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Editorial

MULTIDRUG RESISTANCE IN TUBERCULOSIS

Multiple drug resistance in tuberculosis-MDR-TB or simply MDR-has become the new buzz word in the world of tuberculosis workers. The term covers the presence in environment of mycobacterial strains resistant to more than one of the commonly used highly potent antituberculosis drugs, patients who acquire multidrug resistance during the course of their treatment and the newly diagnosed previously untreated patients harbouring tubercle bacilli resistant to many drugs (initial resistance). The latter group of patients are believed to have been infected with MDR strains-nosocomial or from environment-or not divulged their past history of chemotherapy. The scenario should be familiar to us. Nonetheless, the recent focus on MDR appears to be due to the unusually large number of publications in the advanced countries, particularly U.S.A., on MDR, during the last few years, emphasising the rising proportion of MDR patients admitted to hospitals, AIDS Centres, prisons and among alcoholics as well as "skid row" population. Prior to 1980, this proportion had been falling and had come down to a very low level. Thus, in New York, resistance to one or several drugs among the HIV infected previously untreated tuberculosis patients has risen from 10% to 23% in the last decade. And for U.S.A., as a whole, nearly 90% of MDR is found among the HIV seropositive tuberculosis patients, with case-fatality of around 70% in 4 to 16 weeks time, while case fatality among the non HIV/MDR patients is as high as 25%.

In India, the existence of strains resistant to antituberculosis drugs, as in most developing countries, has been known for a long time, The paucity of reports on the subject is, however, due to the comparatively few centres where facilities for culture and drug sensitivity testing are available. Therefore, much of the drug resistance has been presumed clinically : when patients do not improve or the symptoms return after initial relief and/or sputum remains positive for AFB. Also, some of the published reports did not receive due attention because of the highly selected material in the report that was considered unrelated to what was happening in general

According to some fairly reliable evidence, the initial drug resistance in India is mostly to Isoniazid, of a varying order but below 20%, followed by that to Streptomycin (below 10%) and to Rifampicin (around 1%). And, initial MDR does not perhaps exist, or is very low. As regards acquired drug resistance, among the treatment failures, resistance to Isoniazid has been found to vary between 40% and 70%, to Streptomycin between 15% and 30% and to Rifampicin between 20% and 30%. According to a study reported from Gujarat, acquired resistance to Rifampicin increased from 2.8% in 1980 to 37.3% in 1986, apparently as the use of Rifampicin increased rapidly, and 95% of the Rifampicin resistant strains were resistant to Isoniazid or Streptomycin or both. It has also been observed by others that resistance to Isoniazid alone does not affect the results of treatment so much, if proper regimens for treatment or retreatment are prescribed but simultaneous resistance to Isoniazid and Rifampicin limits severely the results of their retreatment.

The disaster like situation reported from New York becomes understandable when it is realised that 19% of the isolates from the newly diagnosed patients were resistant both to Isoniazid and

Rifampicin, and 30% to 44% of the patients were HIV positive. Besides, results of even good chemotherapy among such high risk group cases cannot be promising because non-adherence to treatment among such patients is high. Many of them who do not die away soon automatically become “good chronics” and continue to infect others till eventual delayed death stops the spread of infection from them.

As to how virulent are the MDR strains compared with the naturally occurring sensitive strains of high as well as low virulence is yet a moot point. The immunocompromised (to a varying degree) HIV infected people appear to be confusing the situation all the more because they are increasingly breaking down to initial MDR disease and making such a comparison complex. The likely fate of transition from infection into frank tuberculosis among the HIV infected population will have to be studied carefully, over a suitable time span, as was done earlier in a series of epidemiological studies, before the advent of AIDS.

What could be done to deal with MDR (especially resistance to Isoniazid and Rifampicin, together) with a measurable degree of success? For suspected or proven MDR with information on the sensitivity pattern of the isolates and history of previously taken drugs available, the first step is to choose at least 3 suitable drugs for the first phase of treatment from among a relatively long list of reserve drugs. The fluoroquinolones-Ciprofloxacin and Ofloxacin-should be included in this battery of drugs. If possible, hospitalization could be arranged for the supervised administration and immediate treatment of toxic reactions which are far more common with such drugs. Besides, a longer duration of treatment should be planned and therapy continued for at least six months after sputum conversion. However, in India where culture facilities are scarce and secondary drugs scarcer, such a course of action becomes academic for the large majority of such patients. It is no surprise, therefore, that NTP in India does not contemplate any provision for MDR, leaving the consideration to individual institutions and their patients who can afford to buy those expensive drugs.

The National Tuberculosis Institute (NTI), Bangalore, had in the late sixties found that after a well organised tuberculosis programme was introduced in the city from the Lady Willingdon TB Demonstration and Training Centre, the levels of both initial and acquired resistance against Isoniazid registered a fall when the resistant cases were removed from the pool through delayed mortality over a number of years. The fewer number of resistant cases did not affect the prevalence of infection rate, on account of the much larger number of sensitive infectious cases, who could not come under the purview of the programme, because of its comparative inefficiency and insufficiency of drugs. It appears that the NTI did not pursue that direction of inquiry further. But a South African study spread over a period of two decades also came to the same conclusion. The observation appears to hold the key to the problem of MDR in our context. There should be no compromise on achieving the best results during initial chemotherapy of tuberculosis, when the bacilli are still fully sensitive or are resistant only to Isoniazid. This single step, among many others, should prevent MDR to a large extent and help control tuberculosis, during our life time or at least before AIDS becomes an epidemic. For achieving this objective, we should find the means to ensure uninterrupted and adequate supply of the requisite anti-tuberculosis drugs, and somehow shelve the intractable argument on shared responsibility, in this one crucial respect, between the Centre and the States. Besides, the temptation to differentiate case-finding from case-holding for the purpose of placing emphasis should be resisted. Both have to go hand in hand for achieving success.

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EARLY DETECTION OF RESISTANT *M. TUBERCULOSIS* FROM SPUTUM SPECIMENS

Introduction

Mycobacteria have long been recognized as important human pathogens. *Mycobacterium tuberculosis* is responsible for some 8 million new illnesses and 3 million deaths per year.¹ While tuberculosis remains a major health problem in developing countries, cases have significantly increased in developed countries as well in recent years. Ominously, the emergence of drug resistant strains has reduced the efficacy of treatment almost to the level of pre-chemotherapy era.² The appearance of Acquired Immunodeficiency Syndrome (AIDS) leading to immunosuppression of the cellular immunity has further complicated the situation.

Elimination of tuberculosis in a community is dependent on our ability to eliminate it from individual patients, as the source of almost all infections is the patient with open pulmonary tuberculosis. It is, therefore, crucially important to find infectious patients, as early as possible, and to give them a fully curative course of chemotherapy. In principle, bacterial resistance to one or more drugs is a limiting factor in attempts to cure individual patients and, thereby, eradicate the disease from the community.

Early application of effective treatment is the key to curing cases and blocking the transmission of the disease. Increasing rates of Multiple Drug Resistance (MDR) threaten to defeat an important element of modern tuberculosis control and the substantial reduction in the number of bacilli in the sputum within days after initiation of therapy.

Mycobacterial Drug Resistance

M. tuberculosis acquires resistance to various anti-tuberculosis agents by mutation which occurs at a low but constant rate and varies from drug to drug, such mutations occur in the absence of the drug: exposure to a drug does not induce mutation; it merely allows a mutant already present to flourish by destroying the drug sensitive bacteria which would otherwise compete for nutrients. Clearly, the chance of mutation to drug resistance occurrence is directly proportional to the bacterial load. Any population of *M.*

tuberculosis of more than 10^9 will inevitably contain several mutants resistant to an anti-tuberculosis drug.³

When Streptomycin (S) was first administered to pulmonary tuberculosis patients, it showed dramatic effect on their clinical condition. However, when it was continued for a few months, the tubercle bacilli became resistant to S. The drug was no longer effective, and most of the symptoms returned.

It was found that the emergence of S resistance could be reduced by the combined use of S with Para-amino-salicylic acid (PAS) and that long term use of S twice weekly and PAS daily was able to prevent emergence of drug resistance and showed good clinical efficiency.⁴

Isonicotinic acid hydrazide (INH) was also found to be a very effective drug for tuberculosis and it was shown that cavitary pulmonary tuberculosis could be cured by the long term combined use of INH, S and PAS. INH soon became the cornerstone of all effective regimes for the treatment and prophylaxis of tuberculosis. Modern chemotherapy was soon shown to be capable of achieving long lasting quiescence of the disease or cure if effective drug regimens were prescribed and taken for a sufficiently long time. The success was due to the prevention of the emergence of drug resistance by the use of combined chemotherapy, i.e. by giving more than one drug to the patient.

Drug resistance can emerge to all the antimycobacterial drugs. It occurs most commonly when a single drug is used alone and when the viable bacterial population in the lesions is very large. In tuberculosis, it first appears after at least two weeks and, more commonly, 1 to 4 months after start of chemotherapy.

The occurrence of drug resistance has been widely thought to be due to the overgrowth of sensitive organisms by the mutant resistant bacilli already present in wild strains before they were even in contact with the drug concerned. The fluctuation test of Lumia and Delbrook indicated mutation rates in *M. tuberculosis* of about 2×10^{-8} for resistance to INH & S, 2×10^{-10} for Rifampicin, and 1×10^{-7} for Ethambutol. The proportion of resistant mutants is variable from

one culture to another. And for most drugs, it is much greater for mutants with low MIC than those with higher MIC. In appropriate terms, the ratio of mutants that are able to grow during treatment of sensitive organisms, in wild strains, is about 1 : 10⁶ for INH & S and 1 : 10⁷ for Rifampicin.⁵

Mutation to drug resistance is usually thought to occur in bacterial chromosomes. It seems reasonable to assume that resistance to INH, Rifampicin, Pyrazinamide and other non-aminoglycoside drugs is always chromosomal and that resistance to S and other aminoglycosides in tubercle bacilli is probably also chromosomal. Plasmids have been isolated from mycobacteria other than tubercle bacilli (MOTT) but not in *M. tuberculosis*.⁶

In clinical practice, two types of resistance are recognized : primary & acquired. Primary resistance is due to infection with a strain originating from another patient who had acquired resistance owing to inadequate chemotherapy. So the patient with primary resistance to a drug has never taken that drug in the past but his source of infection must have done so.

The frequency of primary resistance varies from area to area. It is relatively low in most of the technically advanced countries. After examining 16,000 isolate; of *M. tuberculosis* in south-east England between 1977 and 1984, it has been reported that 1.8% of the strains from European & 5.5% from people of Asian origin were resistant to one of the four drugs, but nearly always to INH. The percentage of resistance to two drugs was 0.18% & 0.45% respectively, with one of the drugs being always INH. Resistance to three or more drugs was very rare.⁷ In developing countries, however, the percentage of primary (sometimes called initial) resistance has been 17.6 in South Africa, 22.9 in Thailand and 32.6 in Bolivia. Drug resistance; has frequently been encountered in India. Two important surveys have been conducted by the Indian Council of Medical Research (ICMR).^{8,9} Both the surveys involved collecting isolates from nine different centres and processing of the same at the Tuberculosis Research Centre, Madras. Out of the 1838 cultures examined in the first survey, 14.7 per cent were resistant to INH, 12.5 per cent to Streptomycin and 20.4. per cent to one or both the drugs. No history of prior antituberculosis treatment was obtained from these patients. In the second survey, though the prevalence of drug resistance in

patients with no previous history of chemotherapy was similar, there was an increased frequency 1 among those with history of previous chemotherapy.

From Bangalore¹⁰ and Madras¹¹, the prevalence of drug resistant tuberculosis to any drug has been reported as 21.2 per cent, initial resistance to INH as 17.4, to Streptomycin as 5.7 per cent and to Rifampicin as 3 per cent. From Gujarat, the prevalence of primary drug resistance to INH has been reported as 13.9 per cent and to Streptomycin as 7.4 per cent. While primary resistance to Rifampicin and Pyrazinamide was not reported, it was detected in secondary resistant cases; the resistance increased from 2.8 per cent in 1980 to 37.3 per cent in 1986. In 95 per cent of these Rifampicin resistant isolates, the strain was also resistant to INH or Streptomycin or both.¹²

The determination of the level of initial resistance gives a picture of the type of *Mycobacterium tuberculosis* in the community as well as the success or otherwise of the National Tuberculosis Programme and influences the design of therapeutic regimens and policy decisions on the need for routine sensitivity testing.

In the past, it was reported that resistance to a single drug, especially primary resistance, had little effect on the success of three-drug regimens (S, INH and PAS) and the results were almost as good in such patients as in those with fully sensitive strains.⁶ It was also suggested that in tropical developing countries, pretreatment susceptibility tests had no real benefit since the technique of drug susceptibility testing was elaborate, requiring the skill of a specialist and laboratories of a high standard. The technique itself was not very uniform and mistakes could frequently occur leading to the use of costlier and more toxic drugs instead of cheaper effective drugs. A study carried out in Hong Kong confirmed that the policy of dispensing with susceptibility testing would spare patients a number of unpleasant and serious side effects and, moreover, avoid waste in terms of manpower, laboratory resources, drug costs and hospital beds and yet be equally effective.¹³

New Evidence

In the recent past, new evidence has started accumulating which challenges the older views. It would now appear that there is a significant

prognostic value of primary or initial resistance regarding results of chemotherapy in newly diagnosed, severe, pulmonary tuberculosis cases. In a study from Madras, it was reported that 71% of patients with primary INH resistance had an unfavourable response with bacteriologically active disease at the end of treatment, as compared with only 14% of patients with sensitive organisms on admission to the study. Similar results were obtained in four co-operative studies in East African countries¹⁴ as well as in the United States of America.¹⁵ It has been suggested that INH along with Rifampicin, Pyrazinamide and Ethambutol be used initially when the risk of INH resistance being present is high and adjustment of the regimen be made when drug sensitivity data become available. When single drug resistance is found (usually to INH), treatment must be continued for 12 months with the other three drugs. When multi-drug resistance is met with, prolonged therapy with a four drug regimen (including 2 drugs to which the organism has been shown to be sensitive) is recommended.

Effect of HIV

Till 10 years ago, tuberculosis was rapidly disappearing from the USA. Beginning from 1985, however, the decade long decline has reversed dramatically.¹⁶ Patients with HIV infection have been recognized to be at risk of activation of latent tuberculosis infection and acquisition of new infection, with rapid progression to tuberculosis. Several reports have documented the transmission of MDR strains of *M. tuberculosis*. The occurrence of tuberculosis caused by resistant bacilli among patients with HIV infection has serious public health implications. Common features of these outbreaks include delay in the recognition of MDR strains of *M. tuberculosis* and inadequate isolation of strains. Treatment regimens are often inadequate and patients remain infectious for prolonged periods of time.¹⁷ Restriction fragment length polymorphism (RFLP) analysis of one outbreak indicated that of the 16 MDR isolates, 14 had identical banding pattern. Of the three isolates from drug susceptible *M. tuberculosis*, each had RFLP pattern distinct from the other two and from the isolates of outbreak patients.¹⁸ Surveys of medical house-staff, nurses and other workers in numerous urban hospitals indicate extraordinarily high tuberculin conversion rates in the range of 10

to 30%.

Gayathri et al¹⁹ have demonstrated that the catalase and peroxidase activity resides on the same enzyme and that a single mutation from INH sensitivity to INH resistance in *M. tuberculosis* leads to the loss of INH uptake and loss of catalase, peroxidase and Y-enzyme activity.

It has recently been documented that a single *Mycobacterium tuberculosis* gene, Kat-G, encoding both catalase and peroxidase, restored sensitivity to INH in a resistant mutant of *M. smegmatis* and conferred INH susceptibility to strains of *E. coli*. Deletion of Kat-G from the chromosome was present in INH resistant strains.²⁰

Rifampicin (R) is the second key drug in any effective treatment regimen for tuberculosis. The mechanism of action of R is believed to involve interference with transcription and RNA elongation by binding of the drug to the beta sub-unit of RNA polymerase in a locus formed by the appropriate complexing of the different RNA polymerase sub-units. Substitution of key amino acids would, thus, lead to conformational changes and defective binding of the drug.²¹⁻²² Telenti et al²² have identified, cloned and partly sequenced the rpo B gene of *M. tuberculosis*. On comparing this sequence with those of PCR generated fragments of the rpo B from 122 clinical isolates, it was possible to detect 15 types of mutations in 66 R resistant strains of *M. tuberculosis*. Most were single nucleotide mutations involving 8 codons. All mutations were found to be clustered within the 23 amino acid region of rpo B. PCR single strand conformation polymorphism (PCR-SSCP) allowed for rapid detection, by gel electrophoresis, of single base changes in PCR products without the need for sequencing.

It has now been recommended that drug susceptibility tests should be put up on all culture positive cases. But, routine direct sensitivity tests would still take weeks, if not months, for results to be available. The BACTEC radiometric system offers one possibility of early sensitivity results.²³ The use of DNA probes for detection of Kat-G gene deletion for INH resistance and analysis of RNA polymerase by PCR-SSCP for mutation in the rpo B region for R resistance may be two new methods which could make results available in clinically useful time, at least in tertiary care centres.

Firefly luciferase represents one of the most efficient available biological reporter molecules because it catalyzes the reaction of luciferin with adenosine triphosphate (ATP) to generate photons (light). Jacobs et al²⁴ constructed a shuttle plasmid from a mycobacteriophage so that the gene coding for the production of luciferase could be transferred to *Mycobacterium tuberculosis* culture. Susceptible cultures when grown in media containing INH or Rifampicin resulted in extinction of light production. On the other hand, light signals in drug resistant strains continued to be produced. Luciferase reported phages appear to be a new existing technique which could make drug resistant results available in days rather than weeks. This technology could be adopted in developing countries, either through the use of inexpensive luminometers or of sensitive film technology.

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NEWER ANTBMYCOBACTERIAL DRUGS AND THEIR ROLE IN THE TREATMENT OF TUBERCULOSIS PATIENTS

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Chemotherapy of pulmonary tuberculosis : Current concepts and need for newer drugs

The main lesion in pulmonary tuberculosis, the pulmonary cavity, contains a large number of mycobacteria (about 10^8 colony forming units). Of these, a large bacillary population is located in the thin liquid caseous layer that covers the inner part of the cavitary wall. Here, the bacilli are extracellular which multiply actively because of the availability of oxygen and nutritive substances. There are at least 2 other bacillary populations, one inside macrophages and another inside solid caseous foci; both these populations are limited in size because environmental conditions are unfavourable for their growth.¹ Among the organisms in these 3 populations, which are normally drug sensitive, drug resistant mutants develop at a mean frequency of about 10^{-6} .

In the early sixties, chemotherapy for tuberculosis relied on the administration of 18 to 24 months of 3 drugs : Isoniazid (H), Para-amino salicylic acid (PAS) and Streptomycin (S), the last being given during the initial few months of treatment. The drugs were given together to prevent the emergence of resistance and prolonged treatment was given until the microbial population was eliminated in order to prevent relapse. Despite such prolonged treatment, the relapse rate after discontinuation of treatment was about 10%.⁷

The introduction of Rifampicin (R), in the seventies, brought a marked change in the chemotherapy of tuberculosis.² Rifampicin was effective not only against actively multiplying organisms but also against the dormant or persisting organisms, responsible for relapse after treatment was stopped. Around this time, the bactericidal activity of Pyrazinamide (Z), a drug highly active against organisms located in an acid environment, especially inside macrophages, was

also rediscovered. These developments formed the basis of controlled clinical trials which established the efficacy of 6-month regimens. The high success rate of antituberculosis regimens of only 6 months' duration in the treatment of extensive, sputum positive, pulmonary tuberculosis was clearly demonstrated in a large scale study in East Africa.³ This led to the recommendation by numerous health authorities of the 6-month short course chemotherapy (SCC) for tuberculosis that is presently used in many countries, including India.

The major drugs, whose potency and acceptability have resulted in their widespread use in the current chemotherapy of pulmonary tuberculosis are H, R, Z, S, Ethambutol (Emb) and Thioacetazone (TB I).⁴ The usefulness of a drug in the treatment of tuberculosis depends mainly on its sterilising activity; ability in combination with H to prevent emergence of resistance and early bactericidal activity. The time taken for organs to be sterilised, in experimental animal tuberculosis, and the relapse rate after SCC in man are measures of a drug's action against semidormant tubercle bacilli and its sterilising activity. The ability of a drug to prevent the emergence of resistance is assessed during treatment with the drug concerned in combination with H, and is graded according to the proportion of patients who fail, with the emergence of H resistance; this proportion is about 0.5% for Rifampicin (high activity)⁵ and 13-15% for Thioacetazone (low activity).⁵ The fall in the count of viable tubercle bacilli during the first few days of treatment, when the actively growing bacilli are killed, is termed the early bactericidal activity (EBA) and can be measured *in vitro*, or in man.

Tubercle bacilli in a special population which show spurts of metabolism are particularly susceptible to Rifampicin and the ones in a special population surrounded by an acid environment are susceptible to Pyrazinamide. H and R are highly

effective in preventing the emergence of resistance, H has high EBA, R and Z have high sterilising action, and H, R, Z, Emb and S are highly suitable for intermittent use.⁴

Despite the introduction of effective, nontoxic antituberculosis drugs and therapeutic regimens, pulmonary tuberculosis still affects millions of people in the developing world.⁶ The results of a recent evaluation done by the Tuberculosis Research Centre (TRC), Madras⁷ show that the reality of the tuberculosis problem remains grim even after the implementation of SCC. Of the 3357 smear-positive pulmonary tuberculosis patients initiated on antituberculosis treatment in North Arcot district between April 1986 and March 1988, 2306 had been prescribed a SCC regimen and 1051 had accepted a standard regimen. Only 43% receiving the SCC and 35% receiving the standard regimen had completed 80% or more of their treatment. The overall mortality was 28%. Of the remaining, 31% had active disease and were excreting bacilli, including 65% whose cultures were resistant to H and 12% to R. combined resistance to R & H was seen in 6% and to S & H in 19%. H resistance was significantly higher in those who had been prescribed standard regimens and R resistance was seen even in those who had not received the drug.

Even with the standard regimens, it was possible to achieve 98% cure rate and reduce mortality rate to as low as 1 % in clinical trials.⁸ However, when these regimens were applied under field conditions, less favourable results were obtained, the reason being the difficulty in delivering the presently available complex regimens to patients in tuberculosis control programmes, particularly in developing countries like India.⁹ Partly as a consequence of poor adherence, a sizeable proportion of patients harbour tubercle bacilli resistant to one or more established drugs, which further complicates treatment.¹⁰⁻¹²

Thus, new chemotherapeutic drugs are needed to improve adherence and to treat disease due to drug resistant tubercle bacilli and nontuberculous mycobacteria (NTM). At present, and in the near future, the control of tuberculosis will depend on better case-finding, case-holding and treatment. For this, the availability of well tolerated and rapidly curative drugs that can be delivered by peripheral centres is essential." The new drugs

must be developed with the aim of increasing the sterilising activity of regimens because they might allow further shortening of the period of treatment to less than the current 6 months and, thus, improve adherence and acceptability.

The duration of treatment, the number of drugs used and the frequency of administration of each drug determine the complexity of therapy. Complexity and toxicity often lead to poor adherence. Several strategies have been developed to simplify treatment, to reduce medication errors and to discourage intentional avoidance of the ingestion of one or more drugs. New formulations containing 2 or more drugs, such as combination tablets containing both R and H, and more recently R, H and Z, with bioavailability of the components equivalent to that when single drugs are administered, have been developed. Implants of biodegradable polymeric systems such as polyactic polyglycolic and poly (lactic/glycolic) acid copolymers containing antituberculosis drug, for sustained release of the drug, could help in solving the non-adherence problem. However, with the potency of the present antituberculosis drugs, the implants will have to be very large and the cost of surgical implant and removal after drug cessation increases the cost of chemotherapy.¹³

The financial resources and time needed for developing a new antituberculosis drug and assessing its efficacy *in vitro* and in clinical trials, the decline of tuberculosis in developed countries, the inability of developing countries to purchase expensive drugs and the ready availability of effective antituberculosis drugs are some of the reasons why new agents for the treatment of tuberculosis have not appeared.¹⁴ However, the current increase in the incidence of tuberculosis associated with HTV infection, and the occurrence of multiple-resistant tubercle bacilli have led to a pressing need for the rapid development of new antituberculosis drugs. The second urgent need for new drugs arises because of anticipated future increase in the incidence of R resistant strains. In the treatment of tuberculosis, SCC regimens are effective in patients with strains resistant to H or S, but the results in patients with initial R resistance are poor.⁴

Methods of assessing new drugs

Apart from pharmaceutical firms which

develop them, new drugs have to be assessed independently through *in vitro* studies, animal experiments and clinical investigations to allow unbiased comparisons between drugs and to make such assessments as free as possible from commercial interests. *In vitro* studies include determination of the minimal inhibitory concentration (MIC) and the bactericidal activity against appropriate mycobacterial species. Animal experiments include estimation of blood levels, chemotherapeutic studies, preferably in mouse and occasionally also in guinea pig, and measurement of sterilising activity. In clinical investigations of antituberculosis drugs, a series of single increasing doses of the new drug is given to patients or healthy volunteers, while information on pharmacokinetics and side effects is obtained. The effective dose size is estimated by measuring EBA of the new drug at different dose sizes in comparison with a standard drug such as Rifampicin. These studies have to be followed by pilot and full scale clinical trials.⁴

Promising newer antimycobacterial drugs

Based on all or some of the above mentioned methods of assessment, a few classes of drugs show promise in the treatment of tuberculosis. These include the Rifamycin derivatives, fluoroquinolones, combination of beta lactam agents and beta lactamase inhibitors, and others.

Rifamycin derivatives

The dose required when Rifampicin is given once weekly may lead to a high incidence of serious adverse reactions. This can be avoided by using a Rifamycin with a long half-life which gives sustained blood levels between doses. Many novel Rifamycin molecules with a long elimination half-life have been designed with this aim.² Such long lasting Rifamycins (LLRs) include Rifabutin (LM 427, Ansamycin-Farmitalia), Rifapentine (DL 473-Merrel Dow), CGP 29861, COP 7040 and CGP 27557 (Ciba-Geigy), FCE 22250 (Farmitalia). and R-76-1 (Rifadine) developed in China.

Rifabutin (LM 427, Ansamycin)

Rifabutin is a semisynthetic spiropiperidyl derivative. The discovery of its activity against

M. avium-intracellulare and the emerging problem of disseminated *M. avium intracellulare* infection in patients with AIDS led to the use of the drug in USA on 'compassionate' grounds, before clinical trials, but with disappointing results.^{4,5,16}

Rifabutin was also active against approximately one third of the Rifampicin resistant strains of *M. tuberculosis* tested.¹⁶ All *M. tuberculosis* strains susceptible to Rifampicin were susceptible to Ansamycin, and the MICs were similar. The strains that were highly resistant to Rifampicin were resistant to Ansamycin. However, it showed activity against some Rifampicin resistant *M. tuberculosis* strains. Typical MIC for *M. tuberculosis* strains was observed to be 0.006 mg/l for Rifampicin sensitive strains and 6-16 mg/l for Rifampicin resistant strains. The MICs for resistant strains are much higher than clinically achievable concentration in blood (C max-0.5 mg/l blood after 300 mg given orally; C max 3 mg/l in lung tissues).¹⁷

Dickinson and Mitchison¹⁸ observed that 31% of 35 Rifampicin resistant strains tested had MICs of 0.6 mg/l or less for Rifabutin and could be classified as relatively sensitive. The MICs of Rifabutin were much lower among sensitive *M. tuberculosis* strains. As an explanation for the low MICs of Rifabutin against Rifampicin resistant strains, it has been claimed that the mode of action of Rifabutin is different from that of Rifampicin. Alternatively, this would also suggest that the activity of Rifabutin and other Rifamycins against resistant strains might be proportional to their activity against sensitive strains. These findings raise the possibility that Rifabutin might be of use in the treatment of some patients with Rifampicin resistant strains.

In a study done at TRC, a total of 103 *M. tuberculosis* strains were tested against Rifampicin and Rifabutin.¹⁹ In 42 out of the 52 Rifampicin susceptible strains, Rifabutin showed at least 4-fold higher effectiveness than Rifampicin. The geometric mean MIC of the 52 strains was 1.3 mg/l with Rifabutin compared to 13.3 mg/l with Rifampicin, showing on an average 10-fold higher effectiveness. Of 51 strains resistant to Rifampicin, 11 (22%) were susceptible to Rifabutin.

In animal models, Rifabutin is quite active

against *M. tuberculosis* and its relatively high tissue levels and long half-life suggest that the drug might be effective if given intermittently in tuberculosis. It is about 6 times more active than Rifampicin in experimental infection of mice with *M. tuberculosis* or *M. avium*. The considerable activity in murine disease may reflect high intracellular concentrations in mouse macrophages while caseating lesions in cavitory walls containing numerous acid fast bacilli, characteristic of human pulmonary tuberculosis, might contain lower concentrations corresponding to those in plasma. Thus, it is uncertain whether Rifabutin concentrations in these human sites are sufficiently high for effective antimicrobial activity²⁰

The fall in viable counts of *M. tuberculosis* in sputum collections during the first 2 days of treatment (EBA) suggested that Rifabutin was inactive or less active than Rifampicin in pulmonary cavities.²⁰ This may be due to the low plasma concentrations which are not fully compensated for by the slightly greater antituberculosis activity of Rifabutin *in vitro*.

Recently, a controlled study of Rifabutin in the retreatment of patients with pulmonary tuberculosis resistant to SHR has been reported by the Hong Kong Chest Service/BMRC.²¹ Bacteriological results in the 22 patients studied showed no evidence of sustained benefit in any patient with Rifampicin or Rifabutin. The results suggested that Rifabutin does not have a useful role in the retreatment of patients with multi-drug resistant pulmonary tuberculosis, which includes Rifampicin resistance, except (possibly in a small proportion of patients who have Rifabutin susceptible strains.

O'Brien and associates¹⁷ have reported their findings in patients with pulmonary *M. avium* complex (MAC) disease and drug resistant tuberculosis receiving Rifabutin. Conversion rates were 9% for pulmonary MAC and 35% for drug resistant tuberculosis. In another study, these authors have concluded that some patients with Rifampicin resistant tuberculosis may benefit from the addition of Rifabutin to the treatment regimen.²² However, when resistance to all first line drugs is present, the outcome remains poor. To obtain an irrefutable answer regarding its efficacy, Rifabutin should be evaluated in controlled trials for the treatment of both newly

diagnosed pulmonary tuberculosis and MAC pulmonary disease. The drug's long half-life suggests that it may be especially valuable for intermittent administration. Whether or not this drug is more effective than Rifampicin can be answered only by randomized clinical trials. However, before such trials are initiated, it is important to determine the optimal dose, lest failure to show effect be attributed to a sub-therapeutic dose. It is imperative that controlled studies of various drug regimens that contain higher doses of Rifabutin be undertaken for the treatment of patients with disseminated MAC disease and AIDS.

Rifapentine (DL 473)

Rifapentine, a cyclopentyl Rifamycin has been shown to have antimycobacterial properties. The *in vitro* activity of Rifapentine is 2-4 times that of Rifampicin against a variety of clinical mycobacterial isolates; the range of MICs is 0.025-0.1 mg/l as compared with 0.05-0.8 mg/l for Rifampicin. Rifapentine is bactericidal against actively growing bacilli, with a rate of killing similar to that documented for Rifampicin.

In a TRC study, of 103 strains of *M. tuberculosis* tested, 51 strains resistant to Rifampicin (MIC>128) were also resistant to Rifapentine, indicating complete cross-resistance.¹⁹ The remaining 52 strains were sensitive to both; in these strains Rifapentine has a 2 to 16 fold higher activity than Rifampicin.

In experimental tuberculosis in mouse, Rifapentine has a 50% effective dose (ED 50), 10 times lower than that of Rifampicin. Rifapentine administered to experimentally infected mice once a week is as effective as Rifampicin administered daily, in the initial as well as the continuation phase, and retains considerable efficacy when given once every 2 weeks. In most rat tissues, Rifapentine concentrations are about 40 times greater than that observed for Rifampicin.² All animal toxicity studies including long term assessment have been completed.

Rifapentine has been used in 2 clinical studies in man, one in Britain and the other in Finland, for the treatment of chlamydial urethritis, where it has been given at 600 mg per day in up to 6 consecutive doses without evidence of toxicity. It is taken up into the cytosol fraction of neutrophils

and macrophages and retains intracellular activity. It binds weakly to serum albumin. After administration of a single oral dose of 600 mg to healthy volunteers, peak serum concentrations of 20 μ g/l were observed with Rifampin. The half-life of Rifampin after 1st and 3rd once weekly administration was found to be the same. It has not yet been used in the treatment of human tuberculosis. It is potentially of great value in the chemotherapy of tuberculosis for use in supervised widely intermittent regimens.⁴

Fluoroquinolones

Fluoroquinolones such as Ofloxacin, Ciprofloxacin, Norfloxacin, Pefloxacin, Enoxacin, Lomefloxacin and Sparfloxacin are synthetic compounds active against a wide variety of microorganisms. Their activity is exerted through inhibition of gyrase, an enzyme involved in DNA replication. As expected, there is no cross resistance between these agents and other antituberculosis drugs.²

Ofloxacin (DL 8280)

In 1983, Tsukamura²³ showed that Ofloxacin was active *in vitro* against *M. tuberculosis* as well as against the potentially pathogenic NTM, *M. kansasii*, *M. xenopi*, *M. fortuitum*, and *M. marinum*. While most *Af. intracellulare* serotypes of the *M. avium* complex were inhibited by Ofloxacin, 'avium' serotypes were generally resistant as were most strains of *M. chelonae*. In 1985, Tsukamura²⁴ reported that the drug might be beneficial in the treatment of drug resistant tuberculosis because no cross resistance between Ofloxacin and other antituberculosis drugs was seen *in vitro*. Spontaneous resistance to Ofloxacin appeared to occur in about 1 in 10⁵ organisms, a proportion similar to that for other drugs. It showed considerable bactericidal activity at concentrations a little higher than the MIC. The combined effect with other antituberculosis agents seemed to be additive. The development of resistance to DL 8280 was of an obligatory 2 step pattern. There are 2 phenotypes. The resistance levels of these phenotypes are 5 mg/l and 100 mg/l respectively. There was no resistance beyond 100 mg/l.

Davies et al²⁵ reported in 1987 on the comparative *in vitro* efficacy of Ofloxacin,

Ciprofloxacin, Pefloxacin, Enoxacin and Norfloxacin, on 50 *Af. tuberculosis* strains. The authors concluded that since Ofloxacin and Ciprofloxacin were shown to have the highest *in vitro* activity against mycobacteria, these 2 drugs could be used in the treatment of *Af. tuberculosis* resistant to standard drugs. However, in these studies only *M. tuberculosis* strains sensitive to antituberculosis drugs were tested. A study concluded recently at TRC shows that there is no difference in the activity of Ciprofloxacin and Ofloxacin in drug resistant and susceptible strains.²⁶ The geometric mean MICs were also similar for both of these drugs for both categories of strains tested. None of the strains showed an MIC > 4 mg/l in LJ slopes incorporated with the drugs. Since the mean MIC of Ofloxacin is far below the peak serum level of 10.7 mg/l attainable in normal dosage, this drug may have a role in the treatment of drug resistant tuberculosis.

A few clinical studies have been carried out with Ofloxacin. Tsukamura²⁷ administered Ofloxacin in a daily dose of 300 mg in combination with other drugs for 6-8 months to 19 tuberculosis patients who had failed on conventional therapy and had organisms resistant to most of the commonly used antituberculosis drugs. The patients had been previously treated for tuberculosis for an average of 16 years; all had Isoniazid resistant bacilli, and with one exception had organisms resistant to Rifampicin as well. Ofloxacin had a demonstrable effect in decreasing the number of viable *Af. tuberculosis* organisms in these patients. Moreover, the fact that 12 patients acquired *in vitro* resistance to Ofloxacin and 4 of these 12 patients had culture conversion indicated drug activity. Plasma concentrations in excess of the MICs were generally achieved. No serious toxic effects were shown among this small group of patients.

In the uncontrolled study of Ofloxacin in the retreatment of patients with pulmonary tuberculosis resistant to SHR reported by Hong Kong Chest Service/BMRC,²¹ of a total of 17 patients who had shown no evidence of a sustained benefit with Rifampicin or Rifabutin and subsequently retreated with Ofloxacin, 10 showed a response, disease becoming and remaining quiescent in 3. Ofloxacin appeared to be a better drug to use, in combination with any companion drugs still available.

Ciprofloxacin

Marinis and Legakis²⁷ reported that Ciprofloxacin was active against all strains of *M. tuberculosis* sensitive to S, H, R, and Emb and inhibited almost all strains showing intermediate sensitivity or resistance to one or more of the above agents. Nearly all isolates were inhibited at a concentration of 3.2 mg/l. The same phenomenon was also observed with atypical isolates.

An investigation conducted at TRC on 53 isolates of *M. tuberculosis* sensitive to SHR and 54 isolates resistant to SHR/HR also revealed more or less similar findings²⁹. The percentage distribution of the MICs with the different categories of strains was similar, there being no difference between sensitive and resistant strains, the geometric means being 17 and 3.8 mg/l, respectively.

The expected mean levels of Ciprofloxacin in plasma after oral doses of 250 and 500 mg are 1.45 and 2.0 mg/l respectively. However, the drug may achieve levels in pulmonary tissue in excess of those in serum. In that respect and in the light of the present results, the achievable Ciprofloxacin level is expected to inhibit almost all of the clinically important species of mycobacteria including those showing resistance to one or more of the primary antimycobacterial agents.²⁸

Lomefloxacin

Lomefloxacin is a new difluoroquinolone that has the additional advantage of having a relatively long half-life (7-8 h).³⁰ In a study carried out by Piersimoni et al,³¹ the MICs of Ciprofloxacin, Ofloxacin and Lomefloxacin were determined for 90 *M. tuberculosis* strains isolated from both AIDS and other patients, including 11 (2.2%) which showed *in vitro* resistance to one or more first line antituberculosis drugs. The MIC range for Ciprofloxacin was 0.125 to 4.0 mg/l; for Ofloxacin, 0.25 to 4.0; and for Lomefloxacin 0.5 to 4.0 mg/l. On the basis of these data and also based on studies by Chen et al,³² the authors proposed the MIC of 1.0 mg/l as susceptible break point for both Ciprofloxacin and Ofloxacin and 2 mg/l for Lomefloxacin. These MICs are below the peak concentrations of the drugs in human

serum, that is, 10.7 mg/l for Ofloxacin, 2.9 mg/l for Ciprofloxacin and 4.9 mg/l for Lomefloxacin. These peak concentrations were attained after single oral doses of 600, 1000 and 400 mg respectively. The authors felt that although none of these quinolones had been approved for use against *M. tuberculosis*, combination therapy will probably be recommended for fluoroquinolones as for other antimycobacterial drugs. Lomefloxacin, because of its pharmacokinetic property (long serum elimination half-life) should merit further evaluation as a potential supplementary drug for the intermittent treatment of tuberculosis.

Sparfloxacin

Sparfloxacin is a new difluorinated quinolone with *in vitro* activity and *in vivo* efficacy equal to or better than Ofloxacin and Ciprofloxacin. In a study carried out by Rastogi and Goh, comparison of bactericidal action with reported serum peak concentration has shown that Sparfloxacin has a potential for use against the tubercle bacillus and on 10 strains of *M. tuberculosis*, the MICs by 7H12 broth testing ranged from 0.5 to 1.0, 0.25 to 0.5, and 0.1 to 0.2 mg/l for Ofloxacin, Ciprofloxacin and Sparfloxacin, respectively, whereas MICs in solid medium ranged from 0.5 to 1.0, 0.5 to 1.0, and 0.2 to 0.5 mg/l, respectively.³³

Combination of beta lactam agents and beta lactamase inhibitors

Mycobacteria produce beta lactamase and are resistant to beta Lctam antibiotics. Beta lactamase stable penicillins like Dicloxacillin do not have sufficient intrinsic activity against *M. tuberculosis*. Recently, a group of beta lactam drugs have been produced which, although devoid of antibacterial activity, are potent inhibitors of beta lactamase. The addition of one such inhibitor, Clavulanic Acid, increased the antimycobacterial activity of Amoxycillin/Ampicillin.² The best combination was found to be Ampicillin and Clavulanic Acid in 1 : 1 ratio which gave a MIC 90 value of 11 mg/l. *In vitro* studies have also shown that augmentin (Amoxycillin and Clavulanic Acid) inhibits and kills most strains of *M. tuberculosis* at a concentration of 4-8 mg/l of Amoxycillin and 2-4 mg/l of Clavulanic Acid. Another good combination was Ticarcillin and

Clavulanic acid, which inhibited all strains of *M. tuberculosis* at <32 mg/l, a clinically achievable serum concentration³⁴. Ampicillin/sulbactam combination has been found to have MIC values of 8/8 gm/l in 13 *M. tuberculosis* strains³⁵.

Tuberactinomycin (Tuberactin, Enviomycin)

Tuberactinomycin is elaborated by strain *Streptomyces griseovorticillatus* var *tuberacticus*. This water soluble drug resembles structurally Viomycin. The mechanism of action is surmised to be similar to that of Viomycin, i.e. inhibition of protein synthesis³⁶.

In a study carried out at TRC, cross-resistance was not observed between Tuberactin and Streptomycin, H, Emb, R and Ethionamide.³⁷ However, 15 (54%) of 28 Kanamycin-resistant strains were not susceptible to Tuberactinomycin at 25 mg/l.

Clinical studies with Tuberactinomycin containing regimens have been conducted in Japan³⁸. The rate of sputum conversion by culture after 6 months ranged from 73% to 80% in the Tuberactin regimens compared to 63% in a similar Viomycin containing regimen. In advanced cases, this ranged from 67% to 76% in the Tuberactin regimens compared to 59% in the Viomycin regimen. Thus Tuberactin was better than Viomycin and daily administered regimen was better than biweekly administered regimen.

Amikacin and Capreomycin

The aminoglycoside antibiotic Amikacin is a semisynthetic derivative of Kanamycin A. It has been reported to inhibit *M. tuberculosis* at a concentration lower than that for Kanamycin or Streptomycin and to be more active than either of them in experimental murine tuberculosis and also to be active in experimental tuberculosis in the guinea pig.

In a study carried out by Hoffner and Kallenius,³⁹ out of a total of 585 *M. tuberculosis* strains isolated during a 3-year period in Sweden, resistance to S was seen in 27 (4.6%) isolates. All but one of the S resistant isolates were susceptible to Amikacin and none of the 263 S susceptible isolates tested was resistant to Amikacin. From these results, Amikacin appeared to be an

alternative to S in the treatment of patients with S resistant *M. tuberculosis*.

Earlier, Alien et al⁴⁰ had reported the results of treating 4 patients with Amikacin, each of whom had a long history of previous chemotherapy and had multiple-resistant organisms. The activity of Amikacin was very low, although emergence of resistance indicated that it had some activity. Amikacin was no more active than Kanamycin. Since Amikacin is considerably more expensive than Kanamycin and there appeared to be complete cross resistance between the 2 antibiotics, they had concluded that there was probably no place for its use in the chemotherapy of tuberculosis.

Capreomycin is a polypeptide antibiotic produced by *Streptomyces* spp and has the same pharmacokinetics and toxicities as the other aminoglycosides. It is no more effective than Streptomycin and has an incomplete cross-resistance with Amikacin and Kanamycin.⁴¹

Clarithromycin

Clarithromycin, a newer erythromycin derivative, has been shown to be highly active against multiple drug resistant MAC organisms, besides having promising activity against various, other potentially pathogenic NTM including *M. paratuberculosis**². It has been shown to cause a reduction in the bacillary load and clinical improvement of *M. avium* disease in AIDS patients.⁴¹

Conclusions

From the available information it is clear that among the newer drugs which have antituberculosis activity and are promising, Rifampentine shows extensive cross reaction with Rifampicin, and Rifabutn shows lower MIC values. In only about 10-30% *M. tuberculosis* strains resistant to Rifampicin. Only the fluoroquinolones do not show cross resistance with Rifampicin. The other drugs like Amikacin, Capreomycin and beta lactam antibiotics may not have any additional benefits or have not yet been evaluated fully.

In India, under* the National Tuberculosis Programme, SCC is being implemented at present in about 250 districts. As a result, Rifampicin is

being used freely in these districts in the primary chemotherapy of sputum positive pulmonary tuberculosis patients under programme conditions: A large number of patients who do not seek treatment in government institutions get treated by private practitioners and also by other agencies, often with Rifampicin in addition to other drugs. A similar situation prevails in most of the other developing countries also. In these places, the prognosis for patients harbouring sensitive *M. tuberculosis* strains and also those who harbour *M. tuberculosis* strains with initial drug resistance to S/H will be good if the patients are regular and complete the full course of treatment. However, under programme conditions only about 50% of patients under SCC and about half of them under standard chemotherapy complete the full course of treatment. In respect of patients harbouring strains with initial resistance to Rifampicin, the prognosis is even more bleak. The problem gets more complicated if these patients are also infected with HIV.

The treatment of tuberculosis in immunosuppressed patients is not well established. A regimen of Rifampicin, Isoniazid and Ethambutol for 6 months, supplemented with Pyrazinamide during the first 2 months has been reported to be quite effective and able to bring about sputum conversion in more than 80% after 3 months,⁴³ although relapse has been reported⁴⁴ and adverse reactions to antituberculosis drugs are frequent.⁴⁵ It has been reported that corticosteroids added to the antituberculosis chemotherapy give dramatic clinical improvement.⁴⁶ It is also known that patients can tolerate concurrent therapy with Azidothymidine and antimycobacterial drugs without unacceptable toxicity.⁴⁷ Currently, the American Thoracic Society and CDC⁴⁸ recommend that antituberculosis chemotherapy should be started whenever acid-fast bacilli are found in a specimen from a patient with AIDS or suspected HIV infection. The treatment should be with H, R, and Z, and should be continued for a minimum of 9 months and for at least 6 months after culture conversion. For any person, regardless of age, who has a positive tuberculin test reaction and is HIV seropositive, preventive therapy with H is recommended. Studies have to be undertaken to see if HIV infected individuals treated for tuberculosis, in whom subsequent immunosuppression may lead to relapse, would

benefit from continued administration of one or more antituberculosis drugs.⁴⁹ The ideal duration of treatment remains to be determined because in a recent study with a longer duration of follow-up, 6% of the patients who completed the 1 year treatment had relapse.⁵⁰ In this study, relapse was frequent in cases of poor adherence with premature discontinuation of treatment. In India, the guidelines for treating tuberculosis in HIV positive persons and chemoprophylaxis using Isoniazid alone or in combination with other drugs have to be worked out.

In essence, the time has come for rational thinking and judicious use of the available alternative drugs in the treatment of failure patients. It is even more important to acknowledge the fact that at present there is no better substitute than regular treatment with the currently available antituberculosis drugs and regimens.

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PREVALENCE OF PULMONARY TUBERCULOSIS IN A PERI-URBAN COMMUNITY OF BANGALORE UNDER VARIOUS METHODS OF POPULATION SCREENING

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Summary. The role of Initial screening of population methods for bacteriological examination in tuberculosis case-prevalence surveys was studied in a population of 56,293 around Bangalore City. Symptom questioning by sociological investigators (SQCS) general health workers (FHW) and Mass Miniature Radiography (MMR) Were used as the screening methods, independently of each other. Person found eligible for testing of the basis of the respective screening methods were subjected to smear end culture examination of sputum. It was found that FHWs had identified the same proportion of persons with general symptoms as well as chest symptomatics among the population as the SOCS, and that prevalence rates of culture as well as smear positives cases were about the same by any of the three methods.

Prevalence rates of smear positives cases obtained through symptom questioning, either by SOCS or FHWs, were more or less similar to the estimate obtained by the more comprehensive screening method of MMR and/or symptom questioning. The culture positive prevalence rate following MMR screening only was 0.25%, which was lower than the rates observed in other surveys. The paper discusses the possible hypothesis that could explain the observation. It also presents correction factors to compute rates comparable to the best estimate i.e., that obtained through comprehensive screening by MMR and/or symptom questioning, followed by sputum culture.

Radiography (MMR) followed by sputum culture of the X-ray abnormal is the customary method for arriving, at the prevalence rate of bacteriologically positive cases in a community.¹² Some State Tuberculosis Centres (STCs) may be interested in carrying out tuberculosis prevalence surveys in their areas, in order to evaluate the effect of antituberculosis measures implemented by them, but have neither a mobile MMR unit nor culture facilities. Mobile MMR unit, in any case, is costly equipment which is difficult to maintain and operate. Therefore, simpler means of screening the population and establishing the diagnosis by smear examination of sputum only, as alternative survey methods, because of their applicability, have to be investigated.

A previous study had shown that the method of screening the population, for sputum test, had influenced the estimate of the case prevalence rate.³ Therefore, adjustments in rates had to be made for the variation in screening methods in order to render the prevalence rates comparable.⁴ In that study, screening of the population was carried out through questioning for chest symptoms by trained social workers, besides the MMR.³ It is possible that services of trained social workers may be difficult to obtain. It is, therefore, necessary to investigate whether symptom questioning by general health workers could be used and the prevalence rates obtained could be compared with those arrived at on the basis of symptom screening by trained social workers.

The objectives of the present investigation are to study the prevalence of:

- (i) chest symptomatics, as identified by general health workers compared to that by trained

Introduction

Screening of the population by Mass Miniature

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- social workers, and of
- (ii) bacillary cases, as obtained on screening of population through identification of chest symptomatics by social workers or general health workers compared to that by MMR.

Methodology

Sampling

The area selected for the study was the five kilometre wide belt beyond a radius of 19 kilometres drawn on the map from the centre of Bangalore City. For the purpose of this study, it is termed as "peri-urban area" because of the predetermined geographical proximity and consequent socio-economic association with the city centre. Villages in the selected belt could be different as a class from the Bangalore district rural area in general. All the villages in the area were listed from the census book. The selection of 60 representative villages was carried out on the basis of a simple random sample.

Field Work

Thirty of the villages selected at random were to be covered by social investigators (SOCS) and the other 30 by general health workers customarily engaged in population enumeration for the epidemiological studies of the Institute (FHW). A mobile 70 mm photofluorographic unit (MMR) was additionally used to screen the population in all the 60 villages.

Each village was visited in advance and the village leaders were contacted. On the appointed day, the team set up an examination centre for work in the afternoon/evening. All the persons were registered by the Census Takers by making house to house visits, irrespective of whether the village was allotted to SOCS or FHW for symptom questioning.

Following registration, the census cards for villages listed for symptom questioning by SOCS (symptom screening-SOCS) were passed on to the SOCS workers, who went from house to house and questioned persons aged 15 years and over on the presence of cardinal chest symptoms (C.S.) of any duration namely, cough, pain in chest, fever and haemoptysis. Presence of other symptoms (O.S.) was also recorded. Prior to actual questioning, rapport with the interviewee was

established by employing sociological field techniques. A memory recall period of two months was allowed for recording the symptoms. Leading questions were put regarding duration of symptoms, if not volunteered. From persons having cough, chest pain and fever of two weeks and more duration or haemoptysis on any occasion (C.S.2), one specimen of sputum was collected. The cards of persons interviewed were then passed on to the MMR team.

Similarly, for the villages earmarked for symptom questioning by FHW (symptom screening - FHW), the cards of registered persons were passed on to the FHW team members who went from house to house identifying symptomatics by simple direct questioning and collecting one specimen of sputum from those identified as C.S.2. All the cards were subsequently passed on to the MMR team.

The registered persons aged 15 years and more in all the 60 villages were then subjected to a 70 mm photofluorography examination by the MMR team (MMR screening). Screening of individuals by any method was, therefore, independent of other methods. From persons having photofluorographic abnormality, as interpreted by any of the two readers independently, a specimen of sputum was collected, in case it had not been done earlier on the basis of symptom identification either by SOCS or FHW. All the sputum specimens were subjected to smear and culture examination for *M. tuberculosis*.

Field work for the study was conducted between August 1986 and October 1989.

Definitions

Peri-Urban Area

See under 'Sampling'.

Screening

The process of subjecting the entire community to a standard prior examination for identifying the eligibles for sputum examination (e.g., MMR, symptom questioning, etc.).

Case

Person having abnormality on MMR or identified as C.S.2, (by SOCS or FHW) and in whom *M. tuberculosis* was grown on culture of sputum.

Table 1. Age-sex distribution of registered population

Age group	Registered persons			Percentage of registered to total Population		
	Male	Female	Both Sexes	Male	Female	Both sexes
0-14	10541	10099	20640	36.2	37.1	36.7
15-24	5822	5537	11359	20.0	20.4	20.2
25-34	4400	3988	8388	15.1	14.7	14.9
35-44	3121	2646	5767	10.7	9.7	10.2
45-54	2414	2289	4703	8.3	8.4	8.4
55+	2809	2627	5436	9.7	9.7	9.7
Total	29107	27186	56293	100.0	100.0	100.0
≥15	18566	17087	35653	63.8	62.9	63.3

Smear Positive Case

Any culture positive case who was also smear positive.

Best Estimate

The prevalence rate of cases arrived at by combined screening of the entire population by any of the two screening methods (MMR and/or symptom questioning) and subjecting those eligible to sputum culture examination.

Study Population

In all, 56,293 permanent residents were registered in 60 villages (Table 1). Of them, 35,653 (63.3%) being 15 years and over in age, formed the study population. Females constituted 47.9% of the latter.

Coverage

In the 30 villages under symptom screening by SOCS, 15,260 persons and in 30 FHW villages, 14,881 persons were interviewed. Of the 35,653 person registered in all the 60 villages, 84.5% could be interviewed (Table 2). The coverage by MMR screening was comparatively less, being 76.9% only.

Of the persons eligible for sputum

examination, the tests were carried out in 94.5%, 90.4% and 84.4% for villages under SOCS, FHW and MMR screening respectively (not on Table).

Findings*A. Symptoms**SOCS Screening*

In the villages under symptom screening by SOCS, 15,206 of the 15,260 persons were satisfactorily interviewed (99.6%); 62% of them were found to have no symptom at all, 17.7% had chest symptoms of any duration (C.S.) and 20.3% had symptoms pertaining to any other system (O.S.) (Table 3). Of the 2,697 persons found to have C.S., 2,106 (78.1%) had chest symptoms for two weeks or more or haemoptysis (C.S.2).

FHW Screening

For symptom screening by FHW, 2,520 of the 14,756 persons satisfactorily interviewed had C.S. (17.1%). Of the C.S., 2,146 (85.2%) had C.S.2 (Table 3). The prevalence rates of C.S. as well as C.S.2, in persons aged 15 years and over, as identified by symptom screening (SOCS), were similar to those on symptom screening (FHW).

Symptoms Related to Age

Of persons satisfactorily interviewed by SOCS,

Table 2. Population covered under two independent screening methods

Age group	Interviewed by SOCS ¹			Interviewed by FHW ²			Coverage by Interview by SOCS/FHW (%)	MMR		
	Male	Female	Total	Male	Female	Total		Male	Female	Total
15-24	2385	2504	4889	2198	2398	4596	9485 (83.5)	4337	4222	8559 (75.3)
25-34	1711	1751	3462	1524	1900	3424	6886 (82.1)	2991	3281	6272 (74.8)
35-44	1205	1239	2444	1190	1235	2425	4869 (84.4)	2243	2242	4485 (77.8)
45-54	986	1084	2070	954	1048	2002	4072 (86.6)	1822	1955	3777 (80.3)
55+	1248	1147	2395	1174	1260	2434	4829 (88.8)	2244	2063	4307 (79.2)
Total (15+)	7535	7725	15260	7040	7841	14881	30141 (84.5)	13637	13763	27400 (76.9)

¹SOCS - Social Investigators²FHW - Field Health Workers

Figures in brackets indicate percentage out of total registered in 60 villages in respective age group of Table 1.

Table 3. Symptoms among persons interviewed by SOCS and FHW, by age

Age group	SOCS						FHW			
	Satisfactorily interviewed ¹	Symptomatics			Satisfactorily interviewed ¹	Symptomatics				
		OK	OS	CS		CS \geq 2 Weeks	OK	OS	CS	CS \geq 2 Weeks
1	2	3	4	5	6	7	8	9	10	11
15-24	4871	3968	459	444	304	4543	3691	423	429	318
		(81.5)			(6.2)		(81.2)			(7.0)
25-34	3446	2414	500	532	415	3390	2458	400	532	445
		(70.1)			(12.0)		(72.5)			(13.1)
35-44	2439	1444	496	499	392	2406	1564	386	456	393
		(59.2)			(16.1)		(65.0)			(16.3)
45-54	2060	983	570	507	392	1991	1180	368	443	390
		(47.7)			(19.0)		(59.3)			(19.6)
55+	2390	619	1056	715	603	2426	1115	651	660	600
		(25.9)			(25.2)		(46.0)			(24.7)
Total	15206	9428	1081	2697	2106	14756	10008	2228	2520	2146
		(62.0)	(20.3)	(17.7)	(13.8)		(67.8)	(15.1)	(17.1)	(14.5)

OK - No symptoms

CS - Chest symptoms

OS - Other symptoms

1

Figures in brackets indicate percentage to number interviewed satisfactorily.

Table 4. Prevalence of Cases by different screening methods and by sex

Screening method	Males			Females			Both sexes		
	Examined	Cases	Smear + [@]	Examined	Cases	Smear + [@]	Examined	Cases	Smear + [@]
A. MMR- All Villages	13637	57 (0.42)	27 (0.20)	13763	12 (0.09)	4 (0.03)	27400	69 (0.25)	31 (0.11)
A ₁ MMR – SOCS Villages	7107	33 (0.46)	17 (0.24)	7017	5 (0.07)	1 (0.01)	14124	38 (0.27)	18 (0.13)
A ₂ MMR-FHW Villages	6530	24 (0.37)	10 (0.15)	6746	7 (0.10)	3 (0.04)	13276	31 (0.23)	13 (0.10)
B. Symptom and/or MMR (SOCS)	8436	35 (0.41)	17 (0.20)	8054	10 (0.12)	4 (0.05)	16490	45 (0.27)	21 (0.13)
C. Symptom and/or MMR (FHW)	8100	33 (0.41)	12 (0.15)	8184	10 (0.12)	4 (0.05)	16284	43 (0.26)	16 (0.10)
D. Symptoms (SOCS)	7535	20 (0.27)	13 (0.17)	7725	8 (0.10)	4 (0.05)	15260	28 (0.18)	17 (0.11)
E. Symptoms (FHW)	7040	26 (0.37)	11 (0.16)	7841	8 (0.10)	4 (0.05)	14881	34 (0.23)	15 (0.10)

Figures in brackets are prevalence rates.

[@] Smear positives out of Cases i.e. culture positives.

prevalence rate of C.S.2 rose with age, the highest being 25.2% in 55+ age group and the lowest 6.2% in 15 to 24 years age group (Table 3). The proportion of persons without any symptom pertaining to any system, was highest in 15 to 24 years age group (81.5%), declining with the rise in age to 25.9% in 55+ years aged population. Of the persons who had no symptoms, 6.6% were in age group 55+ and 42.1% in those aged 15 to 24 years. The proportion of C.S.2, in each age group as observed for SOCS and FHW was similar, as also the trend of rise with age.

B. Cases

The prevalence of culture positive cases (“cases” in Table) as detected by the various screening methods is shown in Table 4. Persons positive on smear only are excluded from this presentation, being very few in number.

By MMR Screening

By employing MMR screening, 57 cases were detected among 13,637 males (0.42%) but only 12

in 13,763 females (0.09%) examined ($r < 0.01$). The overall prevalence was 69 cases (0.25%).

By Symptom Screening (SOCS)

On symptom screening by SOCS, 20 cases were found among 7,535 males (0.27%) and another eight in 7,725 females (0.10%) examined ($p < 0.05$). The overall prevalence was 28 cases (0.18%).

By Symptom Screening

On symptom screening by FHWs, 26 cases were detected among 7,040 males (0.37%) and eight in 7,841 females (0.10%) examined ($p < 0.01$). The overall prevalence was 34 cases (0.23%).

By Multiple Screening

When eligibility for sputum examination by MMR and/or symptom screening (SOCS) was adopted, the prevalence rate in males was 0.41% and in females 0.12% ($p < 0.05$). The overall

prevalence rate of cases was 0.27%, (45 cases out of 16,490 covered). Similar prevalence rates were observed for MMR and/or symptom screening (FHW), and for each of the individual screening methods.

Prevalence of Smear Positive Cases

Of the respective cases, 31 were smear positive among MMR screening (44.9%), 17 among symptom screening-(SOCS) (60.7%) and 15 among symptom screening-(FHW) (44.1%) groups. The ratio between the smear positive cases to culture positive cases 1 : 2.3 for MMR or symptom screening (FHW) and 1 : 1.6 for symptom screening (SOCS). There was, however, no significant difference between these proportions. There was also no difference in the proportions of smear positive cases diagnosed either on MMR and/or symptom screening (SOCS) or on MMR and/or symptom screening (FHW) methods (46.7% and 37.2% of culture positive cases respectively).

The prevalence rates of smear positive persons, with or without culture confirmation, following

screening by any of the methods, were found to be similar to smear positive cases confirmed on culture, being in the range of 0.10% to 0.13%.

Age-wise Prevalence Rates

The prevalence rates of cases as well as their age-wise distribution in symptom screening (SOCS) groups was not different from that in symptom screening (FHW) or MMR groups (Table 5). There was significant increase in prevalence rates with increasing age, by any method.

C. Efficiency of Methods

Even though there was no difference in the prevalence rates of cases by each of the screening method individually, yet it was considered essential to study the performance of the different screening methods among the same persons examined by two screening methods. Such a measurement of efficiency of the methods is required for computation of correction factors in the estimates of prevalence rates obtained by each method.

Table 5. Age-wise prevalence of cases by different screening methods

Screening method	Age group	15-34		35-54		55+			
		Examd	Cases Smear+@	Examd	Cases Smear+@	Examd	Cases Smear+@		
A. MMR - All Villages	14831	13 (0.09)	9 (0.06)	8262	32 (0.39)	13 (0.16)	4307	24 (0.56)	9 (0.21)
A ₁ MMR - SOCS Villages	7724	7 (0.09)	6 (0.08)	4231	19 (0.45)	7 (0.17)	2169	12 (0.55)	5 (0.23)
A ₂ MMR - FHW villages	7107	6 (0.08)	3 (0.04)	4031	13 (0.32)	6 (0.15)	2138	12 (0.56)	4 (0.19)
B. Symptom and/or MMR (SOCS)	9084	11 (0.12)	7 (0.08)	4855	22 (0.45)	9 (0.19)	2551	12 (0.47)	5 (0.20)
C. Symptom and/or MMR (FHW)	8845	9 (0.10)	4 (0.05)	4840	16 (0.33)	7 (0.14)	2599	18 (0.69)	5 (0.19)
D. Symptoms (SOCS)	8351	9 (0.11)	6 (0.07)	4514	11 (0.24)	6 (0.13)	2395	8 (0.33)	5 (0.21)
E. Symptoms (FHW)	8020	7 (0.09)	4 (0.05)	4427	12 (0.27)	7 (0.16)	2434	15 (0.62)	4 (0.16)

Figures in brackets are prevalence rates.

@Smear positive out of cases i.e., culture positive.

Table 6. Prevalence of cases for SOCS and FHW interviewed villages with the concurrent use of two screening methods (MMR & Symptoms)

(x) Culture Positive Cases*

Age group	Examined by both MMR and SOCS interview (No.)	SOCS villages			Examined by both MMR and FHW interview (No.)	FHW villages		
		Cases diagnosed by				Cases diagnosed by		
		MMR and/or symp.	MMR alone	Symp. alone		MMR and/or symp.	MMR alone	Symp. alone
1	2	3	4	5	6	7	8	9
15-34	6991	9 (0.13)	7 (0.10)	7 (0.10)	6282	8 (0.13)	5 (0.08)	7 (0.11)
35-54	3890	19 (0.49)	18 (0.46)	9 (0.23)	3613	15 (0.41)	14 (0.39)	10 (0.28)
55+	2013	12 (0.60)	12 (0.60)	8 (0.40)	1973	13 (0.66)	12 (0.61)	10 (0.51)
Male	6206	33 (0.53)	32 (0.52)	19 (0.31)	5470	26 (0.48)	24 (0.44)	19 (0.35)
Female	6688	7 (0.10)	5 (0.07)	5 (0.07)	6403	10 (0.16)	7 (0.11)	8 (0.12)
All	12894	40 (0.31)	37 (0.29)	24 (0.19)	11873	36 (0.30)	31 (0.26)	27 (0.23)

(y) Smear Positive Cases

15-34	6991	6 (0.09)	6 (0.09)	5 (0.07)	6282	4 (0.06)	3 (0.05)	4 (0.06)
35-54	3890	7 (0.18)	6 (0.15)	5 (0.13)	3618	6 (0.17)	6 (0.17)	6 (0.17)
55+	2013	5 (0.25)	5 (0.25)	5 (0.25)	1973	4 (0.20)	4 (0.20)	3 (0.15)
Male	6206	16 (0.26)	16 (0.26)	13 (0.21)	5470	10 (0.18)	10 (0.18)	9 (0.16)
Female	6688	2 (0.03)	1 (0.01)	2 (0.03)	6403	4 (0.06)	3 (0.05)	4 (0.06)
All	12894	18 (0.14)	17 (0.13)	15 (0.12)	11873	14 (0.12)	13 (0.11)	13 (0.11)

* Irrespective of smear results.
(Percentage in brackets)

Table 6 presents the data for 12,894 persons examined by both MMR and symptom screening (SOCS) at the same time, as well as that of a separate group of 11,873 persons covered by both MMR and symptom screening (FHW) at the same time. The prevalence rate of cases was found to be 0.31% for the SOCS group of villages, when the persons were examined by the two screening methods at the same time. Compared to this, the respective prevalence rates were 0.19% and 0.29% by symptom screening (SOCS) only or by MMR only, considered separately. In the FHW group of villages, on the other hand, with symptom screening (FHW) or MMR considered separately, the prevalence rates were 0.23% and 0.26% respectively, compared with the prevalence rate of 0.30% obtained on applying MMR and symptom screening (FHW) concurrently. There was no difference in males or females for FHW or SOCS groups.

Of the 40 culture positive cases in the SOCS group of villages, detected cm sputum culture of all those eligible either by MMR and/or symptom questioning (SOCS), 24 could have been picked up following symptom questioning (SOCS) alone and 37 following MMR screening alone. The efficiency of the former was thus 60% compared to 92.5% for MMR (Table 6). In respect of 36 cases detected similarly in the FHW group of villages, 27 could have been picked up following symptom questioning (FHW) alone and 31 on account of MMR screening. The efficiency of the former method was 75%.

The proportions of smear positive cases among the total cases detected following symptom questioning (FHW or SOCS) were similar (13 smear positive cases detected by symptom screening (FHW) out of 36 cases by the best method in the FHW group; in comparison to 15 by symptom screening (SOCS) out of 40 cases in the SOCS group) (Table 6).

D. Correction Factors

Table 7 presents the prevalence rates obtained with the use of the best method compared to those by other methods, and the correction factors which could be applied to the latter, to obtain the best estimates. The prevalence- rate of culture positive cases following symptom screening (SOCS) could be multiplied by 1.63, to obtain the

best estimate. For symptom screening (FHW), the correction factor could be 1.30.

Discussion

The study was intended to provide data on the basis of which community surveys using simpler population screening methods and diagnostic tools, other than MMR and sputum culture, could be carried out to quantify the tuberculosis problem. The findings presented in this report appear to show that for screening by symptom questioning for estimating prevalence rate of the disease in the community, one need not involve highly trained social workers, as was done in an earlier study³. FHWs could identify the same proportion of chest symptomatics, with or without eliciting the stipulated duration of symptoms, as the SOCS could do. Further, the prevalence rates of culture positive as well as smear positive cases following symptom screening (FHW) were similar to those following symptom screening (SOCS). It should be pointed out, however, that each member of the FHW group in this study had long years of experience of working in community surveys and may have picked up the interview techniques on a par with the SOCS. It is also possible that the differences were not observed because fairly simple techniques were followed in this survey.

It could also be relevant to examine the reliability of diagnosis by smear examination only since culture examination facility may not be available every time, as there were only a few smear positive culture negative cases in this study.

Our findings may be of interest to those state level Tuberculosis Centres which may neither have MMR screening nor sputum culture facility, but would like to measure the extent of the problem in their area. However, it is to be kept in mind that even though dependence on MMR and culture can be substantially reduced by changing the method of survey, the workload in reaching and registering the population in villages would still remain unchanged. Moreover, sputum examination load following symptom questioning in the study was higher than that for MMR; 14% of the populations being eligible for sputum examination by symptom questioning (either SOCS or FHW) compared with only 4% when MMR screening was employed.

In an earlier paper, influence of age and sex on

Table 7. Correction factors to compute prevalence rate of cases by best method of screening the population (MMR and/or screening) from rates arrived at through symptom screening (FHW or SOCS)

Age group	Correction factors			
	SOCS villages		FHW villages	
	Culture positive ^a	Smear positive ^b	Culture positive ^c	Smear positive ^d
1	2	3	4	5
15-34	1.30	1.86 (1.29)	1.18	2.17 (1.00)
35-54	2.13	3.77 (1.38)	1.46	2.41 (1.33)
55+	1.50	2.40 (1.00)	1.29	4.40 (1.33)
Male	1.71	2.52 (1.24)	1.37	3.00 (1.13)
Female	1.43	3.33 (1.00)	1.33	2.67 (1.00)
All	1.63	2.58 (1.17)	1.30	2.73 (1.09)

Note : 1. Correction factors computed as under from rates given in columns of Tables 6 (x) and (y).

- a) 3/5 of Table 6 (x)
- b) 3 of Table 6 (x)
5 of Table 6 (y)
- c) 7/9 of Table 6 (x)
- d) 7 of Table 6 (x)
9 of Table 6 (y)

2. Figures in brackets are correction factors to estimate smear positive cases diagnosed through screening of population by MMR and/or symptom concurrently (either SOCS or FHW) computed as under:

Figures in brackets of Col. (3), estimates based on Col. 3/5 of Table 6 (y)

Figures in brackets of Col. (5), estimates based on Col. 7/9 of Table 6 (y).

the efficiency of screening by different methods was suggested for further study³. It was indicated that age-sex correction factors could be worked out depending on comparability of the test methods with the best possible one. Such correction factors are now made available by the results of this study. Data from the same population subjected concurrently to two screening methods were analysed to achieve the desired comparability. The efficiency of the

estimate arrived at following one method of screening was judged against the best estimate arrived at by screening the same persons by both the methods.

From the computed correction factors shown in Table 7, it could be observed that among persons screened both by symptom questioning (FHW) and MMR, as well as symptom questioning (SOCS) and MMR, the overall prevalence rates obtained on smear examination could be

multiplied by 2.73 and 2.58 respectively to obtain the best estimates of cases in the community, (i.e. through screening by MMR and/or symptom screening, followed by culture). The culture positive case rate obtained by symptom screening (FHW), however, could be multiplied by 1.30 to obtain the best estimate and by 1.63 to obtain the same in the case of symptom screening (SOCS).

It could be seen from Table 6, that for smear positive cases, the prevalence rates obtained through any of the two symptom screening methods and in each of the age groups studied, both sexes, and overall, showed considerable similarity irrespective of the screening method used. Hence, the observed smear positive prevalence rates need not be corrected for comparability, irrespective of the method used (Table 7). The finding is in line with the hypothesis that smear positive cases are more likely to get picked up by any method of screening than the culture positive cases.

Epidemiological Situation

The culture positive prevalence rate of cases, on MMR screening of villages under SOCS and FHW combined was 0.25%, the interval estimates for the 95% confidence limits being 0.19% to 0.31%. This rate appears to be lower than most estimates of case prevalence rates obtained in other surveys². This could be due to the fact that only a single specimen of sputum was collected in this study. It is known that out of cases found on culture of two specimens of sputum, only 80% are detected on examination of the first specimen⁵. Correcting the estimate after making allowance for the said methodological variation, the prevalence rates in 15 years and over population would be as 0.34% (interval estimates 0.27% to 0.42%). It is, however, still lower than the prevalence rate of 0.56% in same age group, at I and IV surveys in Bangalore rural areas in 1961 and 1968², (interval estimates not presented in the published reports but calculated for this paper : Survey I : 0.57% (0.48 to 0.65) : Survey IV : 0.56% (0.54 to 0.59).

The case prevalence rates in urban and rural populations, in various areas in India were found to be similar in the survey carried out by the Indian Council of Medical Research¹. No time

trend rates in urban and rural populations have been studied so far. For the past 24 years, however, the National Tuberculosis Institute, Bangalore, has been carrying out a series of tuberculosis prevalence surveys in the rural areas of Bangalore district to understand the natural dynamics of tuberculosis^{2,6,7}. According to Grigg, the hypothetical time trend curves of tuberculosis in the urban and rural areas may intersect and when the trend is declining in urban areas, it could be ascending in rural areas⁸. **In other words, notwithstanding the similar disease rates observed at one point in urban and rural population groups¹, the two curves could still represent different epidemiological time trends. Therefore, the hypothesis that the epidemiological time trend of tuberculosis in Bangalore city and its adjoining areas (peri-urban) could be different from that in the rural areas deserves to be studied over a period of time.** In view of this, the results from the present study may be of some significance in providing data for constructing the epidemiological situation model for the area, supplemented by information on the risk of infection.

It is necessary here to stress that the villages selected in the study had a predetermined association with the city centre in terms of the fixed geographical proximity. Even though, in the absence of socio-economic data, the area could not be classified as urban, the villages were considered hypothetically different from the remaining rural areas of the district, studied earlier^{2,6,7}. Hence, this sample of villages was designated as representing a peri-urban belt. It becomes interesting to note, then, that the prevalence rates of cases were lower in the study area than in the rural samples in the adjoining taluks of the same district^{2,7}, despite the industrial growth during the last two decades in and around Bangalore City.

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E. COLI REACTIVE ANTIBODY IN HUMAN TUBERCULOSIS SERA

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Summary. Using ELISA technique, we could demonstrate the presence of two Independent antibody sub-sets in a number of serum specimens from TB patients and healthy volunteers with respect to their immunoreactivity to the antigens of *M. tuberculosis* or *E. coli*. The titers of these antibodies varied over a wide range in individual serum specimens : TB sera contained significantly elevated levels of anti-mycobacterial antibody than the control sera. No such difference could be demonstrated in the titers of anti-*E. coli* Antibody between these two group. No significant correlation was found between the titers of anti-mycobacterial and anti-*E. coli* antibodies in TB serum specimens. In competition inhibition assays, *E. coli* antigens did not compete with mycobacterial antigens for anti-mycobacterial antibody in TB -sera. However, a poor competition was manifested between mycobacterial and *E. coli* antigens if the antibody were derived from circulating immune complexes (CIC) and not from serum. Our results suggested that the two antibody types present in the sera of TB patients may have been generated in response to two co-existing infections, and are independent of each other. The significance of the presence of such antibody types with respect to pathogenesis and immunodiagnosis has been discussed.

reactive or simply co-existing; if cross reactive, whether it can influence and modify the immunopathological events of tuberculosis; whether it can interfere with the immunodiagnosis of tuberculosis, and so on. Though several workers have noted the presence of such an antibody in TB sera and immune sera,^{4,5,6,7} little attention has been paid to resolve these questions.⁸ As pointed out by Grange, attempts to study the influence of other infections on the course of tuberculosis have been few, using lymphocytes and, more so, using antibody.⁸ Our studies attempted to analyze the nature of immunoreactivity of TB serum antibodies as it might be helpful in resolving the nature of the antigenic stimuli that resulted in their generation. We selected *E. coli* for our studies, as of late, there have been several reports on antigen sharing between mycobacteria and enterobacteria, especially *E. coli*.^{3,9,10,11,12}

Material and Methods

Serum specimens : TB patients diagnosed by attending physicians at the OPD of Lala Ram Samp TB hospital, New Delhi (Courtesy : Dr R. Sarin) and randomly selected for this study were all fresh cases including both sputum positive and negative cases as well as females and males. Blood specimens collected from the students of School of Life Sciences, Jawaharlal Nehru University, New Delhi, were used as normal controls. The specimens were collected by venipuncture and sera were prepared according to a standard protocol.¹³

Antigen preparation : The cell extract of *M. tuberculosis* (H37Ra) cultivated on Sauton's medium was prepared by repeated cycles of freezing and thawing, followed by sonication and ultra centrifugation.¹⁴ *E. coli* (k 12) was cultured in Luria broth (Humedla) overnight at 30°C with

Introduction

Mycobacteria share antigens not only among themselves, but also with several unrelated organisms,^{1,2,3} Serum specimens of tuberculosis (TB) patients contain antibody which react with several of these organisms. Presence of such an antibody in TB sera may raise some important questions on the nature of the antigenic stimulus that causes its generation; whether it is cross-

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constant stirring. Cells were harvested (3,000 rpm/20 minutes), washed and sonicated in the presence of phenyl-methyl-sulphonyl fluoride. Antigen was prepared as described.¹⁴ The protein content of antigen preparations was determined by protein-dye-binding assay.¹⁵

Isolation of Antibody from Circulating Immune Complexes (CIC antibody) : CIC from TB sera were isolated using 2.4% PEG.¹⁴ To release the bound antibody from the complexes, the CIC were treated with 0.1 M glycine-HCl buffer (pH 2.8) for 20 minutes at room temperature. After incubation, the pH of each specimen was neutralized with 1M K₂HPO₄ solution. The final volume of the specimen was usually the same as that of the original serum from which CIC were isolated. These preparations were used as a source of CIC antibody in the experiments.

Enzyme Linked Immunosorbent Assay (ELISA): The protocol of ELISA¹⁶ was briefly as follows : the assay wells were coated with antigen (lug/well) and blocked with 3% BSA. The diluted serum or antibody preparations were added to wells, incubated and washed, followed by incubation of the wells with rabbit anti-human antibody coupled to horse radish peroxidase. After washing, substrate solution was added to the wells and enzyme reaction was terminated after appropriate period of incubation. Absorbance values were scored and normalized by comparing with the standard serum control that was included in all the experiments.

Competition Inhibition Assay : Aliquots of appropriate dilutions of TB sera or the CIC antibody were preincubated with different concentrations of *E. coli* or *M. tuberculosis* sonicate extracts for 20 minutes at room temperature in the presence of a mixture of 1%

BSA (Sigma) and 5% fetal calf serum (Seralab). After incubation, the specimens were added to appropriate wells that were previously sensitized with antigen and blocked. The plates were incubated for 1 hour at 37°C and the levels of antibody binding to the coated antigen were determined in ELISA, as described above.

Results

Antibody titers in TB sera

Anti - *E. coli* and anti - *M. tuberculosis* (H37Ra) antibody titers in each of 25 TB serum specimens were estimated by using ELISA. The titers of individual sera were plotted against each other (Figure 1). All the TB sera were found to have antibody that reacted with *E. coli*. However, the titers varied from patient to patient, over a wide range, both in absolute terms and in relation to anti-mycobacterial titers. Certain TB sera reacted stronger to *E. coli* than to *M. tuberculosis*. No significant correlation was found between the titers of these two antibody subsets ($r = 0.095$). To see whether anti *E. coli* antibody could also be detected in the sera of normal subjects, and if so, whether these titers would differ from those of TB sera, we estimated anti-*E. coli* and anti-mycobacterial antibody titers in 15 TB and 15 normal sera, using ELISA. The results have been presented in Table 1. TB and control groups differed significantly from each other with respect to anti-mycobacterial antibody ($p < 0.0005$) but not to anti *E. coli* antibody.

Competition Inhibition Assays

The nature of immunoreactivity of the two subsets of antibodies found in TB and normal sera

Table 1. Relative anti-*M. tuberculosis* and anti-*E. coli* antibody titers in TB and normal human sera^a

S. No.	Group	Antigen coated in ELISA wells	
		<i>M. tuberculosis</i>	<i>E. coli</i>
1.	Tuberculosis	0.740 ± 0.290	0.572 ± 0.339
2.	Normal	0.259 ± 0.043 ^b	0.646 ± 0.306 ^c

^aAnti-*M. tuberculosis* and anti-*E. coli* antibody titers of 15 tuberculosis and normal sera each were estimated using ELISA. The figures in the table represent the mean ELISA absorbance ± standard deviation values.

^bAnti-*M. tuberculosis* titers in TB and normal group are significantly different by Student's t-test ($P < 0.0005$).

^c Anti-*E. coli* titers between these two groups did not differ significantly.

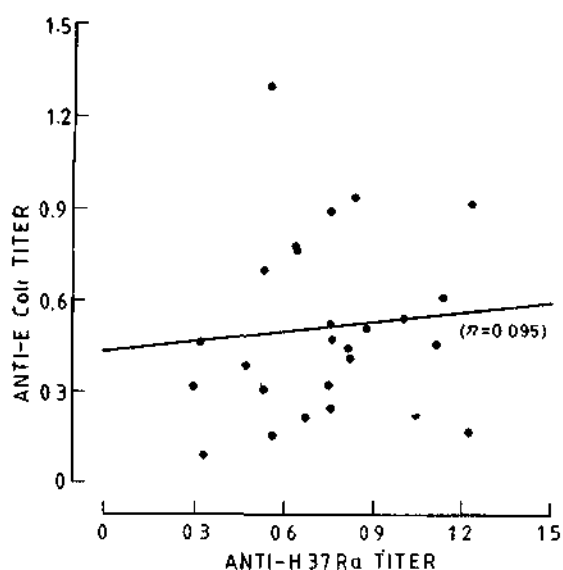


Fig. 1 Relative anti-H37Ra and anti-*E. coli* antibody titers in 25 TB sera: Each data point represents an individual TB serum and respective titers of individual sera are plotted against each other. The regression curve is drawn according to the principle of least squares.

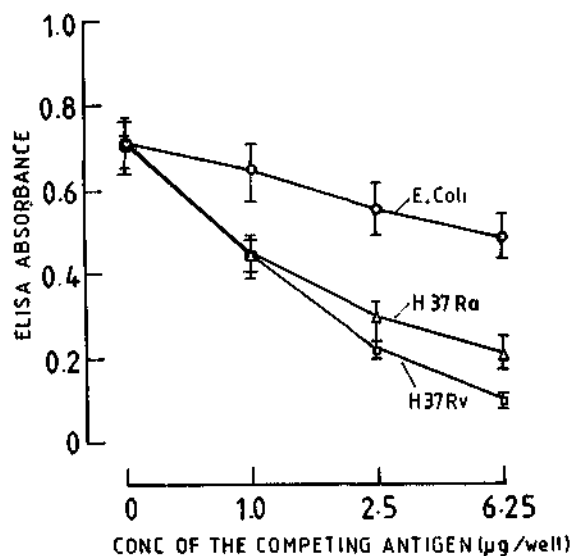


Fig. 3 Competitive inhibition of CIC antibody binding to coated H37Ra sonicate antigens by the antigen preparation of *E. coli*, H37Ra and H37Rv: The mean absorbance values of TB sera were calculated at each competing antigen concentration and plotted against the competing antigen concentrations. The ranges of standard errors of mean have also been shown.

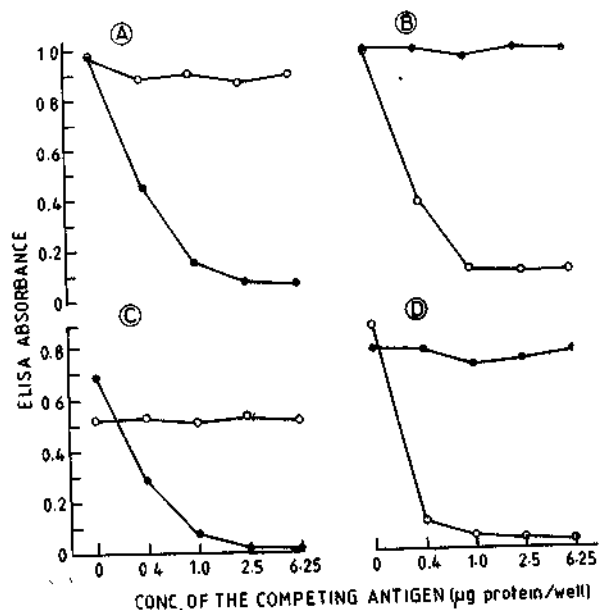


Fig. 2 Competitive inhibition of serum antibody binding to coated antigens by homologous antigen preparations: concentrations of H37Ra (closed circles) or *E. coli* (open circles) sonic extracts and levels of inhibition in antibody binding to coated antigens as a result of competition are shown. Each data point represents the mean of three replicate assay wells.

was analyzed in competition inhibition assays. Five TB and five control sera were used for this purpose. The results obtained with all the sera were identical and the representative results of one TB and one control serum are shown in Figure 2. The results demonstrate the specific nature of immunoreactivity of either antibody subset, both in TB and control sera. Only homologous antigen preparations could inhibit the binding of one subset of antibody to the corresponding antigen. We could also show a progressive inhibition of antibody binding to the immobilized antigens with the increasing concentrations of the competing antigens. Even at a concentration as high as 6.25 µg protein/well, heterologous antigens did not compete for the antibody. Identical results were obtained with 26 TB and 33 normal sera using the competing antigens at highest concentration used in the earlier experiment (Table 2). However, in a similar experiment using antibody derived by dissociating CIC isolated from eight individual TB sera, we noted a competition between the antigens of *E. coli* and *M. tuberculosis* (Figure 3). *E. coli* competed with H37Ra antigen immobilized in the wells, for dissociated CIC antibody. However, this competition was

Table 2. Competitive inhibition of binding of TB serum antibody to the immobilized antigen^a

Competing antigen	Antigen coated in ELIS A wells	
	<i>M. tuberculosis</i>	<i>E. coli</i>
None	(1) 0.883 ± 0.142	(4) 0.882 ± 0.093
<i>M. tuberculosis</i>	(2) 0.128 ± 0.101 ^b	(5) 0.861 ± 132 ^d
<i>E. coli</i>	(3) 0.868 ± 0.147 ^d	(6) 0.097 ± 0.1 18 ^c

^aAppropriate dilutions of 26 individual TB sera were preincubated with 5ug protein/well of competing antigen before adding to the assay wells.

^b(1) & (2) are significantly different by Student's t-test, $p < 0.0005$

^c(4) & (6) are significantly different, $p < 0.005$

^d(1) & (3) and (4) & (5) are not significantly different from each other.

relatively poor compared to H37Ra used as a competing antigen itself. H37Rv appeared even better in this respect.

Discussion

Antibodies in TB sera are known to react with antigens of quite unrelated organisms.^{1,2,7,17} The results of our assays also demonstrated that all our TB sera reacted with mycobacterial and *E. coli* antigens. As the antibodies in the antisera are polyclonal in origin, the reactivity of TB sera to *E. coli* antigens could be due to the presence of certain antibody types which are cross-reactive or the presence of antibodies with restricted specificity to *E. coli*. We used competition inhibition assay to solve this question. The results of the assays did not support the hypothesis of cross reactive antibodies in TB sera. Though all the TB sera reacted with the antigens of both *M. tuberculosis* and *E. coli*, the binding of these antibodies to either of these antigens could be inhibited only by homologous antigen preparation. In other words, binding of the TB serum antibodies to *E. coli* antigens was inhibited, only by *E. coli* but not by *M. tuberculosis* and vice versa (Figure 2 and Table 2). These data indicate the presence of two independent sub-sets of antibodies in TB sera with restricted Immunoreactivity. No significant correlation was observed between the titers of these two antibody sub-sets (Figure 1). Therefore, it appeared that the *E. coli* reactive antibodies present in our TB sera were probably produced in response to independent *E. coli* infection,

If the anti-*E. coli* antibody species in the TB patient sera were in response to a general *E. coli* infection, it should also be true for normal subjects drawn from the same population. This hypothesis was tested by estimating anti-*E. coli* and anti-mycobacterial antibody titers, in both TB and normal subjects. In normal subjects, the presence of high levels of anti-enterobacterial antibody has been reported previously.^{18,19,20,21} The results of our assays demonstrated the presence of anti-mycobacterial and anti-*E. coli* antibodies in the control group too. However, the anti-mycobacterial titers were considerably lower, while the anti-*E. coli* titers were roughly of the same magnitude as seen in the TB group. The difference between the mean values of these two antibody titers in the control groups was statistically significant by Student's t-test ($p < 0.0005$). Conversely, the difference in the mean values of the anti-*E. coli* titers between TB and control groups was not statistically significant (Table 1). It is important to note that a significant increase in anti-mycobacterial antibody levels in tuberculosis was not accompanied by a parallel rise in anti-*E. coli* antibody titers in this group. All these observations led to the hypothesis that the tuberculosis sera in our study contained two sets of antibody that were perhaps generated in response to two independent infections.

However, the lack of competition seen between *M. tuberculosis* and *E. coli* for TB serum antibody is surprising on account of abundant literature available on antigen sharing among mycobacteria, enterobacteria and several other organisms,^{2,3,9}

These proteins are highly immunogenic and a large part of the immune response of the host is directed against them.³ Though hard to explain, our observation may be interpreted as follows : The sera used in our experiments may not have recognized those epitopes common to both *M. tuberculosis* and *E. coli*. For example, though the 71/70 kDa antigen of mycobacteria has been shown to be strongly immunogenic in experimental animals,¹² a group of TB sera were shown to contain no antibody against this protein.²² Another possible explanation could be the difference in the avidity between the antigens and the cross reacting antibody.²³ However, the complete absence of competition between these antigens implies that such a phenomenon is unlikely. The *E. coli* antigens did demonstrate a competition, though relatively a poor one, with mycobacterial antigens, when the source of the antibody was immune complexes isolated from TB sera and not the TB sera (Figure 3). There is evidence that the antibody present in the complexes is of low avidity.²⁴ This suggests that a species of antibody which can react with certain epitopes, both on *E. coli* and *M. tuberculosis*, may possibly be present in the immune complexes. The presence of such an antibody in immune complexes may be immunologically significant as CIC are known to induce granuloma formation in tuberculosis.^{25,26,27}

The present study is concerned with serum antibody in tuberculosis, while the role and significance of serum antibody in mycobacterial infections is uncertain.^{8,28} The TB serum antibody was used to investigate a possible co-infection of the population with *M. tuberculosis* and *E. coli* in the developing countries, leading to generation of different sub-sets of antibodies in sera of variable specificity that may or may not cross react with one another. Concern has also been expressed that exposure to one infection may influence or modify the immune response of the host to another.^{29,30} It has further been suggested that repeated contact with a non pathogenic organism like *E. coli* may influence and modify the immuno pathological interactions between the host and a pathogenic organism such as *M. tuberculosis*.^{2,9} The existence of a species of antibody, whether cross-reactive or co-existing in a population, may be of importance also for immunodiagnosis of tuberculosis.^{8,10} Our study emphasizes the need to study the possible

influence of several co-existing, sub-clinical, and recurring infections on the outcome of interaction between the host and the mycobacterial pathogen. Such studies utilizing TB serum antibody have not been made.

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PRELIMINARY STUDIES ON THE INHIBITION OF SURFACE GROWTH OF ISONIAZID AND STREPTOMYCIN RESISTANT STRAINS OF MYCOBACTERIUM TUBERCULOSIS H₃₇ R_v BY TRIFLUOPERAZINE

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Summary. The calmodulin antagonist drug Trifluoperazine inhibited completely the growth of *Mycobacterium tuberculosis* H₃₇ R_v at a concentration of 4 ug/ml when grown as a surface culture on Youmans and Karlson's synthetic medium. The drug also suppressed completely the growth of *M. tuberculosis* strains TRC C1193 and SO111 resistant to Isoniazid and Streptomycin, at concentrations of 15 ug/ml and 8 ug/ml respectively. This suggests that Trifluoperazine inhibits the growth of *M. tuberculosis* susceptible and Isoniazid and Streptomycin resistant strains, completely when grown as surface culture under lower oxygen tension. The antipsychotic drug Trifluoperazine or some other calmodulin antagonist or phenothiazine compound, therefore, could be considered as a potential antituberculosis drug.

There are many hurdles to be overcome between a drug's potential seen in the laboratory and when the drug can be approved for clinical use.

Introduction

The presence of calmodulin like protein (CAMLP) has been demonstrated in mycobacteria¹ and it has been observed that in glucose grown *Mycobacterium phlei*² and *Mycobacterium smegmatis*³ there is a positive correlation between the levels of CAMLP, phospholipids as well as lipids and growth. And, that the calmodulin antagonist drug Trifluoperazine inhibits the incorporation of ³²Pi into total phospholipids.¹⁻³ We have recently shown that Trifluoperazine inhibits the growth of *Mycobacterium tuberculosis* H₃₇ R_v and Isoniazid

resistant strain of *M. tuberculosis* (TRC C1193) as shake cultures,⁴ i.e. under higher oxygen tension. It is not known whether this drug would be equally effective when the pathogenic mycobacteria are grown as surface cultures, with lower oxygen tension. Further, its effect on Streptomycin resistant strain was also not studied at that time.

Material and Methods

Chemicals : Trifluoperazine was purchased from Sigma Chemical Co. St. Louis, U.S.A. All the other chemicals were of the highest purity available locally.

Cultures: *M. tuberculosis* H₃₇R_v was originally obtained from Trudeau Mycobacterial Culture Collection, National Jewish Hospital, Denver, U.S.A. *M. tuberculosis*, TRC C1193 and S 0111 resistant to Isoniazid and Streptomycin respectively were kindly given by Dr. C.N. Paramasivan, Deputy Director (Microbiology), Tuberculosis Research Centre, Madras.

Growth of mycobacteria : The mycobacteria were grown as surface cultures on Youmans and Karlson's medium⁵ for four weeks in test tubes. The experiments were done three times, each time in duplicate.

Assessment of antituberculosis activity : Aqueous solution of Trifluoperazine was sterilized by filtration through 0.22 µm millipore membrane filter and added to the growth medium (5 ml in each tube) in the concentration range of 2-20 µg/ml. A growth covering entire surface area and also the side of the tube was classified as 3+; covering 3/4 of the surface area 2+; covering half the area 1+; very slight growth ± and no growth -. Growth was observed every week.

Table 1. Effect of Trifluoperazine on the growth of *M. tuberculosis* H₃₇R_v and Isoniazid and Streptomycin resistant strains of *M. tuberculosis*

Strain	Drug Conc., ug/ml	Weeks after inoculation			
		1	2	3	4
<i>M. tuberculosis</i>	0	+	++	+++	+++
H ₃₇ R _v	(control)				
	2	+	+	++	+++
	4	-	-		
	8	-	-		
<i>M. tuberculosis</i>	0	++	+++	+++	+++
Isoniazid Resistant	(control)				
	10	-	±	++	
	15	-	-		
	20	-			
<i>M. tuberculosis</i>	0	++	+++	+++	+++
Streptomycin Resistant	(control)				
	4	+	++	+++	+++
	6	-	+	++	
	8	-	-		
	10	-			

Results and Discussion

With 2 ($\mu\text{g/ml}$) of Trifluoperazine (Table 1) there was a slight inhibition of growth of *M. tuberculosis* H₃₇R_v upto 3 but not by 4 weeks. However, at 4 $\mu\text{g/ml}$, and higher doses of the drug, there was a total inhibition of growth. When the activity was tested against *M. tuberculosis* strains resistant to INH and Streptomycin, the minimum inhibitory concentration was found to be 15 and 8 $\mu\text{g/ml}$ respectively (Table 1). These results show that the MIC of Trifluoperazine for inhibiting the growth of susceptible strain (4 $\mu\text{g/ml}$) is lower than that for *M. tuberculosis* INH resistant (15 $\mu\text{g/ml}$) and Streptomycin resistant (8 $\mu\text{g/ml}$) strains.

The studies assume significance from many aspects. Since a calmodulin antagonist inhibits the growth of the human pathogenic strain *M. tuberculosis* H₃₇R_v and also strains resistant to two anti-tuberculosis drugs, it appears that calmodulin like proteins have a role in the metabolism of mycobacteria which is vital for their growth. Further, that calmodulin antagonists could be potential antituberculosis drugs. It is for the first time that we have shown that a

calmodulin antagonist having the phenothiazine group possesses antituberculosis activity.⁴ It is true that the MIC of Trifluoperazine of about 4 $\mu\text{g/ml}$ is higher than that of Rifampicin (2 $\mu\text{g/ml}$) and Streptomycin (4 $\mu\text{g/ml}$) as reported by Abbruzzese et al.⁶ However, the MIC of Trifluoperazine is more or less the same for susceptible *M. tuberculosis* H₃₇R_v, whether grown as surface (4 $\mu\text{g/ml}$) culture (Table 1) or shake (5 $\mu\text{g/ml}$) culture.⁴ Yet, the MIC of Trifluoperazine for Isoniazid resistant strain (15 $\mu\text{g/ml}$) was higher than that of the Streptomycin resistant strain (8 $\mu\text{g/ml}$), when grown as surface culture for Isoniazid resistant strain and in shake culture, the MIC of Trifluoperazine was only 8 $\mu\text{g/ml}$.⁴ This might imply that higher oxygen tension in shake cultures could be favourable for the antituberculosis action of Trifluoperazine. It is hoped that this study would promote the synthesis of better phenothiazine drugs or other camodulin antagonists with a fewer MIC and lower side effects. Its effect in animals would have to be investigated first.

Since Trifluoperazine is already in use in humans for psychotic disorders and is given for a

long time,⁷ its antituberculosis property could pave the way for human trials, if animals experiments are encouraging. In this connection it is to be noted that Trifluoperazine has been reported to inhibit the growth of influenza virus⁸ and the germination of spores of *Bacillus cereus*.⁹

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The mycobacteria were grown on Youmans and Karlson's medium with and without Trifluoperazine, as surface cultures. Growth was monitored every week for 4 weeks and graded as described in material and methods.

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ISOLATION OF TUBERCLE BACILLI UNDER FIELD CONDITIONS

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Summary: Tuberculosis prevalence surveys reveal that storage and transport of sputum specimens from the field to a central laboratory adversely affect the cultivability of tubercle bacilli and emphasize the need for devising a simple culture technique that could be adopted in the field itself. The single step culture technique developed at the Institute of Thoracic Medicine, Madras, uses a transport medium as a "digestant-decontaminant" which has been found to retain the viability of tubercle bacilli for three days at room temperature. This study shows that sputum specimens can be collected in this medium and transported from far away without cold storage and that such a procedure will not affect the cultivability of tubercle bacilli. The single step method was employed in the TB detection camps conducted in some of the remote tribal areas in Tamil Nadu because of its technical simplicity. Thus, it is technically possible to put up cultures even under adverse field conditions with almost the same efficiency as in a well organised laboratory.

Introduction

It is a known fact that the main disadvantage of direct smear microscopy is its comparatively low sensitivity. And a fairly large proportion of bacillary cases cannot be picked up by this basic diagnostic technique. Culture of tubercle bacilli gives a higher positive yield, and is a more conclusive evidence of tuberculosis. Because of its precise but expensive and laborious nature, culturing of tubercle bacilli has been limited to well equipped laboratories only. However, a number of attempts have been made to isolate tubercle bacilli from sputum specimens collected during large scale prevalence surveys of tuberculosis either by transporting the specimens from far away places to a central laboratory or by

putting up the cultures in the field itself and bringing the inoculated slopes to the central laboratory for further processing. The findings from some of these WHO/UNICEF assisted tuberculosis surveys revealed two important points; one, that cultivability of tubercle bacilli is adversely affected by storage and transportation;¹ secondly, that if the cultures are put up in the field itself, and the inoculated slopes are kept at room temperature before incubation in the central laboratory, the delayed incubation will not have any adverse effect on the isolation of tubercle bacilli.^{2,3} These findings strongly emphasize that it is necessary to devise a simple culture technique that could be adopted in the field itself.¹ The Institute of Thoracic Medicine, Madras, has developed a single step culture technique to isolate tubercle bacilli using a transport medium which is a digestant and decontaminant,^{4,5} at the same time.

Since the viability of tubercle bacilli is retained upto three days in the mentioned transport medium at room temperature,^{4,6} in this study two attempts were made. One, that sputum specimens were collected in the transport medium itself and the mixture was transported from a far away place, without cold storage to a central laboratory for processing. And second, this single step method was applied, because of its technical simplicity, in TB detection camps conducted by the Institute of Thoracic Medicine in some remote tribal areas in the hilly regions of Tamil Nadu. The feasibility of applying the culture technology successfully under such adverse field conditions was studied.

Material and Methods

1. *Effect of transportation on the isolation of tubercle bacilli*

Three hundred sputum specimens were

collected from the inpatients of Government Hospital for Thoracic Medicine, Nagercoil, situated about 800 Kms away from Madras. Each specimen was shaken well with the "transport medium" and divided into two aliquots, A and B. Next morning, group 'A' specimens were cultured, as per the single step method,⁵ and smears were made from the deposits at the Nagercoil Hospital under ordinary laboratory conditions. This formed the control group. Group 'B' and the control group specimens were properly arranged in metal racks, placed in a cardboard box, closed, and tied lightly. The parcel was carried the same evening by train which reached Madras on the following morning i.e. 48 hours after sputum collection. On the day of receiving the parcel, the B aliquots were cultured at the Institute of Thoracic Medicine, by the single step method and smears made from the deposits under the same laboratory conditions that existed at Nagercoil Hospital. All the cultures and smears were then given code numbers. The cultures were incubated at 37°C for six weeks and the smears were stained by the cold staining method and examined for the presence of acid-fast bacilli.⁷

Preparation of "transport medium" and single step culture technique⁷

Tri sodium phosphate	200 g
Ammonium sulphate	5 g
Magnesium sulphate	500 mg
Ferric ammonium citrate	250 mg
Distilled water	1000 ml

All the above chemicals were dissolved in water by heating. The solution was filtered and sterilised at 121°C for about 15 minutes. After cooling, Penicillin, 100 U/nil was added. About 10 ml of this medium was distributed into sterile screw-cap McCartney bottles and stored at room temperature (In routine practice, a red mark was made on the bottles about half an inch above the liquid level. These bottles were supplied to patients who were instructed to open the cap and expectorate sputum into bottle upto the red mark and close cap again).

Culture Technique : The sputum-transport medium mixture was shaken well and allowed to stand overnight at room temperature for digestion, decontamination and concentration. On the

following morning, the supernatant fluid was decanted and one loopful of the deposit was inoculated on to a slope of Lowenstein-Jensen medium and incubated at 37°C for six weeks.

2. Application of single step culture under field conditions

The single step technique was employed in TB detection camps in some of the remote tribal areas in Tamil Nadu. Since each of these camps lasted for more than three days (3-5 days), cultures were put up in the field itself.

A wooden table with two metal racks, one containing transport medium bottles and another containing L J Medium slopes, a plastic bowl with lysol solution to discard the supernatant fluid, microscope glass slides, a spirit lamp and a match box constituted the 'Culture Laboratory' in the field. (In one camp, since no suitable work bench was available, the culture laboratory was set up on the floor in a corner of the room). In all, 304 sputum specimens collected from symptomatics were cultured by the single step method and smears were made from the deposits. The inoculated slopes and the smears were packed in a cardboard box as described previously, and brought to Madras. At the Institute of Thoracic Medicine, the slopes were incubated at 37°C for six weeks and the smears were stained by the cold staining method and examined for the presence of acid-fast bacilli. All the positive cultures were tested for the production of niacin.

Results

The first group of 300 sputum specimens (early morning specimen) were collected from the inpatients of Nagercoil Hospital. This group consisted of both new and old cases who were undergoing anti-TB treatment, with various regimens, for varying periods. The findings of this study are given in Table 1.

The second group comprised 304 sputum spot specimens collected at the TB detection camps and examined for smear and culture. Table 2 shows the bacteriological findings.

Discussion

The WHO expert committee on tuberculosis recognised in 1973 the need for research in

Table 1. *Effect of transport on the isolation of tubercle bacilli (300 sputum specimens)*

	Before transit (24 hours)		After transit (48 hours)	
	No.	%	No.	%
Smear Positive	127	42.3	126	42.0
Culture Positive	98	77.1	97	77.0
Culture Negative	27	21.3	29	23.0
Smear Negative	173	57.7	174	58.0
Culture Positive	19	10.9	18	10.3
Culture Negative	139	80.3	134	77.0
Total Contaminations	17	5.7	22	7.3

Table 2. *Application of single step culture under field conditions (304 sputum specimens)*

	No.	%	Niacin Positive	
			No.	%
Smear Positive	8	2.6	-	
Culture Positive	7	87.5	7	100
Culture Negative	1	12.5	-	-
Smear Negative	296	97.4	-	
Culture Positive	2	0.7	2	100
Culture Negative	280	94.6	-	
Total Contaminations	14	5.0	-	

preserving sputum specimens during transit, on methods of decontamination, and less expensive procedures for application in case-finding programmes.⁸ The "transport medium" devised at the Institute of Thoracic Medicine, Madras has hardly any deleterious effect on tubercle bacilli, and the single step method was found to be superior to the Petroff's concentration method, the N-acetyl-L-Cystein-Sodium hydroxide method, the Cetyl pyridinium chloride - Sodium Chloride method and Nassau's Swab method which are commonly used in different parts of the world.^{4,5}

Since none of these sophisticated techniques is applicable under field conditions, a comparative study was not possible.

When sputum specimens have to be transported from distant places, the two important points to be considered are : preservation of the specimens in such a way that the tubercle bacilli are not affected and material reaches the laboratory in a condition fit for processing. Secondly, that specimen bottles are properly packed to prevent breakage or leakage during transit.

In this study, by using the "transport

medium”, both these requirements were satisfactorily achieved, since the results obtained before and after transit were almost similar (Table I), it is evident that transportation of sputum specimens without any cold storage has no adverse effect on the isolation of tubercle bacilli or on the contamination rate if the cultures are put up within three days.

The criteria for an ideal culture technique for field application are : it should be simple and efficient, should not require any instrument or electricity, and should be suitable for use under make-shift arrangements. The single step method described satisfied all these requirements. In fact the findings from the TB detection camps are highly encouraging, even though the number of specimens examined is small because the main objective of the study was to find out if it is technically possible to put up cultures under adverse field conditions with the same efficiency as in a well organised laboratory.

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MYCOBACTERIURIA IN PULMONARY TUBERCULOSIS PATIENTS IN MADRAS, SOUTH INDIA

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Summary. Three consecutive, entire, early morning urine specimens, collected from each of 137 bacteriologically confirmed pulmonary tuberculosis patients aged more than 12 years were processed for culture of *M. tuberculosis* by the usual centrifugation method. Of the 411 urine specimens, 5 yielded *M. tuberculosis*. About 50 ml each from 405 of the above specimens, from 135 patients, was also processed for culture by a filtration method and *M. tuberculosis* was isolated from only one of them. In all, mycobacteriuria was present in 5 (3.6%) of 137 patients (95% confidence interval being 1.2% to 8.4%). Of these patients, 92 had no history of previous chemotherapy and 3 (3.3%) excreted tubercle bacilli in Urine (95% confidence interval being 0.6% to 9.3%).

could yield a better recovery of tubercle bacilli which are likely to float during centrifugation. So, the objectives of the present study were : (1) a systematic investigation for the presence of mycobacteriuria in PT patients in Madras to get an estimate of renal involvement in them, and (2) to employ a filtration method on an experimental basis, in addition to the established centrifugation method, to recover tubercle bacilli from urine.

Material and Methods

One hundred and thirty-seven bacteriologically confirmed PT patients aged more than 12 years, admitted (except one) consecutively in a clinical trial were included in the study. From each of these patients, before starting chemotherapy, three consecutive, entire, early morning urine specimens were collected and processed for culture of *M. tuberculosis* by the centrifugation method using multiple media.⁵ The entire left over centrifuged deposit was inoculated into Selective Kirchner's liquid medium (SKLM). A total of 411 specimens were processed.

Of the 411 specimens, 405 from 135 patients were also processed by a filtration method as described below:

To about 50 ml of the urine, sodium dodecyl sulphate was added to give a final concentration of 2 mg/ml and then incubated at 37°C in a water bath for 30 minutes. This was filtered, using a syringe, through a filter membrane (Pore size : 0.45 micron; diameter : 25 mm; from Microdevices Pvt Ltd., Ambala, India) which had been treated with 0.028% Malachite Green solution and assembled in a filter holder (Laxbro, India). Then, through the same assembly, 10 ml of 1% sodium hydroxide was filtered slowly (in about 10 minutes) followed by 20 ml of sterile

Introduction

In patients with pulmonary tuberculosis (PT), mycobacteriuria has been reported to vary from 3.6% to 14.8%.¹⁻⁴ In USA, Bentz et al¹ observed mycobacteriuria in 4.7% of 275 untreated PT patients; in India, Chadha and Shahi² recorded it in 3.6% of 55 patients with unspecified period of treatment for PT; Agarwal et al³ reported it in 6.4% of 110 patients without giving any information about previous therapy; and Challu et al⁴ noted a higher rate of 14.8% in 236 untreated PT patients. These reports from India indicate a variation in the estimates of mycobacteriuria in PT patients and this may perhaps reflect lack of a systematic approach.

In mycobacteriology laboratories, urine specimen is usually centrifuged and the deposit is processed for culture. Filtration of urine and processing of the filter membrane for culture

distilled water. The filter membrane, in the first 120 specimens, was implanted on a selective 7H11 (S7H11) agar plate and incubated. Since plate contamination was encountered frequently, the membranes in next 84 specimens were implanted on S7H11 agar slopes. As selective Kirchner's liquid medium supports the growth of even paucibacillary inoculum,⁵ the filter membrane in last 201 specimens was cut into two halves, one half being implanted on a S7H11 agar slope and the other half transferred to about 7 ml of SKLM. The media were examined weekly for visible growth of tubercle bacilli and subcultured for identification by the standard procedures followed in this laboratory.

For computing the confidence intervals, the "square root transformation method" as recommended by Radhakrishna et al⁶ was followed.

Results and Discussion

Of the 137 patient (irrespective of previous treatment), 5 (3.6%) excreted tubercle bacilli in the urine (95% confidence interval being 1.2% to 8.4%). History of previous treatment was available for 130 patients and 92 of them (70.8%) had denied previous anti-TB treatment. Three (3.3%) of the 92 untreated patients excreted tubercle bacilli in urine (95% confidence interval being (0.6% to 9.3%).

M. tuberculosis was isolated in 4 patients in one of the 3 specimens by the centrifugation method. In the 5th patient, tubercle bacilli were recovered from 2 different specimens, one by the centrifugation method and the other by the filtration method.

In all, of the 411 urine specimens from 137 patients, 5 yielded *M. tuberculosis* by the centrifugation method and all the isolates were obtained from the subcultures into SKLM which were inoculated with the entire left over centrifuged deposit after inoculating a loopful of it onto 3 media, namely, Lower stein Jensen (LJ) medium, LJ with pyruvate (LJP) and S7H11 slopes. In one specimen, LJP gave a positive culture in addition to SKLM. However, Challu et al⁴ reported isolation of *M. tuberculosis* in 11.6% of 233 specimens from LJ slopes; inoculated with a loopful of centrifuged deposit.

Of the 405 specimens from 135 patients tested by the filtration method only, one was culture positive and that was obtained on S7H11 agar slope, whereas Challu et al⁴ had reported isolation of *M. tuberculosis* in 12.6% of 95 specimens, collected one each from their patients.

Clogging of filter membranes was experienced in 10% of urines while Challu et al⁴ excluded 141 (60%) of 236 specimens from analysis due to clogging and inadequate quantity of urine. This could perhaps be due to the larger volume (100 ml) of urine used for filtration in their study. In the present study, 14 of 285 (4.9%) cultures were contaminated (excluding the first 120 cultures), while Challu et al⁴ observed it in 33.7% of 95 cultures using 5% oxalic acid for decontamination and LJ medium slopes for culture in their filtration method. In spite of lower rate of contamination by employing a milder treatment procedure (1% NaOH), examination of multiple urine specimens and using 2 media for culture, the filtration method did not yield additional positives in the present study.

It is to be mentioned that in a preliminary experiment filtration method was found to recover *M. tuberculosis* H37Rv from all of 13 normal urine specimens (about 20 ml) artificially seeded with about 50 to 500 viable bacilli. The failure to recover bacilli from urine specimens in the present study may perhaps be attributable to the presence of such a low number of bacilli that the portion taken for filtration might not have contained any bacilli.

All the 5 patients with mycobacteriuria were male (out of 107) and were more than 23 years old (range 23-55). The pretreatment radiographic findings showed that 3 patients had extensive bilateral lesions while the other 2 had lesions in the right apical region. The Mantoux reaction was 10 mm or above in all of them. Two had received anti-TB treatment previously for 6-9 months and the other 3 had none. Smear and culture results of pretreatment sputum from 4 of them showed that they were "heavy positives"-with confluent growth from at least one of the 4 specimens. However, the 5th patient produced only a single colony from one of the 3 sputum specimens examined. Before admission to the study, one of them had complaints of burning micturition, haematuria, oliguria, pain in lumbar region and

tenderness in renal angle; another patient complained of frequency of micturition and nocturia; the remaining 3 patients had no symptoms. Absence of urinary symptoms and normal intravenous pyelogram have been reported in patients with mycobacteriuria.¹

Of these 5 patients, one was not followed up as his pretreatment sputum smears were negative for acid fast bacilli and, thus, he became ineligible for admission to the chemotherapy study. One patient refused hospitalisation and no information could be collected on his renal function. The remaining 3 patients were referred for further investigations, immediately after the urine culture results were known. Intravenous pyelogram was normal in these 3 patients. Ultrasonogram was normal in 2 patients and in one suggestive of bilateral renal disease.

The isolates from urine and sputum specimens of 4 patients were sensitive to Streptomycin, Isoniazid and Rifampicin. In the fifth patient, while the urine isolate was sensitive to Streptomycin, Isoniazid and Rifampicin the sputum isolate was sensitive to Streptomycin and Rifampicin but resistant to Isoniazid.

Four patients received short course chemotherapy and 2 responded to treatment. One patient showed radiographic and clinical deterioration due to non-tuberculous etiology, at the end of the 24th month, and developed pneumothorax at the 36th month. Another patient with initial Isoniazid resistance developed resistance to Rifampicin at the end of chemotherapy and the treatment was changed. Later, he had a favourable response to treatment. Three urine specimens, each collected at the end of treatment from the above 4 patients, were all negative for culture of *M. tuberculosis*.

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SPINAL SUBDURAL TUBERCULOUS GRANULOMA

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Summary. Subdural tuberculous granuloma of the spine is very rare. Five such cases are presented and features of interest in these cases have been discussed. The results of surgical excision and antituberculosis therapy are presented.

Introduction

Extra-osseous tuberculous granuloma in the spine may be extradural, intramedullary or subdural (intradural extramedullary).¹⁻¹² The last variety is the least frequent.^{4,5} The localised variety of subdural granuloma is amenable to surgical excision. Five cases of localised spinal subdural tuberculoma comprise this report. The clinical, radiological and surgical features of these are summarised in the case reports below.

Case Report

Case 1

A female, 32 years old had weakness of left leg followed by paralysis of both legs with retention of urine. She was being treated for tuberculous abdomen for 1 month. On examination, there was flaccid areflexic paraplegia with retention of urine and diminished sensations below D12. X-ray spine was normal, CSF proteins were 4.75 gm%. Cisternal myelography showed intradural total block at D5 level.

Laminectomy was done from D5 to D8 and a subdural extramedullary granuloma was removed. On histopathological examination, tuberculous granulation tissue with caseation was reported. Anti-tuberculosis chemotherapy was continued. Her condition improved and she became ambulant.

One year later, she developed total paraplegia following a fall. Cisternal myelography showed a total block at C7. Surgical reexploration revealed arachnoiditis from C7 to D8. She remained disabled despite full antituberculosis treatment and physiotherapy.

Case 2

A male child 11/2 years old had low back pain with yellowish discharge coming through a dermal sinus at the back and minimal weakness of both legs.

X-ray of lumbar spine showed widening of lumbar canal and spina bifida of L5 and SI. The dermal sinus was entering a dense vascular intradural tumor which was infiltrating cauda equina. Tuberculous granulation tissue was found histopathologically. The child recovered fully after surgery and specific therapy.

Case 3

A male 28 years old had progressive weakness of legs of 40 days' duration. He was treated 4 months back for tuberculous meningitis. On examination there was spastic hyper-reflexic paraplegia with sensory loss below D10. Spine X-ray was normal. CSF proteins were 1 gm%, Cisternal myelogram showed a block at D4 lower border.

Laminectomy was done at D5 D6. A subdural granuloma was removed. Histopathologically, the mass was tuberculous granulation tissue.

Following anti-tuberculosis therapy the patient became ambulant with residual weakness in lower limbs. Late follow up showed complete recovery.

Case 4

A female 30 years old presented with progressive paraparesis and precipitancy of

micturition of 18 months duration. She had been treated for tuberculous meningitis 10 months ago with anti-tuberculosis drugs.

On examination, there was bilateral optic atrophy; spastic paraplegia (Gr. 1 power) with sensory at D5 level, with flexor spasms. The CSF protein was 3 gm %, X-ray of dorsal spine was normal. Cisternal & lumbar myelograms showed an intradural block from D2 to D10. (Figs. 1 & 2).

After laminectomy, a fleshy, tough granuloma with caseation on the dorsal aspect of the cord (D2 to D10) was removed. Histopathologically, the mass was tuberculous in nature.

The patient is improving in the immediate post operative period or anti-tuberculosis treatment.

Case 5

A female 30 years old presented with weakness of both lower limbs of 6 months' and paraparesis of 3 months duration. There was just Gr. IV power in both lower limbs. Areflexia was present in both legs.



Fig. 1 Cisternal myelogram showing the upper limit of the intradural lesion at D2 (Case 4)

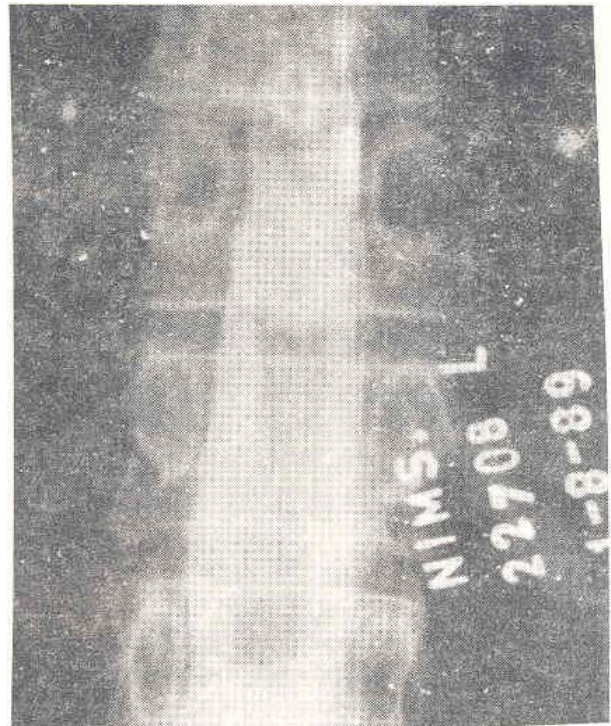


Fig. 2 Lumbar myelogram showing the lower limit of the lesion at lower border of D10 (Case 4)



Fig. 3 Spinal CT Scan of lumbar spine showing intradural isodense and mildly hyperdense lesion within the duramater (Case 5)

X-ray examination of spine & chest was normal; lumbar puncture was dry. The CT Scan showed an intra dural SOL in the lumbar region. (Figure 3).

Laminectomy showed that duramater & epidural tissue were thickened and opaque. There was an intradural fleshy mass engulfing the nerve roots which proved tuberculous histopathologically.

Tuberculosis chemotherapy was begun and 4 months after surgery, considerable improvement was recorded

Discussion

Intraspinal non-osseous tuberculomas are about 40 times less frequent than intracranial ones.¹⁻¹¹ Dastur found localised subdural tuberculomas in 4 of 74 cases of spinal tuberculoma.⁵ Until 1984, about 15 cases of subdural tuberculous granuloma had been reported.³⁻⁴ In 1988, Mathuria et al reported another 4 cases.¹² In the diffused type, the granuloma tends to surround the cord and extends over many segments.^{4,5,7,8} This entity has been described under titles wch as tuberculoma of cord, tuberculous granuloma and leptomenigitis. The localised variety, on the odier hand is very rare.^{1,13} Cases 3 and 4 of Mathuua et al were of the diffused type and 1 and 2 of localised type.

Tuberculosis of the spinal cord and its coverings usually results from hematogenous spread.^{7,8,14} Discrete lesions may be found in every conceivable anatomical plane.⁵

The lesion may be primary or secondary and healed or active meningitis. In the former type, a small tuberculous focus on the surface of the spinal cord flares up into a localised granuloma with variable involvement of arachnoid and piamater. In the latter, the proliferative lesion is a manifestation of localisation of the meningitic process.¹⁵ Cases 3 and 4 of our series are of this type. In Case I, the patient was treated for tuberculous abdomen. In Case 2, there was no evidence of tuberculosis elsewhere. The congenital dermal sinus was thought to be the source of the disease which had localised intradurally.⁶

The noteworthy feature of Case 4 is the longitudinal extent of the lesion, from D2 to D10 (Figures 1 and 2). Though the mass was situated only on the dorsal aspect of the cord, was well

demarcated, and was not encircling the cord like that of the diffused variety, it was extending over several segments, unlike the other cases of classical localised subdural granuloma. In Case 5, tuberculous histology was identified in the epidural tissue and in the duramater, in addition to the major intradural (subdural) mass. Simultaneous occurrence of lesions in both intra and extramural planes is very rare. Dastur found combined intra and extramural lesion only in one case.⁵

In contrast to the diffused type of subdural granuloma and arachnoiditis, in both of which the outlook is poor, in the localised variety of subdural tuberculoma the results of surgery are gratifying.^{5,12,15} Four of our five cases showed improvement. In one patient (Case 1), however, the result was poor due to the development of arachnoiditis at the site of the previous surgery.

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HYPERSENSITIVITY REACTION TO ISONIAZIDR.L. Agrawal¹, S.K. Jain² and D.K. Agrawal³

(Original received on 19.10.92; Accepted on 8.2.93)

Summary. An unusual case of Isoniazid induced hypersensitivity reaction in the form of serum sickness, parotitis and paralytic ileus is reported.

Introduction

Hypersensitivity reactions are a common toxic manifestation during the long course of antituberculosis treatment. Isoniazid is known to be the least toxic among the antituberculosis drugs. Neurological manifestations in the form of peripheral neuropathy¹, toxic psychosis² and convulsions³ are common with Isoniazid. Hypersensitivity reactions due to Isoniazid may manifest in the form of fever, skin rash and hepatitis.⁴ A case of pyrexia, erythematous rash, glandular swelling in right axilla, arthralgia, albuminuria, parotitis and paralytic ileus due to Isoniazid is reported.

Case Report

S., a 45 years old female was admitted on 2nd April 1990 with complaints of fever, skin rash, swelling in right axilla and right side of face, gradually increasing distension of abdomen with swelling and pain in right knee joint for two weeks prior to admission. She was put on antituberculosis drugs (Streptomycin, Isoniazid, Rifampicin, and Ethambutol) four months earlier to treat her pulmonary tuberculosis. She had continued treatment regularly for two months and had responded well, but developed drug induced jaundice afterwards. All antituberculosis drugs were stopped for two weeks till jaundice subsided. After recovery, she was kept on Ethambutol and Isoniazid which she continued regularly till her

present admission. She had no history of taking other drugs or any allergic manifestations in the past.

General examination revealed moderate pallor, body temperature 101°F. erythematous macular serpigenous rash on the borders of both hands and feet, and right parotid gland both enlarged and tender. Tender lymphadenopathy was also present in the right axillary region. A single diffuse swelling was present in the right knee joint which was mildly tender. Occasional crepitations were noted in the right infraclavicular region of the chest. Gaseous distension of abdomen was present and bowel sound was absent. Liver was enlarged two cm. soft and tender.

Hemogram revealed absolute eosinophilic count 1200 cells/cu mm and liver function test was within normal limits. Antinuclear factor was negative. Urinalysis showed albumin, granular, hyaline casts and red blood cells. Skiagram chest (PA view) revealed a heterogenous opacity in right upper zone. Gaseous distension was observed on plain X-ray of abdomen.

After stopping all antituberculosis drugs, she was put on intravenous fluid with steroid I.V. for three days followed by oral steroids, tapered within a fortnight. Seven days after her complete recovery she was given Isoniazid 300 mgm daily following which she developed similar symptoms, i.e. fever, skin rash, arthralgia after six days. Isoniazid was stopped and she was discharged with advice to continue Rifampicin and Ethambutol. No untoward reaction was seen upto five months of follow up.

Discussion

Hypersensitivity reactions are a common manifestation of all antituberculosis drugs.⁵ Serum

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sickness occurs mainly with Streptomycin and Para-aminosalicylic acid.⁶ Isoniazid is a potent producer of antinuclear antibodies but rarely causes clinical manifestations of systemic lupus erythematosus.⁷ Eosinophilia, albuminuria, presence of casts along with reducing substance in urine may be occasionally produced due to Isoniazid.⁸ Isoniazid induced serum sickness has also been reported.⁹ Common clinical manifestations of serum sickness include fever, cutaneous eruptions, arthralgia, lymphadenopathy and albuminuria. The reported case had serum sickness, parotitis, and paralytic ileus, due to Isoniazid and not Rifampicin or Ethambutol.

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CEREBRO-SPINAL PSEUDOCYST COMPLICATING VENTRICULO-PERITONEAL SHUNT

Y.K. Sarin¹, M. Zaffar² and A.K. Sharma³

(Original received on 28.1.93; Accepted on 20.5.93)

Summary. An unusual complication following ventriculo-peritoneal shunt done for hydrocephalus associated with tuberculous meningitis is reported.

Introduction

In our country, the occurrence of neurotuberculosis in pediatric age group is common. Surgeons are occasionally called in to help patients having hydrocephalus associated with tuberculous meningitis. Over the last two decades, it has been recognised that shunting of cerebrospinal fluid (CSF) alleviates the neurological problem of such patients. However, the possibility that the shunt might disseminate the disease into the system where the CSF is being diverted has to be faced. We report an unusual case of the spread of tuberculosis to the peritoneal cavity and formation of a pseudocyst following a ventriculo-peritoneal shunt done for hydrocephalus associated with tuberculous meningitis.

Case Report

A 2 years old male child was admitted to the Department of Pediatric Medicine, Medical College, Jaipur with clinical diagnosis of tuberculous meningitis : clinical stage III. The CSF studies had shown a protein content of 140 mg%, chlorides 580 mg%, glucose 20mg% and 200 cells/hpf (majority being lymphocytes). The CT scan of brain showed moderate hydrocephalus. He was started on anti-tuberculosis drugs (Rifampicin 10mg/kg/day, Pyrazinamide 25mg/kg/day, Isoniazid 10mg/kg/day and Prednisolone 0.5mg/kg/day). The child's neurological status did

not show any clinical improvement even after two weeks of therapy. A pediatric surgical consultation was sought. Ventriculo-peritoneal shunt was performed using a low-pressure Chhabra's device. The CSF was under moderate pressure after the shunt procedure. The child showed steady improvement and recovered fully within a period of two weeks.

The child was later lost to follow up but was readmitted after 6 months with blocking of the peritoneal end of the shunt. General condition of the child, then, was very poor. He was semicomatose, with bilateral fixed pupils and a left spastic hemiplegia. Fundus examination revealed bilateral papilloedema. There was marked neck stiffness and Kernig's sign was positive. Abdominal examination revealed a large cystic swelling occupying the right upper abdomen; ultrasound examination revealed a large area of fluid collection measuring 10 x 8 x 6 cms. in the right hypochondrium and right lumbar region surrounded by a loop of bowel. On

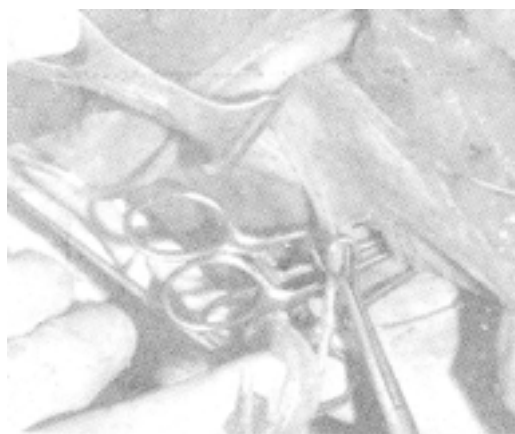


Fig. 1. Cerebrospinal fluid pseudocyst after deroofing (Note its glistening surface and the ventriculoperitoneal shunt entering it).

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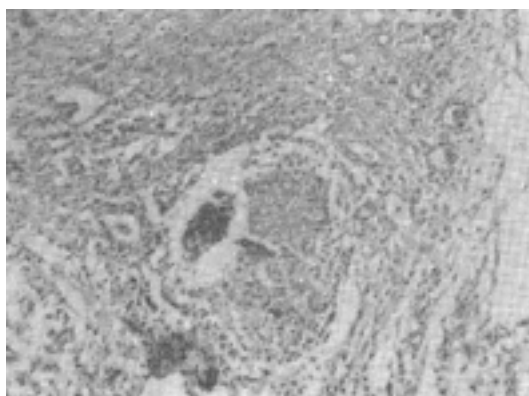


Fig. 2 Microhistograph showing tuberculous granuloma with the typical epithelioid cells and Langhans giant cells in the pseudocyst wall (H & E stain, x 250).

abdominal exploration, a large cyst occupying the right side of the abdomen was found (Fig. 1). The rest of the peritoneal cavity looked normal with no features to suggest tuberculosis. The cyst wall was partially excised. There was a free flow of CSF from the shunt and the peritoneal end was re-sited in the peritoneal cavity. Unfortunately, the child died on the second post-operative day.

The examination of CSF from the cyst showed a protein content of 96 mg%, chlorides 620mg%, glucose 20 mg% and 108 cells, mainly lymphocyte; no acid-fast bacilli were seen. Histopathology of the resected pseudocyst wall revealed evidence of tuberculosis (Fig. 2).

Discussion

Ventriculo-peritoneal shunting of cerebrospinal fluid is standard therapy for the management of hydrocephalus associated with tuberculous meningitis. The introduction of shunt under the umbrella of anti-tuberculosis drugs results in remarkable regression of neurological deficits and improvement in level of consciousness in most of the cases^{1,2}. Most of the authors have previously affirmed that there was no fear of tuberculous dissemination through the shunt device even when the procedure was carried out during the active phase of the disease¹⁻⁴. Review of literature revealed only a single instance of dissemination through the shunt device previously. Suwanwala in 1968 had reported miliary dissemination in one case following a ventriculo-atrial shunt⁵. The

present case has yet again confirmed the possibility of such dissemination. However, unlike the widespread miliary dissemination following a ventriculo-atrial shunt⁵, the dissemination was localised by the formation of a pseudocyst in our case.

Pseudocyst formation following ventricular-peritoneal shunt done for hydrocephalus due to causes other than tuberculous meningitis has been reported to be around 1%^{6,7}. The pathophysiology is not clearly understood, although infection, high protein content of CSF, peritoneal adhesions and an inflammatory response to the catheter material have been implicated⁸. We feel that pseudocyst formation is an example of the normal defence mechanism to localise the infection descending into peritoneal cavity through a ventriculo-peritoneal shunt.

The present case is the second documented case of the spread of tuberculosis through a shunt device. This is probably the first reported case of CSF pseudocyst formation following ventriculo-peritoneal shunt for hydrocephalus associated with tuberculous meningitis.

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FORUM

Sir,

Tuberculosis workers are very lucky to have the Indian Journal of Tuberculosis as a link between them and the Tuberculosis Association of India to solve the problems that face the workers now and then.

It is very fortunate that the Journal has progressed so effectively so that we get the scientific views in a very efficient manner. The Editorial Board is gifted with deep knowledge of the subject and a beautiful manner of expression to bring home to us the matters attractively. A tuberculosis worker needs help in the problems of diagnosis, treatment and prevention of tuberculosis. The problems of all respiratory diseases are mixed up with those of tuberculosis and these have to be sorted out. All these aspects are now and then dealt with by the Journal.

One of the prevalent and difficult problems facing us is the effect of smoking and tobacco chewing on health. A chest physician has to face it very often. May I suggest that more effective coverage of prevention of smoking and management of the results of heavy smoking including pulmonary emphysema is done by the Journal.

Bhagat Singh Alag
Jabalpur.

Sir,

I am a social worker doing Tuberculosis relief work since 1939.

In 1947, pre-partition days, a number of refugees came and took refuge in a nearby Dharamshala. A very beautiful girl, Pushpa, 12 years old, came to my office along with her grandmother, from Montgomery whose language I could not understand. Pushpa spoke in chaste Hindi.

“Bhupaji mere do bhai TB se mar gae hain, tisre ko bhi ho gai hai kuch madad karo”

I was deeply touched and immediately wrote to Pandit Jawaharlal Nehru. A reply came to me after a few days.

“Send the names of all such patients. We will give them Rs. 50/- p.m. each”.

Fifty rupees, forty five years ago had a great meaning. Pushpa's third brother could not be saved and a few months later Pushpa also caught the disease and died in the Silver Jubilee TB Hospital

There were no medicines then. The introduction of new anti-TB drug “RIFAMYCIN” has revolutionised the anti-TB campaign. However, the drug is in short supply in all the TB Hospitals & Clinics, probably due to the cost involved.

In national interest I request you to kindly reproduce this letter in part or in full in your esteemed journal.

Kailash Chand Jain,
New Delhi.

Sir,

In the article entitled “Non-Tuberculous Mycobacteria Isolated from an Epidemiological Survey in Rural population of Bangalore District” by Dr. M.M. Chauhan published in *Indian Journal of Tuberculosis*, 1993, 40, 195, there are some interesting as well as alarming findings such as only 1.1% of sputum samples being found to be positive for *M. tuberculosis* out of 4015 sputum specimens collected from symptomatic, tuberculin positive persons. At the same time, the isolation rate for non-tuberculous mycobacterial (NTM) species was 2.5% which seems to be quite high. The author further mentions that on culture there were less than 20 colonies in all 101 NTM isolates. Most of the reports published from various workers show higher rate of isolation for *M. tuberculosis* than NTM. We have reported an isolation rate of 1.05% of NTM out of 4554 sputum samples collected from 3943 individuals, while the rate of isolation was 12.2% for *M. Tuberculosis* (Sankar et al *Ind. J. Chest Dis. & All. Sci.* (1988) 31 (1): 9. Temporary colonization by NTM in the respiratory tract is not uncommon and about 5% of the healthy individuals were found to have such colonization (Kotian et al, *Ind. J. Tub.* 1983, 30,149).

For the induction of tuberculin sensitivity the

organisms (NTM) have to infect and multiply in the body of the host to cause sensitization against tuberculin. Thus the number of colonies to be seen on LJ medium has to be more than 20, if not in all 101 cases but in most of them. This seems to be actually a case of contamination from the time of the collection of specimen till the inoculation of the media. The author

ascribes BCG failure to infection by NTM. Further, I would like to suggest, on the basis of these results, that it may not be advisable to interpret the results as BCG failure or induction of tuberculin sensitivity in general population by NTM.

N.K. Jain,
New Delhi.

ERRATA

In the July 1993 issue of the Indian Journal of Tuberculosis :

Page	Column	Para	Instead of	Read
120	1	7	There were 2 S-containing without R regimens (one of 5 months' and the other of 7 months' duration) containing 3 drugs.....	There were 2 S-containing regimens without R (one of 5 months' and the other of 7 months' duration) containing 3 drugs.....
121	2	4	With double-drug SH resistance, 70% of 30 patients who did not receive R had an unfavourable response, as compared to a similar proportion of patients (70% of 33) who received R intermittently for 2 months;..... intermittently for 6 months ($p < 0.0001$)	With double-drug SH resistance, 70% of 30 patients who did not receive R and a similar proportion of patients, (70% of 33) who received R intermittently for 2 months had an unfavourable response, as compared to 17% of 46 patients who received R daily for 2 or 3 months ($p < 0.0001$), and 26% of 91 patients who received R intermittently for 6 months ($p < 0.0001$).

**CHANGING SCENARIO OF
PHARMACEUTICALS**

Health care reforms either already introduced or being planned in Europe, Japan, and U.S.A. have begun to change the face of the pharmaceutical industry the world over. GATT negotiations currently going on under the Uruguay round are likely to affect the industry still more, but the picture is not at all clear at present. A radical change, however, is expected.

The overall effect of the reforms in health care practices in large consumers of drugs-countries such as Germany and Italy - has hit the sales of drugs by around 10 percent. The knee-jerk reaction of the industry is to hike the prices. In U.S.A., the tendency has been for consumers to group together and buy drugs cheaply in bulk. Besides, very large users such as Medicare, Medicaid and Health Maintenance Organizations (HMOs) have become far more cost conscious than were the traditional health insurance companies. This has led to waning of interest in the merits of certain drugs compared with other drugs and increased consciousness of their market price. And, "discount battles" among the large multinationals have caused a selective fall in prices, especially of antibiotics. The implications of how the drug multinationals will adjust to these changes and its effect on the consumers, especially in the developing countries is hard to foresee. However, in the U.S.A., thousands of sales representatives who used to go round distributing "samples" to convince practitioners of the merits of a particular "brand name" medicine are on the way out. M/s Merck have announced its intention of manufacturing selected generic medicines without their well known patented names.

In India, the 1986 National Drugs Policy is still under discussion and reformulation, perhaps because of the need to await the detailed implications of the Dunkel draft proposals before GATT. However, it has been made known that prices of essential and life-saving drugs shall be kept under control. Already, a steep hike in the prices of non-essential drugs, which are not under

price control, has occurred and many manufacturers have either stopped or slowed down on supplies of essential drugs in the market. As the current "liberalisation policy" proceeds, pushing up the cost of imported basic formulations, the cost spiral is likely to go up sharply and certain essential but cheap drugs, like INH, for treating tuberculosis may not be freely available.

CONDOM TO PREVENT AIDS

The use of condom to prevent pregnancy, sexually transmitted diseases and now the unbeatable AIDS has focussed attention on certain important attributes of condom.

Reported condom breakage rates during use range from 1% to 18% : The major causes of condom breakage relate to the quality of condom and user practice. A recent study of different batches and ages of condoms tested by the "mean air burst volume" and "elongation at break" has shown that these correlated well with the breakage rates during use. Condom age (along with heat and humidity) leads to a decrease in air burst volume (normally around 30 litres). And elongation at break, due to stiffening, varies with type of condom and time elapsed after manufacture. Quality control at the factory can mimic the effect of shelf aging by applying regulated heat in an oven. Since condom breakage does not occur randomly and is found more among certain users, the importance of user practice and extent of lubrication assume importance. It is well documented that the application of oil-based lubricants may actually damage condoms : in one laboratory study, 60 seconds' exposure to a mineral oil decreased the condom strength by about 90% (measured by air inflation test).

Research on making condoms strong and more reliable is centred on thickness of condom, use of latex and non latex material, incidence of slippage and effect of education on its use. Factors in the evaluation of female condom will have to be different.

HIV, TB, AND PCR

The discovery of a cellular enzyme called "DNA Polymerase" and the progress made in the identification and characterization of the specific nucleic acid fragments (Southern Blot technique) have led to the evolvement of a very useful, though highly sophisticated and expensive, laboratory technique called Polymerase Chain Reaction or simply PCR.

The DNA Polymerase helps in nature in the replication and repair of cellular DNA by attaching additional nucleotides to the shortened nucleotide primer. The PCR allows the specific amplification of the discrete identified fragments of DNA by the added Polymerase, several fold by mimicking the natural DNA replication.

The PCR, conceived and elaborated in the late eighties, thus, can start with a single molecule of the genetic material and generate millions of molecule copies, in a few hours, through its exponential amplification/replication. PCR has in fact revolutionized molecular biology and enabled scientists to address the issues of specificity and sensitivity by characterizing the nucleic acid molecules present in a provided specimen, even in a minute picogram quantity. The specimen can be any biological tissue piece or similar material.

The Primer directed enzymic amplification of the specific DNA is done through the repetition of a set of three steps, in succession, carried out under different controlled temperature conditions *in vitro* : Denaturation, Annealing of Extension Primers and Extension/Amplification, as a single cycle of PCR. The exponential accretion of "PCR - amplified" product is not an indefinite process.

After 30-50 cycles, the amplification efficiency declines to a non-specific amplification and the process must be stopped at that stage.

The field of application of PCR technology has been rapidly expanding. The research application comprises mainly of cloning and sequencing for laboratory experimentation. Several diseases such as HIV, neonatal HIV, human T-cell leukemia, human papilloma cervical cancer of viral origin, rubella, mumps, Epstein Barr virus, hepatitis B and hepatitis C virus, tuberculosis, chlamydial and protozoal infections, etc. can be correctly diagnosed by PCR through the genomes of the different pathogens and their sequences. In diagnostic use, PCR is extremely sensitive as it can identify even a single molecule of the concerned nucleic acid compared with the Dot Blot test which needs a high quantity of the pathogen genome. It is well known that the ELISA test, which is extensively used for serosurveillance of HIV infection, based on the presence of anti-bodies in serum, is not so specific. Therefore, all ELISA positive sera have to be confirmed by the Western Blot Test. It is also known that HIV virus can completely shut itself off within the human body and its presence in new born babies of HIV positive mothers may not be known till they are 18 months old, on account of mother's antibodies. PCR can not only recognise the HIV genome but even whether the anti-HIV drugs are proving effective. In neonates, if PCR test shows that the baby is free of HIV, breast feeding has to be stopped immediately to prevent subsequent infection from the infected breast milk of the HIV positive mother. Thus, PCR can prove a boon when the diagnosis of tuberculosis and several of the diseases caused by viruses is in doubt.

NEWS & NOTES

44TH TB SEAL CAMPAIGN

The 44th TB Seal Campaign organised by the Tuberculosis Association of India and its affiliates in the States was inaugurated on 2nd of October, 1993, by Hon'ble Dr. S.D. Sharma, President of India and Patron, Tuberculosis Association of India, at Rashtrapati Bhawan. The 44th TB Seals were presented to Rashtrapatiji for release by Dr. A.K. Mukherjee, Director General of Health Services & Chairman, T.A.I., in the presence of representatives of the Tuberculosis Association of India, Delhi TB Association and some special invitees.

HEALTH VISITORS' COURSE

The 1994-95 TB Health Visitors' Course will commence in July, 1994. The Course will be of nine months' duration and will be held at the New Delhi TB Centre. The minimum qualification for admission to this Course is 10 +2 with Science and/or Hygiene subjects in Matriculation (equivalent to 10th Standard). Application forms for admission to the Course can be had from the Secretary-General, Tuberculosis Association of India, 3, Red Cross Road, New Delhi-110 001. The last date for receipt of application is 30th April, 1994.

CHANCHAL SINGH MEMORIAL AWARD-1994

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 pages (approximately 6,000 words excluding charts and diagrams) on a subject relating to TB. 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-100 001, before the 31st of July, 1994.

ESSAY COMPETITION-1994

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 the 1994 competition is "Gastro-intestinal tuberculosis". 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-110 001, before the 31st of July, 1994, with a certificate that the author is a final year medical student.

REFRESHER COURSE ON TB & CHEST DISEASES

The TB Association of Andhra Pradesh and the Vizianagaram Dist. TB Association organised a Refresher Course on "TB & Chest Diseases" on 9th October, 1993 at Zilla Parishad Medical Hall, Vizianagaram. The course was inaugurated by Shri V. Nagi Reddy, Collector & District Magistrate while Dr. A.V. Rama Chandra Rao, District Medical & Health Officer, Vizianagaram, presided over the function. About 50 doctors attended the course.

TB OFFICER'S CONFERENCE

The Rajasthan TB Association organised a two-days' workshop of Tuberculosis Officers at TB Demonstration Training Centre, Ajmer, on 15th and 16th September, 1993. The work shop was inaugurated by Dr. B.L. Gupta, Director Medical & Health Services, Rajasthan.

INTERNATIONAL DECLARATION OF HEALTH RIGHTS

The Johns Hopkins School of Hygiene and Public Health Baltimore (U.S.A.), one of the foremost and reputed "citadels" of public health research, education and practices has begun

promoting as International Declaration of Health Rights, since 1991. Formulated by the school's faculty, students and alumni, the declaration embodies the highest aspirations of any public health professional's calling.

Without question, we have unprecedented opportunities for improving the health of the people. The debate is now rising about how medical care is to be delivered, where, and by whom to how a society should regards sickness, medical treatment, prevention and positive health. The resurgence of Tuberculosis in the developed world, and how to prevent AIDS in affluent societies have opened many a closed mind to public debate on issues which were regarded as concerns of the developing countries alone.

HEALTH CHECK-UP CAMPS

A general health check-up camp was organised at Yennaram, Ramannapet Mandal, Nalgonda District, Andhra Pradesh, on 25th July, 1993, under the auspices of the TB Association of Andhra Pradesh and the Cosmopolitan Employees' Cultural Association, Hyderabad. The camp was inaugurated by the District Medical and Health Officer, Nalgonda and presided over by the Mandal Revenue Officer Shri R. Ramachandra Rao. The Sarpanches and Village heads also attended the camp.

Around 700 persons were examined for presence of any morbidity including tuberculosis by a number of experts who volunteered their services free.

Another health check-up and BCG Vaccination Camp was organised at Afzal Sagar, Mallepally, Hyderabad, on 17th October, 1993. Shri Syed Sajjad, M.L.A., inaugurated the Camp. Dr. R. Ram Prasad, was Chief Guest of the day. Dr. N.

Kumar Rao, Director of State TB Centre, Irramnuma, Hyderabad, gave the presidential address. Medical officers from the State TB Centre and Mahaveer Hospital participated in the camp. The Director, State TB Centre, Hyderabad deputed the technicians for the sputum examination, as well as for X-ray examination of the chest with M.M.R. Mobile X-ray plant. BCG vaccination was also given to the children. In all about 180 symptomatics were examined by X-ray and sputum; 52 children were offered BCG vaccination.

17TH EASTERN REGIONAL CONFERENCE

The 17th Eastern Regional Conference on Tuberculosis and Respiratory Diseases of International Union Against Tuberculosis and Lung Disease, was held in Bangkok (Thailand) from 1st to 4th November, 1993. Shri Ashok Sachdeva, Secretary General, Tuberculosis Association of India, attended the Conference as representative of the National Association and presented a paper entitled "Role of Tuberculosis Association of India in Fight against TB". The Secretary General also attended the Council Meeting of the Eastern Region of the IUAT-LD, during the Conference and was taken as a member on the Executive Committee of the Eastern Region of the IUAT-LD.

HONOURED

Shri Ashok Sachdeva, Secretary-General, Tuberculosis Association of India, was presented with the *Scroll of Honour* by the Lt.-Governor of Delhi and Patron-in-Chief of Delhi Tuberculosis Association, at its Annual General Meeting held on 15.10.1993.

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ABSTRACTS

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ISONIAZID INDUCED PERIPHERAL NEUROPATHY IN THE TREATMENT OF PULMONARY TUBERCULOSIS

Rajeswari Ramachandran, R. Parthasarathy, S. Sivasubramanian, P.R. Somasundaram, P. Venkateshan and R. Prabhakar Neurology India, 38, 517, (1990)

Isoniazid induced peripheral neuropathy was observed in 48 patients among 2502 treated for pulmonary tuberculosis, admitted to 10 different clinical trials at the Tuberculosis Research Centre, Madras, over a period of 20 years. This predominantly sensory neuropathy was four times more common among slow acetylators than rapid acetylators, and was dose related, i.e. 0.3% with 5-7 mg/kg daily and 25% with 13-14 mg/kg daily. But when isoniazid was given intermittently, in the same high dosage of 13-14 mg/kg, the neuropathy was only 1.3%. The onset of neuropathy was earlier in patients receiving 13-17 mg/kg than those receiving 5-9 mg/kg, the means being 3.6 and 6.6 months, respectively. The resolution took 14.9 months, on an average, with high Isoniazid dosages, compared with 7.0 months with the low dosage. Pyridoxine 6-10 mg supplement with every dose of Isoniazid reduced the incidence of neuropathy, irrespective of the acetylator phenotype.

R.R.

EVIDENCE FOR *IN VIVO* GENERATION OF CYTOTOXIC T CELLS

Faizel Lorgat, Mustapha M. Keraan, Pauline T. Lukey, and Stanley R. Ress : Am. Rev. Respir Dis.; 1992, 147, 418.

Cytotoxic T Cells (CTL) are currently thought to play an important role in protection against mycobacteria. It is thought that these cells lyse parasitized macrophages and release the

organisms, exposing them to alternative immune mechanisms.

The hypothesis was that *M. tuberculosis* is a bacterial pathogen capable of survival and replication within human macrophages. Cytotoxic T cells are considered important for the eradication of infected macrophages.

Pleural effusion lymphocytes from patients with tuberculosis pleuritis were stimulated *in vitro* with PPD, and proliferation and cytotoxicity were assessed by thymidine incorporation and chromium release respectively.

This study shows significantly enhanced antigen specific cytotoxicity by pleural effusion lymphocytes obtained from patients with tuberculous pleuritis compared with autologous peripheral blood lymphocytes and normal donor peripheral blood lymphocytes, as well as non-tuberculous effectors.

In future, accelerated proliferative and cytotoxic cellular immune response occurring only in TB patients will be suggestive of TB (diagnostic) and can be used for therapeutic purposes by accelerating recovery if *in vivo* cytotoxicity can be boosted by the administration of subcutaneous lymphokines (already demonstrated in leprosy).

V.K.A.

ISONIAZID ASSOCIATED HEPATITIS DEATHS : A REVIEW OF AVAILABLE INFORMATION

Dixie E. : Snider Jr. and Gus J. Garas Am. Rev. Respir. Dis.; 1992, 145, 494.

Soon after the introduction of INH it was realised that it could cause toxic hepatitis and, rarely, death. Reports of death from INH associated hepatitis continue to appear in the literature.

A retrospective study was done to find out

deaths occurring due to INH hepatitis in patients on INH prophylaxis and not on multiple drugs. Data submitted to the Centres for Disease Control (CDC) in U.S.A. by State and local health departments on the number of persons placed on INH preventive therapy and the number and proportion who completed therapy for the years 1972 through 1988 were used.

It was observed that risk is more with advancing age, sex (female) and liver disease. Deaths due to INH hepatitis are less frequent now than in 1970s but are still occurring.

There are a number of limitations in the study and the information obtained is more valuable for generating a hypothesis than for arriving at definitive conclusion.

V.K.A.

COMBINED TOXICITY OF ZIDOVUDINE AND ANTITUBERCULOSIS CHEMOTHERAPY

Diana Antoniskis, Ann C. Easley, Byron M. Espina, Paul T. Davidson and Peter F. Barnes : *Am. Rev. Respir. Dis.*; 1992, 145,430.

HIV infected patients with tuberculosis commonly have CD4 cell count of less than 500/mm³ and are therefore candidates for Zidovudine therapy. Published data evaluating the toxicity of concomitant Zidovudine and antituberculosis agents is limited and, hence, cohort study was designed to determine the same in patients with dual infection.

A group of 24 consecutive HIV infected patients with tuberculosis who received concomitant Zidovudine and antituberculosis drugs were compared with same number of patients who received only Zidovudine.

Mean haemoglobin was lower for TB patients than for controls. The maximum fall in haemoglobin percentage was same for both groups. Severe anaemia developed in more tuberculosis patients than in controls during therapy (50% vs 17%). Concurrent administration of antituberculosis medication and Zidovudine is safe (more than 8 months). Side effects of Zidovudine like leukopenia, granulocytopenia and myopathy were not increased with antituberculosis medication.

Careful monitoring of haematologic toxicity is essential when zidovudine and antituberculosis

medication are administered concomitantly.

V.K. A.

ARTERIOVENOUS DIFFERENCE IN THE HEMOSTASIS SYSTEM IN PULMONARY TUBERCULOSIS PATIENTS

A.I. Makinsky and V.V. Makovetsky. *Problems, of Tuberculosis, Moscow*; 1992, 12, 11.

Effective treatment of the main focus of a specific lesion during preoperative management and improvement of liver function increases the prospects of controlling thromboembolic complication of surgery in pulmonary tuberculosis.

S.C.K.

BCG REVACCINATION IN SCHOOL CHILDREN AT LOW TUBERCULOSIS INCIDENCE

L.V. Ledeva et al; *Problems of Tuberculosis, Moscow*; 1992, 1-2, 15.

School children in Moscow area were divided into 3 groups (after preliminary BCG vaccination in infancy). Group 1 did not receive any revaccination, Group 2 were revaccinated at 12 years and Group 3 at 7, 12 and 17 years. There was no difference in the incidence of tuberculosis among the three groups.

S.C.K.

DIAGNOSTICA E TERAPIA DELLE FLOGOSICAVITARIE ACUTE DEL POLMONE (ASCESSO E CANCO-ASCESSO)

Parola, D. et al: *Lotta Contro La Tuberculosis E Le Malattie Polmonari Sociali*; 1992, 62, 30.

A study of 22 cases with acute pulmonary inflammation and cavitation (usually with fluid level), and average age 55 years, who, apart from usual clinical and bacteriological examination, underwent fiberoptic bronchoscopy with bacteriology and cytology and computerized thoracic tomography. Eight (36.4%) were found to have neoplastic disease while the balance 14 had inflammatory process. The latter responded well to 'standard' treatment.

S.C. K.

DIAGNOSTIC EVALUATION OF ASCITIC ADENOSINE DEAMINASE ACTIVITY IN TUBERCULAR PERITONITIS

Gupta, V.K., Mukherjee, S., Dutta, S.K. and Mukherjee, P. J.A.P.I.; 1992, 40, 381.

ADA activity in serum and peritoneal fluid was studied in patients with proven malignant ascites, cirrhosis and tubercular peritonitis. Whereas there was no consistent pattern of change or otherwise in patients of cirrhosis and malignant peritonitis, distinct rise in activity was noticed in the ascitic fluid of those with tubercular peritonitis. ADA level of 30 units/L in ascitic fluid had a sensitivity of 100% and specificity of 94.1%.

S.C.K.

RANITIDINE-RIFAMPICIN INTERACTION.

Purohit S.D. et al. J.A.P.I.; 1992,40, 308.

In a controlled trial, equal number of patients on Rifampicin regimes were given Ranitidine or placebo. Ranitidine increased basal and post-drug gastric pH without affecting the absorption, metabolism or excretion of Rifampicin. The frequency of gastrointestinal side effect was reduced by about 50%. Hepatic side-effects were not reduced by use of Ranitidine.

S.C.K.

ADULT RESPIRATORY DISTRESS SYNDROME FOLLOWING NON-THORACIC SKELETAL TRAUMA

Ramamurthy, T.V., Bansal, V.P. and Jindal S.K.

J.A.P.I., 1992,40, 314.

Close monitoring of 30 adults with severe non-thoracic skeletal injury revealed 3 who developed ARDS. The authors suggest that such injured persons be observed closely to enable early recognition and treatment of this dreaded complication.

S.C.K.

REDUCED NATURAL KILLER CELL ACTIVITY IN MULTI-DRUG RESISTANT PULMONARY TUBERCULOSIS.

Rateliffe, L.T. et al., Scand. Jour. Immunol.; 1992, 36 (Suppl. 11) 167.

Five patients of active pulmonary tuberculosis, free of HIV infection and with multi-drug resistance were compared with five similar cases, with drug sensitive infection. The resistant patients had highly significant reduction of natural killer cell activity, at each of 4 effector cell : target cell ratios. There was no significant difference in lymphocyte phenotypes, lymphocyte proliferation or PPD specific toxicity. The authors suggest that inherent NK function defect, by failure to reduce the total bacterial count, contributes to emergence of resistant strains by positive selection once drug therapy is begun. Further similar studies are necessary to determine if this suppression of NK activity is really secondary to resistant mycobacterial infection.

S.C.K.

