frame work of the NTP, the role of STC could be defined in terms of the following sections and their functions.

- A. Training section
- B. DTP demonstration unit
- C. Bacteriology section
- D. Treatment organisation section
- E. X-ray section
- F. Monitoring section
- G. Administrative section

### DIFFERENT SECTIONS, STAFFING PATTERN AND KEY FUNCTIONS

SL. No.	Section	Category of staff	No.	Key functions
1	Training	Medical officer	1	Training incharge
		The staff required for training are drawn from different sections	1 each	Provision of manuals, cards and forms (in local language)
2	Demonstration unit	DTO	1	Overall in-charge of programme in the
		MO	1	district Assist the DTO in DTP
		LT	2	Routine work in the laboratory and supervision of PHIs
		TO	3	Routine work of the treatment section and supervision of PHIs
		XT	1	Routine work of the X-ray section
		SA	1	Receipt of records and reports, allotment of index-number, maintenance of reminder charts, supervision charts and others
		Clerk	1	
		Lab attender	1	
		X-ray attender	1	
		Peon	2	
Safai wala	2			
3	Bacteriology	Bacteriologist	1	Training, mycobacterial culture, supervision of level 3 laboratory (see page 45)
		Sr. Lab. Technician	1	
		Lab. Technician	2	
		Registration clerk	1	
		Lab. attender	2	

SL. No.	Section	Category of staff	No.	Key functions
4	Treatment Organisation	Public Health Nurse	1	Training and supervision
		Sr. Treatment-organiser	1	
		Treatment-organiser	1	
5	X-ray	Sr. Radiographer	1	Training and supervision
		X-ray Technician	1	
		Clerk Attender	1	
6	Monitoring/ Assessment	Deputy Director	1	Overall incharge
		Medical Officer	3	Training, monitoring and assessment
		Statistician	1	Training, supervision and assessment
		Statistical Assistant	3	
7	Administrative	Administrative-Officer	1	Supportive
		Accountant	1	
		Store Keeper	1	
		Stenographer	1	
		LDC	2	
		Driver	2	
		Peon	3	
		Chowkidar	3	
		Safai wala	3	
		Vehicles	2	

#### A. Training section

The training section is more conceptual than real because actually the staff from other sections of STC would be participating in the training of DTP key personnel. The training shall comprise theoretical lectures supplemented with demonstrations and practicals, as per the procedures laid down in the DTP manual. The training is conducted at the STC.

##### Functions

(a) To organise and conduct training courses for DTP key personnel.

- (b) Organise training programmes for a short period of about a week for the peripheral health workers consisting of lab. technicians/ microscopists, drug distributors/pharmacists/ compounders.
- (c) Organisation of orientation programmes for district level health administrators is to be done in consultation with state health directorate. This is necessary to make the health administrators more familiar with the NTP, as they are the officials on whose support the implementation and running of DTP largely depends, from the administrative point of view.

- (d) A brief exposure on the salient features of DTP has to be given to undergraduate and postgraduate students of medical colleges. The present teaching of medical and para-medical personnel in tuberculosis continues to be clinically oriented. It is now realised that tuberculosis, being a community problem must be tackled by a community approach. Hence, there is a strong need to give public health orientation. The present nationally accepted policy under NTP makes it necessary that the guiding principles of the tuberculosis control methodology should be incorporated in all the teaching curricula. These should be demonstrated from a centre within each state where high technical standards could be maintained. This especially calls for greater effort and close collaboration with medical colleges, nursing schools etc.
- (e) General practitioners who provide primary health care facilities also need to be oriented to NTP.
- (f) Annual seminars for DTOs and District Health Officers are conducted with the objective of reviewing the performance of DTPs and suggest relevant measures for improvement.
- (g) Establishment of a demonstration DTP unit is essential for training purposes. DTP key personnel will be imparted training for a duration of four to five weeks as per the recommended curriculum. The DTP unit has to run on the lines recommended in the manuals and attain high standards for the purposes of demonstration. This will also help in getting experience and knowhow required to suggest methods for solving various problems and constraints encountered in the field.
- (h) Apart from regular training courses, STC would also organise short term orientation courses and refresher courses for workers already participating in TB control work.

#### **B. DTP demonstration unit**

This unit, like any other DTP, constitutes an organisational unit of the STC. It will be the

first unit to be established/adopted. In view of the considerable local differences, no definite overall pattern for the urban component of the DTP is recommended. The guiding principle for this part should be to enlist the participation of as many health institutions as feasible in tuberculosis case-finding and treatment for urban patients. The rural part of the programme (to be adopted in the mega cities) is to be used mainly for the training purposes and shall, accordingly be as per the procedures laid down in DTP manuals. Adopted DTP should preferably be away from STC.

#### *Functions*

This unit will be utilised for training purposes.

#### **C. Bacteriology section**

The priority function of the STC laboratory consists of ensuring proper technical standards of diagnostic services under the NTP in the state. This can be achieved through continuous technical supervision and assessment of DTP. Bacteriology section can start functioning even before a full-fledged culture and sensitivity facility is established, which may take some time.

The concept of different levels of laboratories, nation-wide, with defined activities and external & internal quality control mechanisms has been worked out as below:

*Level 1* These are situated at referring centres and are expected to perform sputum collection & smear preparation. The smears are to be sent to a nearby higher level laboratory.

*Level 2* These are situated at microscopy centres and are expected to perform sputum collection, smear preparation, staining, grading & recording of results.

*Level 3* These are situated at DTCs. In addition to the duties mentioned in Level 1 and Level 2, they are entrusted with the responsibility of supervising the above laboratories.

*Level 4* These are located at STCs. In addition to microscopy, culture and, sensitivity test for *Mycobacterium tuberculosis* for primary drugs such as Streptomycin, INH, Ethambutol and Rifampicin is performed. Training is imparted for personnel working in Level 3 laboratories. The larger states having sufficient resources can have more than one Level 4 laboratories.

*Level 5* At present the functions of Level 5 laboratories are being performed at NTI. In due course of time, 3 or 4 such centres are intended to be established on a regional basis. The functions of such laboratories are as follows:

- a. Providing culture facilities, identification tests and sensitivity testing of all mycobacteria.
- b. Training of personnel working in Level 4 laboratories.

#### *Functions*

- (i) Bacteriology section will provide culture and identification of the sputum specimens for mycobacteria and sensitivity tests for primary drugs - Streptomycin, INH, Rifampicin and Ethambutol (Level 4 Tuberculosis Laboratory - refer manual for establishment and functioning of TB culture laboratory 1983, NTI publication).
- (ii) Technical assistance in establishing DTC laboratories and further guidance to them (Level 3 Laboratories).
- (iii) Training of laboratory technicians and orientation to the already trained personnel from PHI laboratories (Levels 1 & 2 Laboratories).
- (iv) Studies on drug resistance strains on samples of newly diagnosed cases of tuberculosis and assist the deputy director in assessment.
- (v) Referral laboratory services for specimens received from other institutions in the state.
- (vi) Specimens of the research studies carried

out by the different sections of the STC will have priority for processing over the specimens that are received from other institutions.

#### **D. Treatment organisation section**

There is a chain of factors which influences the satisfactory organisation of treatment offered under NTP. The public health nurse/senior treatment organiser is entrusted the responsibility of training the treatment organisers regarding initiation of treatment/motivation/ defaulter retrieval actions and other aspects.

#### **E. X-ray section**

An experienced senior X-ray technician who can advise on the quality of radiographs and is versatile with the working of MMR machines can be given the responsibilities of guidance and assistance to DTPs, on proper functioning of X-ray units. He should also be able to attend to minor repairs and service problems of MMR machines. He should also be associated with the technical supervision regarding maintenance of equipment and participate in the training of X-ray technician of DTCs. X-ray unit in the adopted DTP will be used for training purpose.

#### **F. Monitoring section**

Monitoring section collects information on case-finding, treatment activity and related information on infrastructure, staff and equipment from DTPs on a quarterly basis. Achievements in those respects are compared with the expectations. Deviations, shortcomings and corrective actions needed are fed back to the concerned authorities. Monitoring shall be carried out according to a uniform procedure to permit comparisons between different areas in the country. Periodic assessments are carried out based on the methodology provided at the time of training in NTI. It must be stressed that the main purpose of assessment is constructive, in the direction of constant improvement. The collected data would be analysed in the monitoring section and drafted into the format of a report with technical recommendations for eventual submission to the programme officer of the state with a copy

marked to NTI, Bangalore. Different staff of the STC would be drafted for assessment, headed by I/c monitoring section (Deputy Director) depending upon the different objectives of assessment.

### *Functions*

Monitoring section will carryout supervision and assessment.

#### *(i) Supervision*

It is an important component of the programme aimed at maintaining proper working standards and maintenance of supplies. Programme officer of the state, designated as State Tuberculosis Officer (STO) is entrusted with the responsibility of supervision. He is technically assisted by the Director of STC who is responsible for programme assessment. However, in some states it is carried out by the STO who also happens to be the incharge of STC (dual function). Such a situation will interfere in the smooth functioning of the programme and that of the STC. It is, therefore, recommended that the STC should be headed by a full time Director. He will be responsible for co-ordinating the activities of different sections of STC and give feed back information of the monitoring data to the programme officer (STO) for effective supervision and assessment of DTPs. Monitoring of DTPs in the state is done through periodic reports received from various DTPs in the state, under intimation to NTI and DGHS, Govt. of India.

#### *(ii) Assessment*

An STC will have DTPs in different stages of development; some with full fledged programmes, others in the phase of implementation and still others where administrative sanction only is available. The requirements in terms of technical guidance and administrative support would differ widely at all stages of development. Attention will have to be focused on continuous assessment of DTPs. As a part of its overall technical responsibility, the STC shall periodically furnish to the health directorate, technical reports containing suggestions for improvement and cover

operational and technical aspects regarding the progress of NTP in the state as reflected through reports on monitoring and supervision.

### **G. Administrative section**

It will take care of the personnel, budget, other financial matters, co-ordination of training courses, the stores, vehicles and their repair, supplies, building etc.,

### **EQUIPMENT AND SUPPLIES**

Equipment already recommended for TB Demonstration Centre would be adequate. These include all the equipment normally required in DTCs but in larger quantities and, in addition, those required for the culture laboratory.

### **STATE TUBERCULOSIS OFFICER (STO)**

Every state has a tuberculosis programme officer who is designated as STO at the state directorate and he is directly responsible for running the tuberculosis programme in the state. The STC shall execute the various technical functions only on behalf of the STO, as it will be difficult for him to be fully conversant with all the technical details, which will be provided by the Director, STC. Close co-ordination between the STO and STC is essential for achieving effective supervision, monitoring and timely corrective action.

### **NATIONAL TUBERCULOSIS INSTITUTE (NTI), BANGALORE**

NTI, hitherto entrusted with the responsibility of training DTP key personnel will in future train the trainers, who in turn will impart training to DTP key personnel. STC staff concerned will also be trained in monitoring and evaluation techniques. Directors, Deputy Directors and Bacteriologist of STCs would be given orientation/training at NTI in their respective areas. NTI personnel will make periodic visits to STCs for giving them technical guidance. The NTI will continue to get periodic reports on the performance of DTPs from respective states.



**HIPPOCRATIC OATH AND  
MEDICAL ETHICS**

In a recent survey, done among medical schools in the U.S.A., it was discovered that 83% of the schools were making the students about to graduate out to take an oath of medical ethics, compared with 74% in 1958. But, it was seldom the Hippocratic Oath, which now is generally regarded as out-dated and no longer relevant. The oaths being administered, however, enshrined the spirit of the Hippocratic Oath if not its letter. In India, such an information may not be available and only a few medical colleges may be observing the practice of administering an oath at the time of annual convocation.

**Hippocratic Oath**

The words of the ever venerated Hippocrates (*circa* 450 B.C.) were as follows:

“I swear by Apollo physician, Asclepius and Hygieia, Panacea, and all the gods and goddesses, making them my witness, that I will fulfill according to my ability and judgement, this oath and this covenant:

- (i) To hold him who has taught me this art as equal to my parents and live my life in partnership with him, and if he is in need of money, to give him a share of mine, and to regard his offsprings as equal to my brothers in male lineage and teach them this art - if they desire to learn it - without fee and covenant, to give a share of my precepts and oral instructions and all the other learnings to my sons and to the sons of him who has instructed me and to pupils who have signed the covenant and have taken an oath according to the medical law, but to no one else.
- (ii) I will apply dietetic measures for the benefit of the sick according to my ability and judgement; I will keep them from harm and injustice.
- (iii) I will neither give a deadly drug to any body if asked for it, nor will I make a

suggestion to this effect. Similarly, I will not give to a woman an abortive remedy. In purity and holiness, I will guard my life and my art.

- (iv) I will not use the knife, not even for sufferers from stone, but will withdraw in favour of such men as are engaged in this work.
- (v) Whatever houses I may visit, I will come for the benefit of the sick, remaining free of all intentional injustice, of all mischief and in particular of sexual relations with both female and male persons, be they free or slaves.
- (vi) What I may see or hear in the course of the treatment or even outside of the treatment in regard to the life of men, which on no account one must speak abroad, I will keep to myself, holding such things shameful to be spoken about.

If I fulfil this oath and do not violate it, may it be granted to me to enjoy life and art, being honoured with fame among all men for all times to come; if I transgress it and swear falsely, may the opposite of all this be my lot”.

If the Hippocratic Oath has become out-dated for modern times, the question arises - is it necessary to administer an oath of medical ethics to new doctors? Many doctors do not even remember if they took an oath at the time of graduation, and if they did, they do not remember what the oath exactly stated. Nonetheless, it is the consensus of opinion, everywhere, that an appropriate oath is essential to stress the need for preserving medical ethics, especially in the confusing modern times.

Besides providing the ethical beacon, the oath's wording should promise and promote the spirit of trust between patients and their doctors and an assurance of quality of service, equally to all. Even though medical students may be absorbing the spirit of the oath throughout their training as doctors, the taking of the oath on the graduation day, among peers and teachers, could prove crucial, especially for those who are able

to preserve the idealism of their youth, when times become difficult later on in life.

The Hippocratic Oath had its focus on an individual patient, prescribed the doctor's duty towards such a patient, as well as to his teacher (and his family) from whom he learnt the art and a general code of conduct for physicians. It left out of its scope, public health, needs of the society, patients' rights and opportunity given to them for self-determination, as well as the vital need for spreading some medical knowledge to as many people as possible. Therefore, attempts have been made from time to time to come up with a more appropriate oath instead of just ignoring the Hippocratic Oath as a dead letter.

Following are some of the other oaths which have been administered from time to time:

1. "*Prayer of Maimonides*" - written by a Jewish physician in the 12th century and amended by a German physician in the 18th century.
2. "*Declaration of Geneva*" - drafted by the World Medical Association in 1948 and amended in 1983. It says:

"I solemnly pledge myself to consecrate my life to the service of humanity.

I will give to my teachers the respect and gratitude which is their due; I will practice my profession with conscience and dignity; the health of my patients will be my first consideration; I will respect the secrets which are confided to me, even after the patient has died; I will maintain by all the means in my power the honour and the noble traditions of the medical profession; my colleagues will be my brothers; I will not permit considerations of religion, nationality, race, party politics or social standing to intervene between my duty and my patient; I will maintain the utmost respect for human life from its beginning, even under threat, and I will not use my medical knowledge contrary to the laws of humanity.

I make these promises solemnly, freely and upon my honour".

3. "*Oath of Lasagna*" - drafted by a pharmacologist of the Tufts University, Boston, in 1964 and being used fairly widely in U.S.A.:

"I swear to fulfil to the best of my ability and judgement, this covenant:

I will respect the hard won scientific gains of those physicians in whose steps I walk and gladly share such knowledge as is mine with those who are to follow.

I will apply for the benefit of the sick all measures which are required, avoiding the twin traps of over-treatment and therapeutic nihilism.

I will remember that there is art to medicine as well as science, and that warmth, sympathy and understanding may out-weigh the surgeon's knife or the chemist's drug.

I will not be ashamed to say that I know not, nor will I fail to call on my colleagues when the skills of another are needed for a patient's recovery.

I will respect the privacy of my patients, for their problems are not disclosed to me that the world may know. Most especially, must I tread with care in matters of life and death. If it is given to me to save a life, the awesome responsibility must be faced with great humbleness and awareness of my own frailty. Above all, I must not play as God.

I will remember that I do not treat a fever chart or a cancerous growth, but a sick human being whose illness may affect the person's family and economic stability. My responsibility includes these related problems, if I am to care adequately for the sick.

I will prevent disease whenever I can, for prevention is preferable to cure.

I will remember that I remain, as a member of society, with special obligation to all my fellow human beings, those sound of mind and body as well as those who are infirm.

If I do not violate this oath, may I enjoy

life, my art and people's respect while I live, remembered with affection thereafter. May I always act so as to preserve the finest traditions of my calling and may I experience long the joy of healing those who seek my help".

4. "Institutional Oath" - The Association of Academic Health Centres in the United States of America has, in 1989, enjoined on all medical schools to draft their own appropriate oath of ethics with the assistance of their own faculties and ensure that it is taken by their students at the time of graduation. The texts of these oaths may vary but the substance of ethics should be served and preserved.
5. "Code of Ethical Conduct" - as prescribed for physicians by the Medical Council of India. It is not in the form of an oath, taken as a group by graduating doctors in the presence of their teachers but it has its own sanctity.

The issue, in short, is that the foundations of medical ethics have to be well and truly laid, irrespective of how. Just because the Hippocratic Oath has become out-dated, and could be forgotten, the goal of preserving the highest values of the medical profession should not be over-looked.

### GENETIC BREAKTHROUGH

Dr. J. Vraig Venter has recently announced to the American Society of Microbiology his almost incredible achievement: the complete genetic decoding of the common living organism - *H. influenzae*. He had been working for the International Genome Project, but had to leave the National Institute of Health at Bethesda (U.S.A) on account of differences on the best way of genetic decoding. He has now completed the remarkable feat privately, on his own.

The decoding has revealed a chain of nearly 2 million DNA bases making up the life

of a simple living organism, each base being a composite chemical unit constituting a gene. Uptil now, geneticists had been recognising genes by studying what function got impaired when mutation caused a change in one of the DNA bases. Now, scientists could start with each DNA base and study what functions it performed. For example, virulent and avirulent strains of a micro-organism, say *M. tuberculosis*, could be compared critically to locate the gene controlling virulence. The mentioned breakthrough may even lay the foundation for gene therapy. Also, some future diseases could be forecast by studying the genetic map of newborn babies. The next step to genetic decoding should be sequencing of genes, the start point and end point of each gene and the order of their placement.

The large scale emergence of drug resistant strains of bacteria in recent years - including multiple drug resistance in tuberculosis (MDR-TB) - has left clinicians and public health officials wondering if the race between bacterial evolution and technological ingenuity in discovering newer antibiotics is finally going to be lost in favour of the disease producing bacteria. A recent study by CDC (Atlanta) has shown that 8% of all enterococci isolated in U.S.A. hospitals are resistant to all the antibiotics, including the latest Vancomycin. Resistance to an antibiotic like Vancomycin has been shown to be carried by a small circle of DNA known as "plasmid" which is quite separate from the rest of the bacterium's genetic make up, and which can move easily from one bacterium to another (jumping gene). The transferability of plasmids, then, enables resistance to spread more rapidly. Perhaps, it took long years before the right combination of genes got assembled together on one plasmid, after which resistance to Vancomycin began spreading more rapidly. The same may be happening in respect of resistance to *Staphylococcus aureus* in hospitals and even MDR-TB. Till genetic research provides the right answers, public health vigilance has to be tightened up if epidemics of resistant strains of bacteria have to be avoided.



## IN MY OPINION ....



Valued readers of the *Indian Journal of Tuberculosis* should get an opportunity to know and appreciate the views of some of our stalwarts in the field of tuberculosis and its Control. We know from personal contacts that often such views are held quite powerfully, born out of long and rich experience. It is possible that a resource which could provide ideas for research as well as for interaction and introspection has been left untapped.

Tuberculosis and its control have been greatly influenced by an extra-ordinary amount of research done in the last few decades. It is a matter of some satisfaction that Indian tuberculosis research has not only been notable but often trail blazing. The contribution thus made to global tuberculosis control efforts has been widely acknowledged. These scientific achievements have been duly reflected in the pages of the Journal.

It also goes without saying that utilization of these researches, done in

India and abroad, has been far better elsewhere than in this country. The IJT has pointedly devoted attention to this aspect for several years: reviews of NTP, studies reflecting social, operational and administrative aspects of the programme, more effective utilization and training of paramedics, etc. have all been stressed. The new strategies of NTP, now being assisted by WHO and World Bank, have been highlighted.

But, as the entire body is always more than the sum total of its constituent parts, so is, perhaps, tuberculosis control. We feel that to all the above-mentioned efforts on the part of the Journal could be added the strongly felt opinions of those tuberculosis workers who have spent a life time in tuberculosis Control or given serious thought to it. We offer this page, and a new platform, to our seniors who have advice to give; have constructive criticism to make on what is going on, and ideas to share with those who must follow in their foot-steps.

**D.R. NAGPAUL**

Dear Sir,

We have read with interest the article regarding the changing trend of HIV infection and tuberculosis in a Bombay area based on hospital statistics by Mohanty and Basheer (*Ind. J. Tub.*, 1995, 41, 117). We have the following queries to make from the authors:

1. What can be the possible reason for a sudden rise in the number and percentage of HIV positive patients in 1991-'92 (almost 3.8 times from that of 1988-89) without showing similar trend in the subsequent years?
2. What criteria were applied for the diagnosis of tuberculosis is not clear from the protocol. It is mentioned that sputum positivity was there in 48.08% of the tuberculous patients. So, how were the rest of the patients diagnosed to be suffering from tuberculosis?
3. How many of the HIV positive patients had full blown AIDS? Pleural effusion and hilar adenopathy have been a relatively common observations in AIDS patients in the West. What can be the possible reason for the paucity of the same in our situation? Similarly, what was the prevalence of concomitant extrapulmonary tuberculosis in this series? Extrapulmonary tuberculosis upto 60% has been reported in India in HIV positive patients'.
4. What is meant by "standard chemotherapy"? The clarification will help a lot to understand the efficacy of antituberculosis therapy in our HIV population and to compare the results with those of others.

#### Reference

1. Tripathy S.P., Rodrigues J.J., Banerjee K, Joshi D.R., Jayaprakash M, Kher S.K. and Thakar M. Tuberculosis in a group of 169 HIV-1 seropositives at Pune, India. (Abstract # Pub 7559), VIII International Conference on AIDS, Amsterdam, July 19-24, 1992. 3, 142.

**P. Bhattacharya, and  
M.L. Gupta**  
Chandigarh

*The authors reply:*

1. There has been a similar jump in the HIV seropositives in Bombay: In 1987, their number was estimated to be 10,000, which increased to 300,000 in 1992. Persons infected with HTV earlier may have developed pulmonary tuberculosis later and their number increased proportionately as time passed.
2. Patients were diagnosed on the basis of clinical presentation, radiological features and sputum smear examination.
3. In the West, extra pulmonary manifestations are more common because of endogenous re-activation of the previous infection. Ours is a high prevalence country with frequent exogenous re-infection and the HTV infected persons may develop pulmonary tuberculosis more often.
3. We used the term "standard" short term chemotherapy for 2HREZ/4HR.

Dear Sir,

I have read with interest the article by Chakarabarti et al. 'Haematological changes in disseminated tuberculosis'- published in the July 1995 issue of the IJT. In their study, the authors did not find a single granulomatous lesion on bone marrow examination. This is surprising, especially when 59% of the patients in the study had miliary tuberculosis. The reason for the low yield was the fact that the authors failed to do a bone marrow biopsy in most of the cases. Although granulomas may be observed in aspirated material, they are more easily found in sections from biopsy specimens<sup>1</sup>. Furthermore, since the *in situ* architecture of the marrow is better preserved in biopsied than in aspirated material, identification of the lesion is better by biopsy. Yield of granulomas in bone marrow biopsy is approximately 66% in patients with miliary tuberculosis<sup>2</sup>. In fact, bone marrow biopsy (not aspirate) is the standard part of the work up for

PUO\ Bone biopsy is also essential for diagnosis of secondary myelofibrosis which is well described<sup>4</sup>.

In their study, five patients had pancytopenia refractory to iron and vitamin therapy. Although no follow up is mentioned by the authors, I feel these were probably due to the granulomatous seeding of the bone marrow resulting in a myelophthisic anaemia. Subsequent bone marrow biopsy done after completion of anti-tuberculosis therapy would have provided the answer.

### References

1. Wintrobe M.M., Lee C.R., Boggs D.R. Clinical Hamatology, 8th ed. Bombay, K M Varghese, 1981, 35.
2. Daniel T.M. Tuberculosis in: Wilson JD, Braunwald E Isselbacher K.J. et al (eds) Harrison's Principles of Internal Medicine 12th ed. New York, McGraw Hill. 1991, 637.
3. Root R.K., Petersdorf R.G. Chills and fever, *he cit* pp 123.
4. Wintrobe M.M., Lee C.R., Boggs D.R. Clinical Hamatology, 9th ed. Bombay, K M Varghese, 1991, 1615.

**Dr. A.S. Puri**  
New Delhi

*The authors reply:*

The objective was not to find the incidence

of granuloma in bone marrow in disseminated tuberculosis. If that were the objective, it will be very helpful if biopsies are done by localization of the lesions by radionucleotide scan. The authors had lots of limitations as many other haematological investigations could not be done in those cases.

Bone marrow biopsy is a standard part of work up of PUO when there may be many other causes of fever (like metastatic carcinoma, lymphoma etc). Our patients were all diagnosed and confirmed cases of tuberculosis.

Tuberculosis causing secondary myelofibrosis and or leukoerythroblastic blood picture is documented. But the authors did not expect myelofibrosis as the characteristic blood picture of myelofibrosis was missing in the study.

The follow up of the patients was done and is mentioned in the article. The mechanism of anaemia and pancytopenia in disseminated tuberculosis (chronic inflammation) is also clearly stated, with references.

The statement that bone marrow yield of granuloma is approximately 66% in miliary tuberculosis is not very clear. Is it after a single biopsy or multiple biopsies from multiple sites or biopsy after localization by scan, or in miliary tuberculosis following primary infection or miliary tuberculosis following post-primary infection? Again, "bone marrow positive"- does it mean granuloma only? We feel it may have been misunderstood and quoted wrongly.

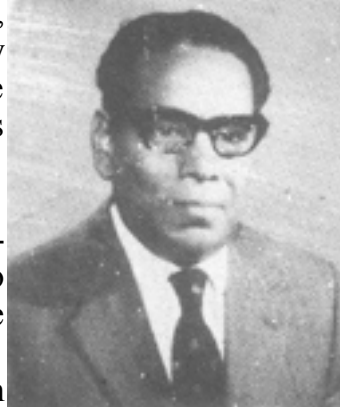
**Obituary****SHRI P.N. RAMAN  
(5.3.1920-13.1.96)**

Shri P.N. Raman, our former Secretary General, passed away on 13.1.96 morning. His sad and untimely demise came as a shock to all associated with the Tuberculosis Association of India, to his colleagues and friends.

Born on 5.3.1920, Shri Raman joined the Tuberculosis Association of India on 15.2.1941 and rose to become its Secretary-General, in which position he stayed upto his retirement in December, 1987.

Possessing rare qualities of hard work, dedication and devotion to this profession, he was a pillar of strength to the organization. With his qualities of selfless service, concern for fellow beings, helping nature, he had endeared himself to one and all.

The Association cannot be adequately grateful to him for all that he has done for its development and places on record its high appreciation of his outstanding contribution to the anti-TB movement as a whole. His contribution to the Tuberculosis Association of India would be remembered for all times to come.



---

## **NEWS & NOTES**

### **46TH TB SEAL CAMPAIGN**

The 46th TB Seal Campaign of the Tuberculosis Association of India was inaugurated on 2nd October, 1995, by His Excellency Dr. Shanker Dayal Sharma, President of India and Patron, Tuberculosis Association of India at Rashtrapati Bhawan. The TB Seals depicting some prestigious locales of India were presented to Rashtrapatiiji by Dr. P.K. Sen, President, Tuberculosis Association of India. Dr. A.K. Mukherjee, Director General of Health Services and Chairman, Tuberculosis Association of India, presented the Special Souvenir brought out on the occasion of the 46th TB Seal Campaign - 1995 to the President of India, in the presence of representatives of the Tuberculosis Association of India and Delhi TB Association. The function was widely covered by media.

### **INAUGURATION OF THE 46TH TB SEAL CAMPAIGN IN STATES**

In Andhra Pradesh, the Seal Campaign was inaugurated by Shri A. Madhava Reddy, Hon'ble Minister for Home, Andhra Pradesh on 2nd October, 1995. Dr. M.S. Rajajee, IAS, Chief Secretary to Govt. of Andhra Pradesh, presided over the function. Shri A.P.V.N. Sarma, IAS, Secretary to Govt. Medical and Health Department, Andhra Pradesh, graced the occasion. About 100 doctors and paramedical personnel attended the function.

In Gujarat, Dr. P.G. Pandya, Additional Director (Health), Gandhinagar, inaugurated the Seal campaign on 14th October, 1995. On this occasion, the first issue of their "News Bulletin" was released by Dr. Ajay Munshi, President, Ahmedabad Medical Association. The function was presided over by Dr. S.B. Trivedi, Chairman, Gujarat State TB Association. About 50 members attended the function.

### **GOLDEN JUBILEE NATIONAL CONFERENCE**

The Golden Jubilee National Conference

on Tuberculosis and Chest Diseases, jointly organised by the Tuberculosis Association of India and the TB Association of Kerala was held at the Kanaka Kunnu Palace, Thiruvananthapuram (Kerala) from 6th to 9th December, 1995.

His Excellency the Governor of Kerala, Shri P. Shiv Shankar inaugurated the Conference, while Hon'ble Chief Minister of Kerala, Shri A.K. Antony presided over the inaugural function on 6th December, 1995. The Hon'ble Minister for Health & Family Welfare, Kerala addressed the function, which was attended by approximately 600 persons.

His Excellency the Governor of Kerala also gave away the various awards of the TAI at the inaugural function.

Over 500 delegates attended the National Conference, a notable feature of which was the CME Programme on 6th December, 1995 which was well attended.

As many as 37 papers were presented, besides the two prestigious orations, viz. the Ranbaxy-Robert Koch Oration delivered by Dr. Arata Kochi, Director, Global TB Programme, WHO, Geneva, on "The Tuberculosis Epidemic in the 90s - Challenge and Response"; and the Lupin-TAI Oration delivered by Dr. Boms Wadia Prof., Chairman & Head, Department of Obstetrics and Gynaecology, Grant Medical College, Bombay on "Correlation of Signs, Symptoms, Clinical, Laparoscopic and Hysteroscopic findings in Pelvic and Abdominal Tuberculosis".

The Golden Jubilee Guest Lecture was delivered by Dr. A.K. Mukherjee, Director General of Health Services and Chairman, TAI.

Dr. P.R.J. Gangadharan, Director of Mycobacteriology Research, University of Illinois, Chicago delivered the talk on "Drug Resistance in Tuberculosis". There was a panel discussion on "Tuberculosis in Children".

The President of the Tuberculosis Association of India, Dr. P.K. Sen was honoured by the Association with Life Time Award for his outstanding life time contribution.

#### **DELHI TUBERCULOSIS ASSOCIATION IN "PERFECT HEALTH MELA"**

The Government of National Capital Territory of Delhi and Heart Care Foundation of India organised "Perfect Health Mela" from 27th October to 5th November, 1995 at the Jawaharlal Nehru Stadium, New Delhi. The main objective of the mela was to project the activities of various NGOs involved in health education, display of community service projects and free health check-up camps. Over 50 governmental and non-governmental organisations actively participated in this mela, and Delhi TB Association was one of them.

The Delhi TB Association put up a stall in which health education panels on tuberculosis were displayed and booklets on the subject were distributed free of cost. Throughout the mela, a video cassette was played on TV showing snort scripts on health education i.e., awareness programme on tuberculosis and AIDS. Over one lakh visitors from all walks of life visited the stall.

#### **AWARENESS CAMPAIGN ON "SOCIETY'S ROLE IN CONTROLLING TUBERCULOSIS"**

The Delhi Tuberculosis Association, jointly with the Regional TB Committee, New Delhi TB Centre and Interact Club of Indraprastha Girls Senior Secondary School, Jama Masjid, organised an essay competition on "Society's Role in Controlling Tuberculosis" on Saturday, the 28th October 1995. Shri G.S. Patnaik, Director of Education, was the Chief Guest.

#### **CHANCHAL SINGH MEMORIAL AWARD - 1996**

The Tuberculosis Association of India awards a cash prize of Rs. 1,000/- to a medical graduate (non-medical scientists working as

bacteriologists, biochemists, etc. in the field of tuberculosis are also eligible) below 45 years of age and working in tuberculosis for an original article not exceeding 30 double spaced foolscap size pages (approximately 6,000 words excluding charts and diagrams) on a subject relating to tuberculosis. Articles or papers already published or based on work of more than one author will not be considered for this award. Papers may be sent, in quadruplicate, to reach the Secretary-General, Tuberculosis Association of India, 3, Red Cross Road, New Delhi-110001, before the 31st of July, 1996.

#### **ESSAY COMPETITION - 1996**

The Tuberculosis Association of India awards every year a cash prize of Rs. 500/- to a final year medical student in India for an original essay on tuberculosis. The subject selected for the 1996 competition is "Tuberculous Lymphadenopathy: Diagnosis and Treatment". The essay should be written in English, typed double spaced, on foolscap size paper and should not exceed 15 pages (approximately 3,000 words including tables, diagrams, etc.). Four copies of the typescript should be forwarded through the Dean or Principal of College/University to reach the Secretary-General, Tuberculosis Association of India, 3, Red Cross Road, New Delhi-110001 before the 31st of July, 1996, with a certificate that the author is a final year medical student.

#### **HEALTH VISITORS' COURSE**

The 1996-97 TB Health Visitors' Course will commence in July 1996. The course will be of nine months' duration and will be held at the New Delhi TB Centre. The minimum qualification for admission to the course is 10+2 with Science and/or Hygiene and some experience in tuberculosis. Relaxation of experience in TB work may however be allowed by the selection committee. Science subjects should normally be upto 10th class. Application forms for admission to the course can be had from the Secretary-General, Tuberculosis Association of India, 3, Red Cross Road, New Delhi-110001. The last date for receipt of application is 30th April, 1996.

### **SYMPOSIUM ON “TUBERCULOSIS IN CHILDREN: DIAGNOSIS, MANAGEMENT AND PREVENTION”**

The Delhi Tuberculosis Association in collaboration with Delhi Medical Association and Indian Paediatrics Association (Delhi Branch), organised a symposium on “Tuberculosis in Children, Diagnosis, Management and Prevention” on 28th September, 1995, at the Delhi TB Association Hall. The symposium was presided over by Dr. Ashok Dutta and Dr. D.R. Nagpaul, was the Chief Guest. More than 100 doctors attended the symposium.

### **REFRESHER COURSE ON TUBERCULOSIS & CHEST DISEASES**

A refresher course on Tuberculosis and Chest Diseases was organised at Vellore on 30th June 1995, under the joint auspicious of North Arcot District TB Association and State TB Association, Tamilnadu. The course was inaugurated and presided over by Thiru M.P. Vijayakumar, District Collector of North Arcot Ambedkar District. About 150 doctors attended the Course.

### **WORKSHOP ON ROLE AND INVOLVEMENT OF NGOs IN NTP IN INDIA**

The Directorate General of Health Services, Ministry of Health and Family Welfare, New Delhi, organised a one day Workshop on the role and involvement of non governmental organisations (NGOs) in NTP in collaboration with WHO, World Bank and INMED on 28 September 1995 in New Delhi. INMED (International Medical Services for Health) is a newly established non-profit international health organisation involved in the control of tuberculosis, worldwide. A large number of NGOs from different parts of the country, actively involved in tuberculosis control services sent their representatives to take part in the deliberations. Dr. Luelmo and Mr. Richard Bumgerner (WHO), Ms. Maria Clark (World Bank) and Dr. Linda Pfeiffer (INMED) along with Dr. B.N. Mittal (DDG, LEP. & TB), Dr. L.B.S. Dey and Dr. Rohit Sarin from the DGHS provided the various inputs to facilitate the discussions. A joint plan of action was evolved and a common understanding about the role,

involvement and commitment expected from NGOs was reached.

### **RESOLUTIONS OF THE NINTH WORLD CONFERENCE ON TOBACCO AND HEALTH**

Following are the resolutions passed at the 9th World Conference on Tobacco and Health:

1. All nations implement the International Strategy for Tobacco Control.
2. The Prime Ministers of Germany, the United Kingdom and the Netherlands be informed by formal letter from the President of the conference and individual letters from conference participants that their governments' action in blocking the implementation of the directive on tobacco advertising in the European Union is an international scandal and is detrimental to the health of all citizens of the European Union, and by example of the citizens in all developing regions of the world who look to the European Union for leadership in public health policy.
3. The International Strategy for Tobacco Control (Resolution 1) should be implemented by all the governments of Central and Eastern Europe. Moreover, the Western Governments which have the headquarters of the transnational tobacco companies (which now control a majority of the tobacco production capacity in the Central and Eastern Europe region) should share the responsibility for ending the tobacco epidemic and assist government in the region to implement the strategy.
4. This conference further resolves that:
  - (a) national governments, Ministers of Health and the World Health Organization should immediately initiate action to prepare and achieve an international convention on tobacco control to be adopted by the United Nations as an aid to enforcement of the International Strategy for Tobacco

Control (Resolution 1) adopted by the Ninth World Conference on Tobacco and Health.

- (b) duty free sales of tobacco products be prohibited,
- (c) leaders of all religious communities be urged to adopt an official position and take action to protect humanity from the danger to health from tobacco,
- (d) an Islamic Council for tobacco control be established,
- (e) national governments should be encouraged to take measures leading to the adoption of generic packaging as a means of reducing inducements to tobacco consumption, and
- (f) in view of the vital importance of information and data exchange, the European Commission is strongly urged to maintain its support for BASP to enable this organisation to continue its major contribution to tobacco control in the European Union.

#### **SAARC TUBERCULOSIS CENTRE (STC), KATHMANDU**

The STC, Kathmandu has reported the following activities:

- 1. The 20th Session of Its Standing Committee was held on 27-29 April, 1995 and the Eighth SAARC Summit Meeting of the Governments of SAARC Member Countries took place on 24 May, 1995.
- 2. A Seminar on Tuberculosis Programme through Primary Health Care was organised on 4-5 April, 1995. Fifteen participants from SAARC member countries attended the Seminar. Six recommendations made by the participants included the establishment of a referral bacteriological laboratory for quality control of laboratory services and exchange of NTP experts between member states.
- 3. Fourth meeting of the Governing Board of

STC was held on 31 January - 1 February, 1995.

- 4. A Trainers' Training Course for DTPs in SAARC was held on 4-9 July, 1995. Seventeen participants and 2 facilitators attended the course.

#### **NEW INITIATIVES OF JAPAN ANTI-TUBERCULOSIS ASSOCIATION**

The Research Institute of Tuberculosis (RIT) under the Japan Anti-Tuberculosis Association has recently started 3 new programmes, namely:

- 1. "International Course on prevention and care of AIDS in Asia",
- 2. "International Collaborative Research Programme on Tuberculosis", and
- 3. International Mobile Seminar on Tuberculosis Control.

The RIT intends to expand and strengthen the activities in the field of international cooperation. Details of the above mentioned courses can be obtained from: Dr. M. Aoki, Director, RIT, 3-1-24, Matsuyama, Kyose-shi, Tokyo 204, Japan.

#### **CHAIRMAN, TECHNICAL COMMITTEE**

Dr. Hoimi Basu, Hony. Genl. Secretary, Bengal TB Association, Calcutta, has been nominated as Chairman of the Standing Technical Committee of the Tuberculosis Association of India for the year 1996-97. Dr. Basu will be the President of the 51st National Conference on Tuberculosis and Chest Diseases to be held at Bangalore in October-December 1996.

#### **IMPORTANT ANNOUNCEMENT - CALL FOR ABSTRACTS**

#### **51ST NATIONAL CONFERENCE ON TB & CHEST DISEASES**

The 51st National Conference on Tuberculosis and Chest Diseases will be held at

Bangalore (Karnataka) in October-December 1996. The exact venue, dates and other relevant details of the Conference will be announced shortly. The subjects selected for this Conference are: (1) Management of bronchial asthma/fiberoptic bronchoscopy, (2) National TB Control Programme including its assessment, (3) Follow-up studies on patients completing short course chemotherapy, (4) Management of treatment failure cases under field conditions, (5) Newer diagnostic methods in tuberculosis/controversies in respiratory diseases, (6) Smoking and tuberculosis, (7) Role of Indian Systems of Medicine, (8) Multi-drug resistance and its management, (9) Improvement of host factors in tuberculosis, (10) Drug interactions, (11) Serum concentration of anti-tuberculosis drugs and multi-drug resistance, (12) Role of NGOs in NTP and (13) Sociological aspects of tuberculosis. Free communications would, as usual, be eligible for presentation.

Those who wish to present papers on the above subjects may kindly send three copies of an abstract of their papers, latest by 30th April, 1996 to the Secretary-General, Tuberculosis Association of India, 3, Red Cross Road, New Delhi-110 001 for consideration by the Programme Committee.

It would be appreciated if the abstracts are

sent by registered post/UPC so as to ensure their safe receipt. The guidelines for the authors are:

(A) For preparing abstracts: 1. The length of an abstract should not normally exceed 250 words, including the heading. 2. The abstract should be as informative as possible, comprising the (a) objectives of the study, (b) methodology of investigation and (c) main findings. In respect of some papers (b) and (c) will comprise the ideal/hypothesis discussed and the conclusion. 3. If analysis is incomplete at the time, a revised abstract should be sent, at least six weeks prior to the Conference for printing in the Programme and Summaries for distribution among delegates. 4. An abstract which is considered inadequate may not be selected by the Programme Committee for presentation of the paper at the Conference.

(B) For preparing project slides/overhead transparencies: 1. Material on the slide should be relevant, minimum,, in bold letters/figures and either printed or typed. 2. Material should normally cover 3/5 of the available space on the slide, with margins on all slides, 3. Blue on white background is better than black and white slides. For multi-colour slides, the preferable colours are red, black and green, 4. Overhead transparencies should preferably not be handwritten. Typed or computer composed printouts could easily be photocopied, to the desired size, onto a transparent plastic sheet for use on an overhead projector.

## ABSTRACTS

Vol. 43 No. 1

January 1996

*Effect of Rifampicin on Theophylline pharmacokinetics in human beings*Sukesh Rao et al; *J.A.P.I.*; 1990, 42, 881.

Theophylline pharmacokinetics were studied in 20 healthy volunteers, after I.V. infusion of 5 mgm Aminophylline per kg body weight, and this was repeated after they had received Rifampicin 450 mgm daily (in single dose) for 6 days. Plasma half life of theophylline fell by a mean of 18.5% after Rifampicin administration, while volume of distribution increased by 23.4% and metabolic clearance by 47.6%. It is suggested that in patients on theophylline treatment, who have to be given Rifampicin, as in tuberculosis complicating COPD, the dosage of theophylline be adjusted and serum levels monitored both when Rifampicin is introduced and when it is withdrawn.

S.C.K.

*Rapid Isolation of Mycobacterium Tuberculosis using egg enriched sheep blood media (EESBM)*Vimal K. Sharma et al. *Ind. Jour. Ch. Dis. & Alii. Sci.*; 1994, 36, 97

One hundred twentyfive sputum specimens (100 smear positive) were cultured on L.J. medium and on EESBM. 11 grew mycobacteria - after 8 days on EESBM and after 6-8 weeks on L.J. medium, while 4 were negative on both. It is concluded that EESBM being as efficient as L.J. medium, but much quicker, can be a useful and cheap diagnostic tool.

S.C.K.

*Acid Fast Bacilli Positivity on buffy coat and bone marrow in Pulmonary Tuberculosis*R. Sen et al. *Ind. Jl. Chest Dis. & Alii. Sci.*; 1994, 36, 101

Buffy coat smears from peripheral blood and bone marrow aspirates of sixty fresh cases of pulmonary tuberculosis were examined microscopically after Ziehl Neelsen staining. - 55% buffy coats and 48% bone marrow smears were positive for AFB. The authors recommend these techniques for early diagnosis in tuberculosis.

S.C.K.

*A study of fungal infection in patients of Pulmonary Tuberculosis*R. Prasad et al; *Ind. Jl. Ch. Dis. & Alii. Sci.*; 1994 36, 104.

Fungal species were detected in sputum smears of 31.55% of 118 consecutive patients of pulmonary tuberculosis, while the yield was 79.66% on culture. *Candida* spp. accounted for 49.15% and *Aspergillus* for 27.12% of the positives, while positive immunodiffusion was seen in 37% of *Aspergillus* cases and 29.3% of those with *Candida* among those whose sputa were positive.

S.C.K.

*Abdominal Tuberculosis: A varied presentation*Naseer Baluch et al. *Pakistan Jl. of Med. Res.*; 1993, 32, 259.

Thirty surgically operated cases of abdominal tuberculosis have been analysed. Seventeen of them presented with subacute intestinal obstruction while the other 13 had acute obstruction. The duration of symptoms ranged from 4 days to one year. Only four of these patients had pulmonary disease, the rest being 'primary'. Diagnosis of tuberculosis was confirmed by positive histology, demonstration of AFB in biopsy specimens and favourable response to anti-tuberculosis chemotherapy. Strictures, single or multiple, were seen in 20 cases, ileocaecal mass in 6, and perforation in four cases.

S.C.K.

*Rifabutin for the treatment of newly diagnosis pulmonary tuberculosis: a multinational randomised comparative study versus Rifampicin*

Gonzalez - Montamer, L.J. et al: *Tubercle and Lung Disease*; 1994, 75, 341

Five hundred twenty fresh untreated sputum positive cases of pulmonary tuberculosis were taken up for study in 6 centres in Brazil, Argentina and Thailand and randomly allotted to one of three regimens. All patients received Isoniazid for 6 months along with Ethambutol and Pyrazinamide for the first 2 months. In addition, 175 (Group I) were given Rifampicin 10 mg/kg, 174 (Group II) Rifampicin 150 mgm daily and 117 (Group III) Rifabutin 300 mgm daily. All cultures (pretreatment) were sensitive to all the drugs used except 3 strains resistant to 1 ug/ml Rifampicin, 2 to 10 ug/ml Ethambutol (all received Rifabutin) and 17 i.e. 3 % to 0.2-0.5 ug/ml Isoniazid. Clinical and radiological improvement was uniformly good in all cases, while bacteriological conversion was of the order of 97% in Rifampicin and Rifabutin 300 mgm groups and 99% in Rifabutin 150 mgm group at 12 weeks, and 96%, 94% and 98% after 24 weeks. Four patients on Rifabutin relapsed during followup spanning 24 months, all within the first twelve months, 2 from each dosage group. Adverse reactions related to treatment were seen in 3% Rifampicin group, 2% of those on Rifabutin 150 mgm and 5% in the Rifabutin 300 mgm group.

It is concluded that Rifabutin 150 mgm daily can be a good and effective substitute for Rifampicin in ATT.

S.C.K.

*Dysregulation of homeostasis of blood T-lymphocyte subpopulations persists in chronic multibacillary tuberculosis patients refractory to treatment*

Base M. et al: *Tubercle and Lung Disease*; 1995, 76, 59

CD4/CD8 lymphocyte ratios were determined in 21 cases of refractory multibacillary pulmonary tuberculosis; 10 newly diagnosed drug sensitive cases and 10 normal healthy

individuals who served as controls. The ratios were 0.69, 0.81 and 1.84 respectively. After appropriate chemotherapy for three months, the ratios rose to 1.54 in the fresh cases, sputum becoming negative in all, while 7 out of 21 chronic cases were converted and the mean CD4/CD8 ratio rose only to 1.05 i.e. far below normal.

It is concluded that dysregulation of T-lymphocyte homeostasis becomes persistent with long standing bacillary load, thus delaying clinical and immunological recovery, even with adequate chemotherapy.

S.C.K.

*The use of sputum induction for establishing a diagnosis in patients with suspected pulmonary tuberculosis in Malawi*

Parry, CM. et al: *Tubercle and Lung Disease*; 1995, 76, 72

Eightytwo sputum negative suspects (the majority did not produce any sputum) were subjected to nebulisation with hypertonic saline to induce sputum production. Sputum specimens could be obtained in 73, of which 47 had never before produced sputum. This induced sputum was smeared for AFB and cultured for mycobacteria. Eighteen tested positive by smear (all culture positive also) and 12 more were culture positive. Sputum induction is a useful inexpensive technique to improve case detection.

S.C.K.

*Transthoracic (percutaneous) fine needle aspiration cytology diagnosis of pulmonary tuberculosis*

Das, D.K. et al: *Tubercle and Lung Disease*, 1995, 76, 84

Fine needle aspirations, under ultrasonic and CT guidance of lungs and pleural shadows in 190 patients yielded 38 cases of pulmonary tuberculosis (positive smears) i.e. 20% against a clinical suspicion of this diagnosis in only 8. Epithelioid granuloma was seen in 4 cases (AFB not demonstrated), granuloma with necrosis and necrosis only, in 17 cases each, with AFB positivity of 38.5% and 60% respectively.

FNAC could be a useful investigation in diagnosis of pulmonary tuberculosis.

S.C.K.

*Incidence of smear positive pulmonary tuberculosis from 1981-83 in a rural area under an active health care programme in south India.*

Ray, D. and Abel, R. *Tubercle and Lung Disease*, 1995, 76, 190.

In a prospective survey, involving total coverage of a rural population of 18,688 in July, 1981, 249 symptomatics were identified, and 242 submitted to examination. Of these last, 45 (18.6%) were sputum smear positive for AFB. By July 1982, 298 symptomatics were detected and barring 5, the rest gave their sputa, yielding 22 (7.5%) positive. Another 252 were identified by July 1983 and 21 (8.6%) gave positive sputa.

Overall prevalence of smear positives, in July, 1981 was 2.41/1000, with males having a higher rate 2.6% than females (all above 9 years). Incidence rates over the two year period was 1.15/1000 during the first year and 1.07/1000 for the second year. The surveyed population increased to 19129 in 1982 and 19570 in 1983. Highest prevalence was in age group 40-59.

S.C.K.

*Protein electrophoretic pattern in healthy and Pulmonary Tuberculosis subjects*

Khan, Jahangir, A. et al; *Pakistan Journal of Medical Research*, 1995, 34, 39.

Protein electrophoresis was performed on sera of 30 healthy controls and 30 patients of active pulmonary tuberculosis. Albumin was decreased while mean total protein level and all globulin fractions were increased in the patients.  $\alpha_2$  and  $\gamma$  globulins showed high level of increase while in  $\beta$  globulins, the increase was less dramatic, but significant.

S.C.K.

*Chronic lung inflammation in victims of toxic gas leak at Bhopal*

Vijayan V.K., Sankaran K, Sharma S.K., Misra NP. *Respir Med* 1995; 89: 105.

Bronchoalveolar lavage (BAL) studies in 20 patients at Bhopal, 1.3±0.4 yr. and 2.7±0.6 yr. after toxic gas exposure had revealed that the lower respiratory tract inflammation had progressed from initial macrophage alveolitis to macrophage-neutrophilic alveolitis. The interval between the two lavages was 1.4±0.6 yr. BAL studies in a new group of 24 patients 5.1±1.0 yr. after exposure had confirmed chronic inflammation of the lower respiratory tract as evidenced by macrophage-neutrophilic alveolitis in these subjects as well. Clinical, radiographic and pulmonary function abnormalities were persistent in a proportion of subjects in both groups.

Fibronectin (FN) levels were estimated in BAL fluid in 41 patients. Elevated FN levels were seen in 12 (29.3%) subjects and 9 of these 12 had radiographic abnormalities. Severely exposed subjects (n=30) had significantly higher BAL fibronectin levels compared to normal subjects and mild/ moderately exposed subjects. The number of patients showing abnormal decline in pulmonary function was higher in patients with elevated FN levels than in patients with normal FN.

Thus, persisting clinical, roentgenographic and ventilatory abnormalities, as well as macrophage-neutrophilic alveolitis along with abnormally elevated FN levels in a proportion of subjects, suggest the possibility that lung fibrosis can occur in subjects exposed to toxic gas at Bhopal.

A.S.