The Indian Journal of Tuberculosis

Vol. 54 **January, 2007** **No. 1**

Editorial

XDR-TB - DANGER AHEAD

(***Indian J Tuberc 2007; 54:1-2***)

XDR-TB, a virtually untreatable form of tuberculosis – extremely drug resistant TB (XDR-TB) - is spreading all over the world, including India. It is a grave public health hazard that is taking alarming proportions.

Multi-Drug Resistant Tuberculosis (MDR-TB) is defined as strains of tuberculosis that are resistant to at least the two main first-line TB drugs – Isoniazid and Rifampicin. During 1990's, MDR TB emerged as a threat to TB control worldwide[6]. XDR-TB or extremely drug-resistant TB is a variety of MDR-TB that is also resistant to three or more of the six classes of second-line drugs. XDR-TB was first described in early 2006, following a joint survey by the US Centre for Disease Control and Prevention (CDC), WHO and the Supranational Reference Laboratory Network (SRLN) as agreed upon by the WHO Global Task Force on XDR-TB[7]. The survey of over 17,000 patients from 48 countries identified XDR TB patients in all regions of the world[1]. In the United States, 4% of MDR-TB cases met the criteria for XDR-TB. A survey conducted by WHO and the US Centre for Disease Control on data from 2000-2004 found that XDR-TB is most frequent in countries of the former Soviet Union and Asia. The XDR-TB strain is mixing with the AIDS virus, causing nearly 100% mortality. If this XDR-TB strain mixes with HIV, the combination is explosive.

In Latvia, a country with one of the highest rates of MDR-TB, 19% of MDR-TB cases met the XDR-TB criteria. Separate data on a recent outbreak of XDR-TB in an HIV-positive population in KwaZulu-Natal in South Africa were characterized by alarmingly high mortality rates. The outbreak of MDR-TB among HIV-infected patients in Durban, South Africa was reported to be the leading cause of death in HIV patients in Sub-Saharan Africa[2,3].

Integration of care and treatment for co-infected patients may improve outcome of both diseases but can be jeopardized by inadequate infection control facilities and practices and rising MDR-TB rates. In a study, out of the total MDR isolates, they detected 53 XDR TB cases (24%) which is alarming. Increased surveillance in rural South Africa revealed high prevalence of MDR and XDR TB with evidence of recent nosocomial and community transmission in HIV co-infected patients. The presence and consequence of high rates of MDR/XDR TB and HIV is a deadly threat to gains in survival achieved by TB DOTS and antiretroviral therapy. Implementation and evaluation of facilities and procedures to reduce community and nosocomial transmission are urgently needed.

There is however further bad news – XDR TB strain is mixing with AIDS virus causing nearly 100% mortality. WHO's[4] Coordinator for TB/HIV and drug resistant programme Paul Nunn calls it "an explosive combination". With the threat extremely serious, Nunn is holding an urgent meeting in Johnesburg to address the management of this deadly strain of TB. At present, South Africa is the worst-affected by this strain.

Nunn informed that 52 of the 53 patients with XDR-TB in South Africa died within 210 days, between January 2005 to March, 2006. The deaths occurred on an average within 25 days even in those HIV patients who were taking anti-retroviral drugs. Even in the United States[5], which has the best medicines available, a third of those who were diagnosed with XDR TB have died.

Nunn said "XDR-TB Problem is relatively small in India right now. But the worrying thing is that it is there. India must prepare its defences immediately like South Africa, India has a severe burden of HIV patients. If this XDR strain mixes with HIV, the combination is explosive. India must immediately undertake a survey to gauge the extent of XDR-TB presence, especially in an HIV burden area like Mumbai" and other places.

Nunn has several other suggestions for India: increase the number of labs to diagnose TB cases, improve management of clinical cases, strengthen basic TB care to prevent the emergence of drug-resistance and increase collaboration between HIV and TB control programmes to provide necessary prevention and care to co-infected patients.

According to India's Health Ministry records, over 3% of the fresh cases suffering from XDR TB while over 12% of old cases undergoing treatment have developed this strain. This new strain is not only resistant to the two first-line therapies – antibiotics Isoniazid and Rifampicin but also to the other six classes of drugs used for second line therapy. TB has also become the single largest killer of AIDS in India.

National AIDS Control Organisation recently revealed that over 60% of all AIDS patients contract and ultimately die of TB. NACO has now decided to scale up and integrate the National AIDS and TB control programmes from 2006.

We have to seriously think, how did we get here and what are we doing about it? The problem is very challenging and serious since we have no new drugs discovered since 1968. Bacilli are now becoming drug resistant. Researches are already under way looking at new drugs, including research into TB vaccines. Clinicians can adopt WHO Guidelines for control of XDR-TB and can prevent XDR-TB by ensuring that these guidelines in their countries are adopted according to the international standards for TB care.

M.M. SINGH

REFERENCES

1. Final Programme of the IUATLD's 37th World Conference on Lung Health held in Paris in October-November, 2006
2. Friedland G, Moll A, Gandhi N.R., Pawinski R, Andrews J, Zeller K *et al*. Outbreak of MDR-TB among HIV-infected patients in Durban, South Africa. *Int J Tuberc Lung Disease* 2006; **10(11 Suppl 1)**: S6
3. Neel R Gandhi, Anthony Moll, Willem A Sturm *et al*. Extensively drug- resistant uberculosis as a cause of death in patients co-infected with TB and HIV in a rural area of South Africa. *Lancet* 2006, Nov (4); **368 (9547**): 1547-80
4. WHO Emergence of XDR TB. WHO concern over extensive drug resistant TB strains that are virtually untreatable
5. Trends in tuberculosis in United States, 1998-2003. *MMWR Morb Mortal Weekly Rep* 2004; **53**: 200-14
6. Dooley SW, Jarvis WR, Martone WJ, Snider DE. Multi-drug Resistant Tuberculosis. *Ann Intern Med 1992*; **117**: 257-9
7. Stop TB Partnership. The global plan to stop TB 2006-2015. Executive Summary 2006

Guest Lecture

MANAGEMENT OF MULTI-DRUG RESISTANT TUBERCULOSIS : PRACTITIONER'S VIEW POINT

Rajendra Prasad

Summary: Multi-Drug Resistant Tuberculosis (MDR-TB) is a growing hazard to human health world wide and threat to control of tuberculosis. Current estimates report the prevalence of primary and acquired MDR-TB in India as 3.4% and 25% respectively. MDR-TB is suspected if sputum is persistently +ve for AFB along with clinical and radiological deterioration after multiple courses of irregular or regular treatment including 4 months of WHO retreatmant regimens under direct observation . Diagnosis is confirmed by drug susceptibility testing from reliable and reputed laboratories under constant quality control. Reports of susceptibility should not be accepted uncritically. Treatment of MDR-TB should be at a specialized centre with standard microbiology laboratory facilities. Though treatment guidelines including standardized, empirical and individualized approaches have been laid down by the WHO but therapy should be tailored to the needs of the particular patient. Treatment of MDR-TB is difficult, complicated, much costlier, challenging and needs experience and skills. All measures should be taken to persuade and encourage patients not to stop treatment despite all its discomforts to prevent morbidity, mortality and transmission of MDR-TB. Current proposal of DOTS Plus by WHO highlights the comprehensive management strategy to control MDR-TB. MDR-TB is a man-made problem and its emergence can be prevented by prompt diagnosis and effective treatment of all TB cases. Adoption of directly observed treatment short course (DOTS) to prevent the resistant/multi-drug resistant strains and careful introduction of second line drugs to treat patients with MDR-TB are the top priorities for the proper control of MDR-TB. ***[Indian J Tuberc 2007; 54:3-11]***

Key words: Multi-Drug Resistant Tuberculosis, Diagnosis, Treatment

INTRODUCTION

Drug resistant tuberculosis has been reported since the early days of introduction of anti-tubercular chemotherapy, but recently multi-drug resistant tuberculosis (MDR-TB), has been an area of growing concern, and is posing threat to global efforts of tuberculosis control. Prevalence of MDR-TB, in a community mirrors the functional state and efficacy of tuberculosis control programme and realistic attitude of the community towards implementation of such programmes[1]. Management of MDR-TB is difficult, much expensive, challenging and quite often leads to treatment failure. The present write-up focuses on the management of MDR-TB.

DEFINITION

Multi-drug resistant tuberculosis is defined as disease due to *M tuberculosis* that is resistant to Isoniazid (H) and Rifampicin (R), with or without resistance to other drugs. Primary drug resistance is defined as drug resistance in a patient who has not received any anti-tubercular treatment in the past, while acquired drug resistance is defined as resistance that develops in a patient who has received prior chemotherapy. Recently the terms "resistance in new cases" and "resistance in previously treated cases," have been proposed for use because of the difficulty to confirm the validity of the patients' past history of treatment. When one is not sure whether the resistance is primary or acquired or unaware of patient's previous treatment, drug resistance is known as initial drug resistance.

MAGNITUDE OF PROBLEM

Global: First information on global magnitude of MDR-TB came in 1997, when WHO - IUATLD reviewed 63 surveys, and reported that range of primary MDR-TB varied from 0-10.8% and acquired MDR-TB varied from 0-48% in various studies[2]. In most regions of the world, the rate of MDR-TB was very low and varied considerably throughout the world, which was due to difference in degree of patients studied, the degree of misuse of drugs, the quality of enquiry regarding previous treatment and inadequate culture and drug susceptibility facilities

The Dr. O.A. Sarma Guest Lecture delivered at the Lucknow Conference, 2006.

Correspondence: Dr. Rajendra Prasad, Prof. & Head, Deptt. of Pulmonary Medicine, K.G. Medical University, Lucknow-226 003. E-mail: rprasad2@sancharnet.in; rprasad2@rediffmail.com. Phone (O) 0522-2255167, (M) 09415021590.

in many parts of the world. Considering the limitations of previous studies, a WHO – IUATLD global project on anti-tubercular drug resistance surveillance spread over 35 countries in 5 continents was carried out during 1994-97[3], which reported the median prevalence of primary and acquired MDR-TB as 1.4% and 13% respectively. In this project, regions with prevalence of MDR-TB greater than 5% were labelled as MDR hotspots. Subsequent second report of this global project[4] conducted from 1996-99, reported the median prevalence of primary and acquired MDR-TB as 1% and 9% respectively. Most of the previous hotspots were confirmed again, while new areas in Russia and China were added. The analysis trend has confirmed that MDR-TB is not a major problem in countries implementing tuberculosis control according to international guidelines for several years. Countries like Botswana, Chile, Cuba, Czech Republic and Uruguay have shown very low prevalence of MDR-TB, confirming that efficient tuberculosis control prevents the development and spread of MDR-TB. The third global surveillance of WHO – IUATLD, carried out in 1999-2002, reported median prevalence of primary and acquired MDR-TB as 1.1% (0-14%) and 7% (0-58.3%) respectively[5]. After analysis, the cut-off value for hotspots was reset to 6.5% in this new report[5].

India: Clinical perception and several isolated reports indicated the development of drug resistance in India, since the beginning of chemotherapeutic era, but they failed to give an idea of national situation as a whole. Pioneering step in this direction was taken by Indian Council of Medical Research (ICMR) in 1965-67 when it conducted two surveys to estimate the prevalence of drug resistance[6,7]. Several studies conducted subsequently in different parts of country, revealed that the total prevalence of primary / initial MDR-TB varies from 0-5%[8-19]. The rates of acquired MDR-TB varies from 6 to 60%[3,9,14,19-23]. The median prevalence of primary and acquired MDR-TB in India according to WHO-IUATLD report on global drug resistant surveillance, conducted between 1996-99 is 3.4% and 25% respectively[4]. Despite the variable results of acquired MDR-TB, the message is very clear that it is not in isolated pockets, but in the country as a whole.

RISK FACTORS OF DRUG RESISTANCE

Three most important risk factors, identified in the causation of drug resistant tuberculosis are- inappropriate previous treatment with anti-tubercular drugs, high prevalence of drug resistant tuberculosis in the community and contact with patients known to have drug resistant tuberculosis. However standardized short course chemotherapy carries a little risk of emergence of MDR-TB. Other factors that may be responsible for increased risk of resistant tuberculosis are : Co-infection with HIV, socio-economically deprived groups in slums, prisons, correctional facilities, day care centres, intravenous drug abusers and other immuno-compromised states as in transplant recipients, anti-cancer chemotherapy recipients and patients with diabetes mellitus.

SOURCES AND CAUSES OF DRUG RESISTANCE

Multi Drug Resistant Tuberculosis is a man made problem. Blame for this goes to the government, the pharmaceutical industry, doctors, patients and their families, each of whom contributes in his/her own way to this problem. The government plays its share by providing poor infrastructure in the National Tuberculosis Control Programme, unnecessary administrative control on purchase with no proper mechanism on quality control and bioavailability tests. The pharmaceutical industry contributes by manufacturing drugs of uncertain bio-availability in fixed dose or inappropriate drug combinations, poor storage condition of drugs and substitution by inferior quality drugs by pharmacies. The doctor, by his lack of knowledge regarding doses, duration of treatment, side effects and standard regimens, frequent change of brand names and poor patient motivation, contributes the lion's share to the problem. In one of the studies where prescriptions of 449 doctors were analyzed, 75% of the doctors were found to have made some prescription error [24]. Added to this is the poor teaching and training facilities for them. Non-compliant patients due to monetary lack, lack of information, side-effects of drugs and social myths and misconceptions often do not adhere to treatment. Co-morbid conditions like diabetes, HIV, psychiatric conditions, the habits of smoking and alcoholism

make the patient more vulnerable. To sum up, drug resistant tuberculosis usually results from inadequate drug therapy in multi-bacillary cases of tuberculosis, addition of single drug in cases of failure, difficulty in obtaining drugs by the poor due to lack of financial resources or social insurances, frequent shortage of second line anti-tuberculous drugs by poor management and/or financial constrains, use of drugs or combination of drugs (FDC) with unproven bioavailability, lack of motivation at the beginning of treatment and inadequate self-administration of drugs without direct observation in the intensive phase of therapy.

DIAGNOSIS OF MULTI-DRUG RESISTANT TUBERCULOSIS

It is needless to emphasize that early diagnosis and treatment of drug resistant tuberculosis is of paramount importance not only from the patient's perspective but also for the community at large. The suspicion of MDR-TB occurs in following situations:

1. History of contact with known cases of Drug Resistant / MDR-TB patients.

2. History of many courses of irregular / regular treatment of tuberculosis.

3. Clinical deterioration is the least reliable evidence of treatment failure. If there is no accompanying bacteriological or radiological deterioration, clinical deterioration is unlikely to be due to tuberculosis.

4. Radiological deterioration in chest radiograph may be a sign of treatment failure. Increase in size of cavities, increase in existing lesion and appearance of new lesion are usually signs of disease progression. One should also realize that deterioration in chest radiograph, may be due to intercurrent pneumonia, pulmonary embolism or supervening carcinoma. Therefore, radiological evidences are less reliable. However, radiological worsening in addition to positive sputum and / or clinical worsening can indicate resistant tuberculosis.

5. Persistent positive sputum smear for AFB even after 4/5 month WHO retreatment regimens.

6. Fall and rise phenomenon in which sputum smear initially becomes negative (or even less positive), and then later becomes persistently positive. This indicates failure usually due to either the patients having ceased to take the drugs or to the development of resistance to all the drugs patient is receiving.

7. Report of sensitivity results indicating resistance to at least Isoniazid and Rifampicin is gold standard for the diagnosis of MDR-TB. However, one has to keep in mind the limitation of highly specific test because the technique is complex and difficult to perform accurately even when skilled personnel are available and laboratory facilities are of a high standard. Further one should also realize that laboratories vary in reliability, error occurs in labs, different sensitivity reports are obtained of the same patient from different laboratories. There is a lack of standardization, coordination and cross-checking with national and supranational reference laboratories in our country. Keeping above background in mind, it is pertinent that sensitivity test result should not be accepted uncritically, they should always be correlated with history, smear results and x-ray and should be used as a guide for future therapy and should not dictate treatment options. Therefore there is urgent need to develop standard laboratories under quality control of national and supranational reference laboratories in our country.

8.Newer molecular techniques like DNA sequencing, Line Probe Assay (LiPA), DNA microarrays, molecular beacons, Single strand confirmation polymorphism, fluorescent Resonance Energy Transfer probes, other PCR based techniques and Mycobacteriophages based assays like FAST Plaque TB and Luciferase receptors phages (LRPs) have been used for identification of resistance associated mutation. The expectation that molecular techniques would surpass conventional methods has yet not been realized because most of techniques still require detailed and systemic evaluation using standard techniques

as references before their application in clinical setting. These techniques might be used as complement to the standard methods in situation of difficult diagnosis but should never be used solely for diagnosis of drug resistance[25].

TREATMENT OF MULTI-DRUG RESISTANT TUBERCULOSIS

The management of multi-drug resistant tuberculosis is an area that has been shrouded in a lot of myths and misconceptions, and therefore utterly chaotic. Though treatment guidelines, including standardized, empirical and individualized approaches have been laid down by the WHO, but therapy should be tailored to the needs of the particular patient.

Basic principles of chemotherapy in multi-drug resistant tuberculosis[26-30]

1. Treatment should be in a specialized centre with standard laboratory facilities.

2. Early diagnosis of MDR-TB and prompt initiation of treatment are important for successful outcome.

3. Designing an appropriate regimen needs experience and skill. Regimen should be based on previous history of anti-tuberculous drugs taken by the patients. Drugs susceptibility test when available from reliable laboratories should be used to guide therapy.

4. Regimens should contain at least four drugs that are highly likely to be susceptible based on drug susceptibility test and/or previous intake of anti-tuberculous drugs by the patient. Often more than four drugs may be started if the susceptibility pattern is unknown, if effectiveness is questionable for an agent(s) or if extensive, bilateral pulmonary tuberculosis is present.

5. Use any first line oral agent to which isolate is sensitive. Use one injectable, one fluoroquinolone and add as many second line bacteriostatic agents as needed to complete the regimens. Injectable agent should be continued for atleast six months.

6. Never add a single drug to a failing regimen.

7. It is ineffective to combine two drugs of the same group or to combine in the prescribed chemotherapy regimen a drug potentially ineffective because of cross-resistance. Cross resistance has been reported between Thioamides and Thioacetazone, Kanamycin/ Amikacin with Streptomycin[25,26]. Rifampicin with Rifapentine, Rifabutin (>70% strains) and among various derivatives of fluoroquinolones. Cross-resistance has also been reported between Ethionamide and Isoniazid, Viomycin and Kanamycin, Viomycin and Capreomycin. Strains resistant to Streptomycin/Kanamycin/Amikacin are still sensitive to Capreomycin.

8. All the drugs should be given in a single daily dose preferably, except PAS which is usually given in two divided doses in order to avoid problems of intolerance. Among Thioamides, Prothionamide is better tolerated than Ethionamide.

9. Intermittent therapy is usually not effective and should be avoided in multi-drug resistant tuberculosis.

10. No drug should be kept in reserve and the most powerful drugs (bactericidal) should be used initially and in maximum combination so as to ensure that the first battle is won and won permanently.

11. Therapy should be under direct observation preferably for 3-4 months or till the sputum conversion.

12. Surgical treatment should be considered as an adjunct to chemotherapy wherever applicable, as results of chemotherapy are very unpredictable.

13. All measures should be taken to persuade and encourage patients not to stop treatment despite

Table 1: Second line anti-tubercular drugs

Drugs	Average daily dosage	Daily dosage (mg)		Type of anti-mycobacterial activity
		Minimum	**Maximum**	
Aminoglycosides Kanamycin Amikacin	15 mg/kg	500-750	1000	Bactericidal against actively multiplying organisms.
Polypeptides Capreomycin	15-20 mg/kg	500-750	1000	Bactericidal
Fluoroquinolone Ciprofloxacin Ofloxacin Moxifloxacin Gatifloxacin Levofloxcin	 20-30 mg/kg 7.5-15 mg/kg	 1500 600 400 400 500	 1500 800 400 400 750	 Weak bactericidal
Thioamides Prothionamide Ethionamide	15-20 mg/kg	500	750-1000	Bacteriostatic
Analog of D-alanine Cycloserine	10-20 mg/kg	500	750	Bacteriostatic
Salicylic Acid: Anti-Folate PAS	150 mg/kg	8 g	10 g	Bacteriostatic
Anti-tuberculosis drugs with unclear efficacy (Not recommended) (Not recommended by WHO for routine use in MDR-TB patients)				
Clofazimine	4-5 mg/kg	100	200	Bacteriostatic
Co-amaxyclav		750	2 gm	Weak bactericidal
Clarithromycin	10-15 mg/kg	1000 mg/day		Bactericidal
Azithromycin	10 mg/kg	500 mg/day		(pH dependent)
Rifabutin Thiacetazone High dose Isoniazid Linezolid	May be useful against some isolates of MDR-TB but sensitive to Rifabutin. High rate of side effect in HIV patients Animal model supports use but conflicting clinical data.			

all its discomforts, as it is the last that stands between patient and death.

DRUGS USED IN MDR-TB AND THEIR TOXICITIES

The second line drugs used for treatment of multi-drug resistant tuberculosis are given in Table 1 with their dosages in decreasing potency from top to bottom against *mycobacterium tuberculosis*. Reserve drugs are frequently associated with very high rates of unacceptable adverse drug reactions, needing frequent interruption and change of regimen. The author reported that 41% patients, experienced some side effects and only 21.1% patients required stoppage or change of drug in their study of 39 patients of MDR-TB[31]. Second line reserve drugs, their toxicities and management are given in Table 2.

REGIMEN FOR MULTI-DRUG RESISTANT TUBERCULOSIS

World Health Organization recommended regimens[26-30] without availability of sensitivity results are given in Table 3, while regimens with availability of sensitivity results are given in Table 4. In Indian

Table 2: Toxicities and their management.

Adverse Effect	Suspected Agent (s)	Suggested Management
Seizures	Cycloserine Isoniazid Fluoroquinolone	Suspend suspected agent pending resolution of seizures Start anti-convulsant therapy. Increase Pyridoxine to 200-mg/ day. Restart suspected agent in lower dose if essential to the regimens. Discontinue suspected agent if it can be done without compromising regimen.
Hearing loss	Kanamycin Streptomycin Amikacin Capreomycin Claithromycin	Document hearing loss. Change to Capreomycin. Increase frequency and / or lower the dose of suspected agent. Discontinue drug if can be done without compromising regimen. The risk of further hearing loss must be weighed against the risk of stopping the drugs.
Psychotic symptoms	Cycloserine Isoniazide Fluroquinolone Ethionamide	Hold suspected agent for short period (1-4 weeks). Initiate anti-psychotic drugs. Lower the dose of drug. Discontinue drug if it can be done without compromising regimen.
Nausea and Vomiting	Ethionamide, PAS,	Assess for dehydration, rehydrate if needed. Initiate anti-emetic therapy. Lower the dose of drug. Discontinue drug if it can be done without compromising regimen.
Gastritis	Ethionamide, PAS	H2 -blokers, proton pump inhibitors, Antacids Hold suspected agent for short period (1-7 days) Lower the dose of drug. Discontinue drug if can be done without compromising regimen.
Hepatitis	Pyrazinamide Ethionamide PAS	Stop all therapy Rule out other potential causes of Hepatitis. Re-introduce drug serially while monitoring liver function with most likely agent introduced last.
Renal Toxicity	Kanamycin Capreomycin Amikacin	Discontinue suspected agent. Consider using Capreomycin if an amino-glycoside had been prior injectable in regimen. Consider dosing 2-3 times a week if drug is essential and patients can tolerate. Adjust dose according to creatinine clearance.
Hypothyroidism	PAS Ethionamide	Initiate thyroxine therapy Completely reversible on discontinuation of drugs The combination of Ethionamide with PAS is more associated with hypothyrodism.
Hypokalaemia and Hypomagnesaemia	Capreomycin Kanamycin Amikacin	Check serum potassium levels. If potassium is low check magnesium and calcium. Replace electrolytes as needed.

set-up, most of the time either sensitivity test results are not available or if available they are usually highly unreliable. Keeping this fact in mind, depending upon past history of anti-tuberculous treatment and resistance pattern, the author himself has used regimen containing Kanamycin, Ethionamide, PAS, Cyclosrine, and Fluroquinolone in treating multi-drug resistant tuberculosis and found to be effective in [31] 71% of patients.

DURATION OF TREATMENT

The optimal duration of therapy for MDR-TB has not been clearly established and duration remains questionable. However, several authorities including the WHO recommend treatment with anti-tubercular drugs for a period of at least 18-24 months after sputum conversion or 12 months after sputum culture becomes negative to prevent relapse. Injectables are preferably used for atleast six months

Table 3: Regimen before (or without) sensitivity test result

INITIAL PHASE		CONTINUATION PHASE	
Drugs	**Minimum duration in months**	**Drugs**	**Duration in months**
Aminoglycoside[a]	6	Ethionamide	12-18
Ethionamide	6	Fluoroquinolone[b]	12-18
Fluoroquinolone[b]	6	Pyrazinamide	12-18
Pyrazinamide	6	Ethambutol +/-	12-18
Ethambutol +/-	6		

[a] Kanamycin, or amikacin, or capreomycin
[b]Ciprofloxacin or Ofloxacin

Table 4: Regimen for multi-drug resistant tuberculosis when sensitivity report available.

Resistance to	**Initial phase**		**Continuation phase**	
	Drugs	Minimum duration in months	Drugs	Duration in months
Isoniazid Rifampicin	Aminoglycoside[e] Ethionamide Fluoroquinolone[f] Pyrazinamide Ethambutol +/-	6 6 6 6 6	Ethionamide Fluoroquinolone[f] Pyrazinamide Ethambutol +/-	12-18 12-18 12-18 12-18
Isoniazid, Rifampicin, Streptomycin, and Ethambutol	Aminoglycoside[e] Ethionamide Pyrazinamide Fluoroquinolone[f] Cycloserine[g]	6 6 6 6 6	Ethionamide Fluoroquinolone[f] Cycloserine[g]	18 18 18
Resistance to all drugs	Aminoglycoside[e] Fluoroquinolone[f] 2 of these- Ethionamide PAS Cycloserine[g]	6 6 6 6 6	Fluoroquinolone[f] 2 of these- Ethionamide PAS Cycloserine[g]	18 18 18 18
Susceptibility test to reserve drugs available	Tailor regimen according to susceptibility pattern[h]			

[d] if Ethionamide is not available or poorly tolerated (even at a dose of 500 mg/day)use ofloxacin
[e] Kanamycin or amikacin, or capreomycin
[f] Ciprofloxacin or Ofloxacin
[g] PAS if Cycloserine is not available or too toxic
[h] Individualized regimen is feasible in designated centres of excellence

and atleast four months after the patients first become and remain sputum smear or culture negative.

MONITORING OF TREATMENT

Sputum specimens should be obtained for smear and culture[29,30] monthly during intensive phase of therapy. After sputum conversion, smear examination and culture are done once in three months till the end of therapy. Markers of response in order of reliability are bacteriology of sputum, radiology followed by the clinical picture.

Surgery

Surgery should be considered in patient with persistent culture positive MDR-TB despite effective medical treatment. If the patient has localized disease,

reasonable lung function and only two or three (weak) drugs available, surgery should be seriously considered. Resectional surgery is done as an adjunct to medical treatment[32]. It has been shown that overall cure rate was substantially higher (81% vs 56%) when surgery was more frequently and aggressively applied[33]. Feasibility and success of surgery appear to be substantially enhanced by nutritional support[34]. In one of the recent studies use of resection surgery and fluoroquinolone therapy was associated with improved microbiological and clinical outcome[35].

CONCLUSION

MDR–TB is a growing hazard to human health world wide. MDR-TB is suspected if sputum is persistently positive for AFB with clinical and radiological deterioration after multiple courses of irregular/regular treatment. Drugs susceptibility test for *Mycobacterium tuberculosis* from reliable and reputed laboratory under constant quality control is gold standard for the diagnosis of MDR-TB. Do not accept sensitivity report uncritically if it is not from a reliable lab. Treatment should be in a specialized centre with standard microbiology laboratory. MDR –TB is a man made problem and its emergence can be prevented by prompt diagnosis and effective treatment of all TB cases. Adoption of Directly Observed Treatment - short course (DOTS) to prevent multi-drug resistant strains and careful introduction of second line drugs to treat patients with MDR-TB are the top priorities for the proper control of MDR-TB.

REFERENCES

1. Multi-Drug Resistant Tuberculosis. *ICMR* 1999; **29 (10 and 11)** : 105-114.
2. Cohn DL, Bustreo F, Raviglione MC. Drug resistant tuberculosis. Review of the world wide situation and the WHO / IUATLD Global Surveillance Project. International Union Against Tuberculosis and Lung Diseases. *Clin Infect Dis* 1997; **24 (Supp l):** S121-S130.
3. Pablos-Mendez A, Biokin N, Rieder HL, Bustreo F, Cohn DL, Lambregts van Weezenbeek CS, Kim SJ, Chaulet P and Nunn P. Global surveillance for anti-tuberculosis drug resistance, 1994-1997. World Health Organization–International Union Against Tuberculosis and Lung Disease Working Group on Anti-Tuberculosis Drug Resistance Surveillance. *N Engl J Med* 1998; **338**: 1641-1649.
4. Espinal MA, Laszlo A, Simonsen L et al. Global trends in resistance to anti-tuberculosis drugs. *N Engl J Med* 2001; **344**: 1294-1303.
5. WHO/ IUATLD global project on anti-tuberculosis drug resistance surveillance (1999-2002). Third Global Report (2004).
6. ICMR, Prevalence of drug resistance in patients with pulmonary tuberculosis presenting for the first time with symptoms at chest clinics in India. Findings in urban clinics among patients giving no history of previous chemotherapy. *Indian J Med Res* 1968; **56**: 1617-1630.
7. ICMR, Prevalance of drug resistance in patients with pulmonary tuberculosis presenting for the first time with symptoms at chest clinics in India II. Findings in urban clinics among all patients, with or without history of previous chemotherapy. *Indian J Med Res* 1969; **57:** 823-835.
8. Krishnaswamy KV and Rahim MA. Primary drug resistance in pulmonary tuberculosis. *Indian J Chest Dis* 1976; **28**: 233-337.
9. Trividi SS and Desai SC. Primary anti-tuberculosis drug resistance and acquired Rifampicin resistance in Gujrat, India. *Tubercle* 1988; **69**: 37 – 41.
10. Chandrasekaran S, Chauhan MM, Rajalaksmi R, Chaudhuri K, and Mahadev B. Initial drug resistance to anti-tuberculosis drugs in patients attending an urban district tuberculosis centre. *Indian J Tuberc* 1990; **37**: 215-216.
11. Chandrasekaran S, Jagota P, and Chaudhuri K. Initial drug resistance to anti-tuberculosis drugs in urban and rural district tuberculosis programme. *Indian J Tuberc* 1992; **39**: 171-175.
12. Paramasivan CN, Chandrasekaran V, Santha, T, Sudarsanam N.M. and Prabhakar R. Bacteriological investigations for short course chemotherapy under the tuberculosis programme in two districts in India. *Tuber Lung Dis* 1993; **74**: 27–31.
13. Gupta PR, Singhal B, Sharma TN and Gupta RB. Prevalence of initial drug resistance in tuberculosis patients attending a chest hospital. *Indian J Med Res.* 1993; **97**:102-103.
14. Jain NK, Chopra KK, and Prasad G. Initial and acquired Isoniazid and Rifampicin resistance to *M. tuberculosis* and its implications for treatment. *Indian J Tuberc* 1992; **39**:121-124.
15. Jena J, Panda BN, Nema SK, Ohri VC and Pahwa

RS. Drug resistance pattern of *Mycobacterium tuberculosis* in a chest disease hospital of armed forces. *Lung India* 1995; **13**: 56-59.
16. Parmasivan CN, Venkataraman P, Chandrashekharan V, Bhatt S, Narayanan PR. Surveillance of drug resistant in tuberculosis in two districts of south India. *Int J Tuberc Lung Dis* 2002; **6**: 479-484.
17. Prasad R, Suryakant, Mukerji PK, Gupta AK, Garg R, Rizvi DM, Jain A, Agarwal SK. Initial drug resistance in patients of pulmonary tuberculosis attending a tertiary care centre. *Indian J Tuberc* 2001; **48**: 159 (Abstract).
18. Sofia V, Balassangameshwara VH, Jagannatha PS, Kumar P. Initial Drug resistant among tuberculosis patients under DOTS programme in Bangalore city. *Indian J Tuberc* 2004; **52**: 17-21.
19. Mahadeo B, Kumar P, Agarwal SP, Chauhan LS, Srikanataramu N, Survillence of drug resistant to anti-tuberculosis drugs in districts of Hoogli in West Bengal and Mayurbhanj in Orissa. *Indian J Tuberc* 2005:**52**; 5-10.
20. Chowgule RV, Deodhar L. Pattern of secondary acquired drug resistance to anti-tuberculosis drug in Mumbai, India – 1991 – 1995. *Indian J Chest Dis Allied Sci* 1998; **40**: 23-31.
21. Prasad R, Suryakant, Mukerji PK, Gupta AK, Garg R, Rizvi DM, Jain A, Agarwal SK. Acquire drug resistance in patients of pulmonary tuberculosis attending a tertiary care centre. *Int J Tuberc Lung Dis* 2003; **11(Suppl)**: S303 (Abstract)
22. Datta M, Radhamani MP, Selvaraj R, Paramasivan CN, Gopalan BN, Sudeendra R, Prabhakar R. Critical assessment of smear-positive pulmonary tuberculosis patients after chemotherapy under the district tuberculosis programme. *Tub Lung Dis* 1993; **74:** 180-186.
23. Prasad R, MDR-TB : Current Status. *Indian J Tuberc* 2005; **52**:121-131.
24. Prasad R, Nautiyal RG, Ahuja RC, et al. Treatment of new pulmonary tuberculosis patients: what do allopathic doctors do in India? *Int J Tuberc Lung Dis* 2002; **6**, 895-902.
25. Hazbon MH. Recent advances in molecular methods for early diagnosis of tuberculosis and drug-resistant tuberculosis. *Biomedica* 2004; **24 (Suppl):** 149-62
26. Treatment of tuberculosis: Guidelines for National programmes. Geneva, WHO 2003 (WHO CDS/TB/ 2003.313)
27. Crofton J Chaulet P, Maher D Guidelines for the management of Drug Resistant Tuberculosis, Geneva,WHO, 1997 (Document WHO/TB/96: 210).
28. Mukerjee JS, Rich ML, Socci AR, et al. programmes and principles in treatment of multi-drug resistant tuberculosis. *Lancet* 2004; **363**: 474-81.
29. Guidelines for the programmatic management of drug resistant tuberculosis, Geneva, WHO 2006 (WHO/HTM/TB/2006.361)
30. Revised National Tuberculosis Programme DOT Plus Guidelines 2006 (Central TB Division, Director General of Health Services, New Delhi)
31. Prasad R, Verma SK, Sahai S, Kumar S, Jain A. Efficacy and safety of Kanamycin, Ethionamide, PAS and Cycloserine in multi-drug resistant pulmonary tuberculosis patients. *Indian J Chest Dis Allied Sci* 2006; **48**:183-186.
32. Iseman MD. Treatment of multi-drug resistant tuberculosis-*New Eng J Med* 1993; **329**: 784-91.
33. Iseman MD, Madsen L, Iseman MC, Ackerson L. Impact of surgery on the management of Multi-drug resistant tuberculosis (abstract). *Am J Respir Crit Care Med* 1995; **151**: A336.
34. Pomerantz M, Madsen L, Goble M, Iseman M. Surgical management of resistant mycobacterial tuberculosis and other mycobacterial pulmonary infections. *Ann Thorac Surg* 1991; **52**: 1108-1112.
35. Edward D. Chan, Valerie Laurel, Matthew J. Strand, Julanie F. Chan, Mai-Lan N. Huynh, Marian Goble and Michael D. Iseman. Treatment and Outcome Analysis of 205 Patients with Multi-drug-resistant Tuberculosis. *Am J Respir Crit Care Med*. 2004;**169**:1103-1109.

Original Article

STATUS OF RE-REGISTERED PATIENTS FOR TUBERCULOSIS TREATMENT UNDER DOTS PROGRAMME

V.Chandrasekaran, P.G.Gopi, T.Santha, R.Subramani and P.R.Narayanan

(Original received on 24.4.2006. Revised version received on 10.8.2006. Accepted on 22.8.2006)

Summary

***Objective*:** To assess the proportion of patients re-registered after default, failure or successful treatment, completion and evaluate their treatment outcome.

Setting: Tuberculosis patients diagnosed were registered for treatment under DOTS in rural area, South India. Patients re-registered during 1999-2004 identified from the TB register were considered for analysis.

***Results*:** Among 273 Category-I patients 'defaulted' 23% and among 112 'failure' cases 68% were re-registered. After 'successful treatment completion' of 1796 cases 6.5% were re-registered as relapse. Corresponding figures for Category II were 20% of 281 defaulters; 23% of 60 failures; 12.9% of 302 'successful treatment completion' patients. Among patients re-registered as 'default', subsequent default was also high (57% vs 37%). Failure in Category II treatment was similar among patients who were re-registered for Category II and initially registered in it for treatment. Median delay for re-registration was >200 days for 'defaulters' and 18 days for 'failures'.

***Conclusion*:** Our findings emphasise the need for continuing motivation and prompt defaulter retrieval action to reduce default at all stages of treatment. 'Defaulters' need to be contacted so that they can be started on treatment without delay. Patients declared as 'successful treatment completion' should be encouraged to report if chest symptoms recur. **[*Indian J Tuberc 2007; 54:12-16*]**

***Key words*:** Re-registration, DOTS, Tuberculosis, Default

INTRODUCTION

The Revised National Tuberculosis Control Programme (RNTCP) based on the World Health Organization's Directly Observed Treatment-Short Course (DOTS) strategy was launched by Government of India in a phased manner since 1993. The progress of patients started on treatment is monitored and treatment outcome given as per international definitions[1]. Patients who are declared as 'failure' or relapsed after being declared as 'cure' or 'treatment completed' and 'default' after receiving one month or more of treatment will be re-registered for treatment under Category II. Not much information is available on the proportion of patients re-registered and their treatment outcomes according to type at re-registration. This paper presents data on the above issues.

MATERIAL AND METHODS

The DOTS programme was implemented in 1999 in the study area of Tiruvallur district, Tamil Nadu covering a population of 580,000. Patients diagnosed with tuberculosis in this area were registered for treatment under DOTS. All patients registered from May 1999 to 2004 were considered for the analyses. **A patient was considered as re-registered for treatment if he/she was already registered previously for treatment in the same Tuberculosis Unit (TU).**

Data collection

The details of the patients registered for treatment under DOTS were collected from the Tuberculosis (TB) register maintained in the TU. A patient started on treatment is assigned a unique TB

Tuberculosis Research Centre, Chennai

Correspondence: Dr. P.R. Narayanan, Director, Tuberculosis Research Centre, Mayor V.K. Ramanthan Road, Chetput, Chennai-600 031
E-mail: prnarayanan@trcchennai.in

number along with the year of registration. When a patient is re-registered, a new TB number will be allotted at the time of re-registration.

Statistical analyses

The data from TB register after scrutiny were computerized, verified keying in twice, edited and corrected for discrepancy and missing information. The Chi-square test was used to test difference in proportions. The level of statistical significance was defined as $P < 0.05$.

RESULTS

From May 1999 through December 2004, 2608 patients were started on Category I treatment, 803 on Category II, 1844 on Category III and 111 on non-DOTS (non-DOTS excluded for analysis). Of 2287 new smear positive patients registered in Category I, 1796 (79%) had 'successful treatment completion', 273 (12%) 'Defaulted', 99 (4%) 'Expired', and 112 (5%) 'Failed' and 7 (<1%) were 'transferred out'. Corresponding figures for 699 smear positive Category II patients were 302 (43%), 281 (40%), 47 (7%), 60 (9%) and 9 (1%), and for 2165 new smear negative and extra pulmonary patients (including 321 smear negative and extra pulmonary TB patients treated with Category I) were 1814 (84%), 231 (11%), 89 (4%), 27 (1%) and 4 (<1%) respectively. A total of 444 (403 smear-positive and 41 smear-negative) patients were re-registered during 1999-2004, including 57 patients who were re-registered twice and eight 3-times and one 4-times in the same TU. Of the patients registered between 1999 and 2004, 273 patients were re-registered of whom 248 (91%) were re-registered within 2-years (Table 1).

Table 1: Year of initial registration and re-registration of patients treated under DOTS

Admission		Re-Registration					
Year	No.	2000	2001	2002	2003	2004	Total
1999	407	32	14	5	1	0	52
2000	970	10	55	31	11	3	110
2001	1046	-	25	55	26	5	111
2002	1097	-	-	18	62	19	99
2003	947	-	-	-	12	51	63
2004	899	-	-	-	-	9	9

Table 2: Proportion of patients re-registered according to Category of patients started on treatment and its outcome (May 1999 –Dec 2004).

Started treatment	Treatment outcome*				Treatment Success	
	Default		Failure			
	Total	Re-registered (%)	Total	Re-registered (%)	Total	Re-registered (%)
Category-I Smear Positive	273	64(23)	112	76(68)	1796	117(6.5)
Category-II Smear Positive	281	56(20)	60	14(23)	302	39(12.9)
New Smear Negative and Extra Pulmonary	231	16(7)	27	21(78)	1844	25(1.4)

* 16 re-registered cases excluded (1 'transfer out', 8 non-DOTS and 7 Category II smear negative)

The proportion re-registered according to initial category and treatment outcome is given in Table 2. From Category I smear positive patients, 64 (23%) of 273 'defaulted', 76 (68%) of 112 'failures' and 117 (6.5%) of 1796 'treatment success' patients were re-registered for treatment. The corresponding figures for Category II patients were 20%, 23% and 12.9% and for new smear negative and extra pulmonary cases 7%, 78% and 1.3%, respectively. We have excluded 1 patient from the 'transfer out', 8 started on non-DOTS treatment and 7 Category II smear negative at the time of re-registration.

The median interval between declaring the treatment outcome and re-start of treatment was 228 days for 109 'default' and 18 days for 105 'failure' and 212 days for 163 'relapse' patients (Table 3).

Of the 57 patients who were re-registered for the second time, 23 were defaulters, 16 relapses, 10 failures, 4 were re-registered as 'new' cases and 4 as others.

Table 3**:** Median interval between declaration of treatment outcome and re-start of treatment

Outcome	Category I Smear positive.		Category II Smear positive		New Smear negative and Extra Pulmonary		Total (No. in first time re-registration)	
	No.	Median days	No.	Median days	No.	Median days	No.	Median days
Default	64	228	56	224	16	438	136 (104)	234 (230)
Failure	76	18	14	72	21	12	111 (104)	19 (18)
Relapse	117	219	39	198	25	192	181 (161)	210 (212)

Table 4: Treatment outcome according to type at the time of re-registration and initially registered as Category II Smear positive cases

Type	Treatment outcome					
	Total	Treatment Success (%)	Default (%)	Died (%)	Failure (%)	Others (%)
Default						
Re-Registered	117	31(26)	67(57)	11(9)	7(6)	1(1)
Initially Category II*	215	98(46)	79(37)	17(8)	16(7)	5(2)
Failure						
Re-Registered	104	37(36)	44(42)	6(6)	17(16)	-
Initially Category II*	34	15(44)	12(35)	3(9)	3(9)	1(3)
Relapse						
Re-Registered	131	64(49)	49(37)	6(5)	11(8)	1(1)
Initially Category II*	95	55(58)	29(31)	4(4)	6(6)	1(1)
Total						
Re-Registered	352	132(38)	160(45)	23(7)	35(10)	2(1)
Initially Category II*	344	168(49)	120(35)	24(7)	25(7)	7(2)

* includes cases registered at the commencement of DOTS, previously treated by private practitioners for one month and above and treated in other TUs

The 'type of disease' at the time of re-registration was correctly classified based on initial treatment outcome for 351 of 444 (79%): 'treatment after default' 87% (128/147), 'failure' 82% (92/112) and 'relapse' 71% (131/184) of patients.

Table 4 describes the treatment outcome of 699 smear positive patients (after excluding 3 patients 'transfer out') of the total 803 Category II patients registered during the study period of which 352 were re-registered and the remaining 344 were initially registered. The successful treatment outcome was significantly lower {132 of 352 (38%) vs 168 of 344 (49%), P< 0.01)} among re-registered patients. This was mainly due to the significantly higher default, particularly among patients typed as 'treatment after default' (67 of 117 vs 79 of 215, p<0.01). However, the failure was similar in the two groups.

DISCUSSION

The overall treatment outcome among Category II patients reported here is similar to another study[2] on re-treatment outcome of smear positive patients. Hence we analysed the outcome of patients initially registered as Category II and re-registered to Category II.

The main findings of the present study were that 68% of the failures and 23% of the defaulters from Category I treatment were only re-registered. This brings forth the need to motivate patients who fail in Category I regimen to restart treatment and prompt default retrieval actions to reduce 'default'. The proportion was much less among Category II failures and defaulters. The RNTCP recommendation to refer failures of Category II to higher institutions for further management could explain the lower re-registration of failure to Category II. We were unable to estimate the proportion of patients re-registered among those relapsed since we do not have the actual number relapsed among successful treatment completion. However, the proportion of relapse cases registered was lower than the relapse rate of 12% reported from an earlier study from the same area[3] where patients with treatment success were followed-up. This stresses the point that patients who have successfully completed treatment need to be advised to report if they develop chest symptoms. The high proportion of defaulters not returning for treatment is of concern for TB control since they may continue to spread the disease and calls for attempts to retrieve these patients and put them on treatment. This would also help to reduce the delay in restarting treatment for these patients.

The higher default rate among the re-registered patients under type 'default' could be because they continue to default suggesting the need for more intense and continuing motivation efforts for these patients. No significant difference in default was observed for those declared 'failure' between the two groups.

RNTCP as a policy emphasizes on the new smear positive patients and their treatment outcome. Our findings suggest that it is essential to monitor re-treatment patients with same vigour to reduce default and improve their treatment outcome.

A limitation of the study was that the defaulted patients were not visited to find out reason and no attempt was made to visit the patients who didn't turn-up for re-starting treatment. Another limitation is that the analysis is based on self-reporting patients who were re-registered in the same TU, but did not cover patients who would have reported to any other TU or to private sector.

In conclusion, the study demonstrated that higher default occurred among re-registered patients resulting in low successful treatment outcome. The fact that only 23% of patients who had defaulted were re-registered and the higher default (57%) among patients re-registered emphasizes the need to motivate them for continuing the treatment. Studies are required to find out reasons for those who did not turn up for re-treatment and evolve methods to ensure their return to treatment.

ACKNOWLEDGEMENTS

The authors thank Mr. S. Radhakrishnan (Senior Treatment Supervisor) for maintaining

Tuberculosis patient register. The authors are grateful for the assistance and cooperation of the Joint Deputy Director Health Services of Tiruvallur district, Tamil Nadu, and all the medical and para-medical staff, including treatment observers who participated in this work. The assistance rendered by the staff of the Statistics division of Epidemiology unit in checking data and arranging for computerization is highly appreciated. The staff of EDP division is gratefully acknowledged for data entry and data management.

This work was funded in part by a grant from the United States Agency for International Development provided through the World Health Organization, SEARO, New Delhi.

REFERENCES

1. Revised National Tuberculosis Control Programme. Technical guidelines for TB control. Central TB Division, Directorate General of Health Services. New Delhi, India: Nirman Bhavan, 1997.
2. Sophia Vijay, Balasangameshwara V H, Jagannatha P S, Saroja V N, Shivashankar B and Jagota P. Re-treatment outcome of smear positive tuberculosis cases under DOTS in Bangalore city. *Indian J Tuberc* 2002; **49**:195-204.
3. Thomas T, Gopi P G, Santha T, Chandrasekaran V, Subramani R, Selvakumar N, Eusuff S I, Sadacharam K, Narayanan P R. Predictors of relapse among pulmonary tuberculosis patients treated in a DOTS programme in South India. *Int J Tuberc Lung dis* 2005; **9(5)**:556-561.

Sixty-First National Conference on Tuberculosis and Chest Diseases

The **Sixty-First National Conference on Tuberculosis and Chest Diseases** will be held at RNT Medical College, Udaipur (Rajasthan) from 23rd to 25th February, 2006. Registration-cum-brochure forms can be obtained from the Secretary General, Tuberculosis Association of India, 3-Red Cross Road, New Delhi-110 001.

Original Article

SOCIAL STATUS MAKES A DIFFERENCE: TUBERCULOSIS SCENARIO DURING NATIONAL FAMILY HEALTH SURVEY - 2

Aarti Kaulagekar[1] and Anjali Radkar[2]

(Original received on 24.4.2006. Final Version received on 2.11.2006. Accepted on 9.11.2006)

Summary

***Background*:** The poorest people are vulnerable to Tuberculosis because of the living and working conditions but they plunge deeper into poverty as a consequence of this disease.

***Aims*:** The present study focuses on the socio-demographic characteristics of patients of TB with specific reference to prevalence of TB and health care seeking behaviour of men and women.

***Methods*:** The data for the study comes from nation-wide National Family Health Survey -2, conducted in 1998-99. Paper looks at the relationship of reporting TB infection and seeking treatment for men and women by various socio – economic characteristics. Multivariate logistic regressions are applied to find the significant factors explaining reporting of TB and treatment-seeking.

***Results*:** In the entire sample 1735 males and 1266 females are reported to suffer from TB. Reporting of TB is significantly (p=0.000) more among males having charecteristics with lower standard of living, scheduled tribes from rural area and illiterate population. It increases with age. Significant difference (p=0.002) is observed between urban and rural female's treatment seeking for TB. In case of females as age increases, treatment seeking goes down (p=0.007). Treatment seeking for currently married women is less frequent than that of all other women.

Conclusions*:** Apart from economic status and living conditions, place of residence (urban / rural) and ethnic identity made people more vulnerable in terms of reporting the disease and access to treatment.[Indian J Tuberc 2007; 54: 17-23]***

Key words: TB, Social status, Gender, Literacy, Scheduled tribes, Treatment seeking

INTRODUCTION

The poorest of people from the poorest of countries are the ones most affected by tuberculosis. Not only are they more vulnerable to the disease because of their living and working conditions, they are also plunged deeper into poverty as a consequence of TB. A person with TB loses, on average, 20 to 30 per cent of annual household income due to illness[1]. Evidence from various researches strongly suggests that there is a close link between TB and poverty[2-5]. The link also highlights the relevance of gender issues in the context of prevalence and treatment of TB in developing countries[6]. A heavier burden of poverty and comparatively lower socio-economic status of women make them more vulnerable to adverse social and environmental conditions. Relatively few studies of gender differences in TB have generally come from Third World countries. These studies suggest differences in diagnosis, treatment and societal perceptions of TB in women, usually to their disadvantage, and reflecting their lower social status in many societies[7-9]. However, Hamid and colleagues[10] found that the gender difference observed in routine TB diagnosis is real, and is not due to lesser accessibility of women to health services.

Gender and TB experts have since been working on an agenda for research into biological, epidemiological, social and cultural differences in the occurrence of TB in men and women and their access to the TB treatment strategy[11]. It is estimated that more than 600 million women worldwide are infected with TB and of these an estimated 3.1 million fall ill each year. TB kills more women annually than all causes of maternal mortality combined. The impact of TB on women is more intense with problems of malnutrition, ill health, HIV infection, repeated childbirth, fear, stigma attached to the disease and

1. Lecturer 2. Lecturer
Inter-disciplinary School of Health Sciences, University of Pune
Correspondence: Dr. Anjali Radkar, Inter-disciplinary School of Health Sciences, University of Pune, Pune - 411 007

the delay in seeking medical care. Although the overall prevalence of pulmonary TB is lower in women, the progression from infection to disease is higher. This could be because of the triple burden of housework, childcare and employment leaving very little time for taking care of her[12]. A recent publication from the National Tuberculosis Institute[13], where all the available literature on the sociological aspects of TB is summarized indicates that there are very few studies in recent times that have studied the socio-economic aspects of the disease.

The present paper aims at estimating the prevalence of TB and differentials by various sub-groups at a national level. It specifically attempts to understand the reporting of TB in the society by gender and other socio-economic and demographic sub-groups of population as well as the differentials in treatment seeking behaviour with reference to various background characteristics.

METHODOLOGY

It is understood that in order to avoid the obvious bias in sampling based on the hospital records, it is necessary to look at TB at community level. The present paper is based on the analysis of the National Family Health Survey – 2 (NFHS-2) data. The NFHS-2, seeks information on the prevalence of TB on a country level from the community. The NFHS-2 has been carried out during November 1998 to March 1999. Information was collected from 92,486 households from 26 states of the country from both rural and urban areas. The sample was weighted stratified random sample, selected on the basis of probability proportional to size. Information about diseases was collected through household questionnaires, which was one of the three questionnaires administered in this survey. Information about all the household members on their personal background and health status was collected from a responsible adult in the family. While analyzing the data, one has to remember that information in the NFHS -2, was reported information and no clinical or laboratory test was conducted to endorse whether the person really suffered from TB or not.

There is all the possibility that the respondent might not have revealed the status of TB in the family owing to the likely stigma associated with diagnosis of TB. Thus one may expect some under-reporting in the estimates of prevalence.

RESULTS

In the entire sample 1735 males and 1266 females reported to suffer from TB. It forms about 6 per thousand of the population covered in the NFHS- 2, which is close to Chakraborty's (2004) estimate of 5.5 persons per thousand[14]. The share of TB infection in females is about 40 per cent. It matches with earlier reports which suggest that responses to TB differ between men and women. Barriers to early detection and treatment of TB may be greater for women than for men[15]. In addition, progression from TB infection to disease may be faster in women of reproductive age than men of the same age[16,17]. Nonetheless, there is an estimated 2:1 male to female ratio in the number of TB cases notified to public health authorities[18].

Reporting of TB

While collecting the information on prevalence of TB and treatment sought after that the questions asked in the NFHS -2 are like,

"Does anyone listed suffer from tuberculosis?"

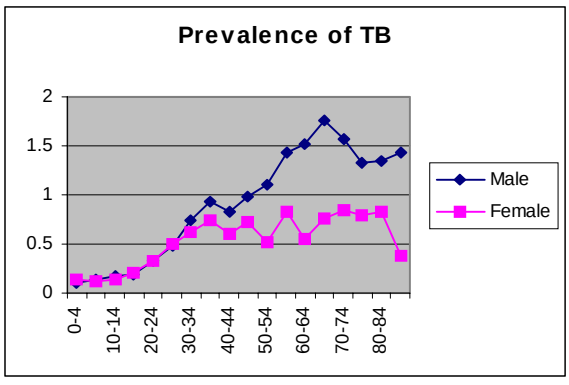

Chart 1: Prevalence of TB by age and sex in India, 1998-99

And
"If suffers from tuberculosis, has (Name) received treatment for tuberculosis?"

It needs to be mentioned here that the reported cases are currently suffering cases. It is not the lifetime experience as such. Also treatment does not necessarily mean TB treatment like DOTS. It just indicates whether the person has taken any treatment for TB.

Socio-economic differences

Among the biological attributes, the current age of a person suffering from TB needs to be looked at carefully. Chakraborty[14] has reported that in the developing countries, females on attaining the reproductive ages, suffer less than the males in the same ages, whereas among children, girls suffer more than boys. It is likely that females are biologically protected after they attain menarche. The prevalence among males and females, as observed in NFHS-2 data, is almost equal in childhood but is on a lower side for old females. Since this is as expected from prevalence survey data available from the recently conducted Chingleput surveys, and other population surveys reported by Chakraborty[14], it is unlikely that the observations under NFHS-2, could be attributed to the under-reporting of TB among females.

As seen from Table 1 reporting of TB by urban males is significantly more (0.50 per cent) than females (0.41 per cent). However, considerable difference in reporting is noted for rural males (0.74 per cent) and females (0.54 per cent). The reporting among rural males is significantly higher than among rural females (p=0.000).

Reporting of TB in males living with poor conditions (1.02 per cent) is over three times more as compared to males with high standard of living (0.30 per cent) while two and half times more among females with lower standard of living (0.73 per cent) as compared to females in high standard of living (0.28 per cent) group. The difference is statistically significant (p=.000). To assess the standard of living, a summary household measure called standard of living index is computed by assigning scores to information on type of house, toilet facility, source of lighting, main fuel used for cooking, source of drinking water, separate kitchen, ownership of house, ownership of agricultural and irrigated land, ownership of livestock and household durable goods.

Table 1: Prevalence (%) of TB by gender and other background characteristics, India, 1998-99

Background characteristics	Prevalence of TB (%)	
	Males	**Females**
Place of residence		
Urban	0.50	0.41
Rural	0.73	0.54
Standard of living		
Low	1.02	0.73
Medium	0.64	0.48
High	0.30	0.28
Ethnic group		
Scheduled tribe	1.07	2.63
Scheduled caste	0.74	0.54
Other backward castes	0.66	0.43
Other	0.47	0.39
Type of house		
Pucca	0.42	0.33
Semi-*pucca*	0.64	0.54
Kachha	0.97	0.65
Cooking fuel		
Causing Smoke	0.76	0.55
Non-smoke	0.40	0.36
Current age		
0 - 14	0.20	0.18
15 - 44	0.66	0.57
45 - 59	1.33	0.91
60 and above	1.82	0.97
Educational level		
Illiterate	1.24	0.80
Below middle school	0.70	0.38
Middle school complete	0.69	0.32
High school complete and above	0.35	0.25
Can read and write		
No	1.25	0.80
Yes	0.59	0.34

Differences in the prevalence of TB among various ethnic groups are significant for both males and females. Prevalence is in descending order for scheduled tribes (1.07 per cent for males and 2.63 percent for females), scheduled castes (0.74 per cent and 0.54 per cent), other backward castes (0.66 per cent and 0.43 per cent) and other advanced caste groups (0.47 per cent and 0.39 per cent). TB worst affects scheduled tribe women (2.63 per cent). It is more evident when compared to advanced caste women. This figure is significantly (p=0.000) higher than scheduled tribe males and any other category. Tribal women's susceptibility to the disease, vulnerability in terms of living conditions remains a point for further exploration. Living conditions and prevalence of TB are associated; information about type of house and main cooking fuel is cross tabulated to find that those who stay in *kachha* houses and use smoke causing fuel for cooking suffer significantly (p=0.000) more by TB.

Table 2: Results of logistic regression for prevalence of TB with selected background indicators, India, 1998-99

Predictor variables	Odds ratio	Sig.
Male Ref – Female	1.559	0.000
Rural Ref – Urban	1.153	0.015
Low SLI*	1.881	0.000
Medium SLI	1.529	0.000
Ref – High SLI		
Scheduled caste	1.149	0.018
Scheduled trite	1.676	0.000
Other backwards classes	1.072	0.184
Ref – Other advanced caste		
Smoke causing cooking fuel Ref – Non-smoke cooking fuel	1.047	0.511
Read and write Ref – Cannot read and write	1.804	0.000
Kachha house	1.264	0.000
Semi-*pucca* house	1.083	0.179
Ref – *Pucca* house		
Constant	0.002	0.000

N=416616
*SLI-Standard of Living

Reporting of TB increases with the age category. As expected 0-14 age group has less cases (0.20 per cent males and 0.18 per cent females) as compared to any other age group. Geriatric TB was more (1.82 percent for males, 0.97 per cent for females) than in other adult age group.

Table 3: Percentage distribution of persons reporting as TB cases and seeking treatment for TB by gender and background and personal characteristics, India, 1998-99

Background and personal characteristics	Proportion of TB cases (%) seeking treatment	
	Males	Females
Place of residence		
Urban	86.96	86.67
Rural	83.61	79.27
Standard of living		
Low	79.94	75.10
Medium	87.10	85.28
High	89.41	87.74
Current age		
0 - 14	81.21	79.45
15 - 44	82.83	84.30
45 - 59	86.80	78.95
60 and above	86.70	73.45
Marital status		
Never married	81.40	80.81
Currently married	85.57	70.24
Widowed, divorced, separated	86.51	84.40
Educational level		
Illiterate	83.52	80.55
Below middle school	86.52	82.21
Middle school complete	84.06	86.67
High school complete and above	85.23	82.89
Can read and write		
No	83.52	80.55
Yes	85.76	82.89
Ethnic group		
Scheduled tribe	77.18	72.51
Scheduled caste	84.11	81.10
Other backward castes	88.60	83.78
Other	86.62	88.70

Education and or ability to read and write can make significant difference (p=0.000) in the prevalence of TB. The difference in prevalence between illiterate (1.24 percent males, 0.80 per cent females) and high school educated (0.35 per cent males and 0.25 per cent females) is more than 3 times. The difference in reporting of TB by illiterate and educated males and females is also noted. A gradual decrease in the prevalence of TB was observed as education increased.

Multivariate analysis for prevalence of TB

The bivariate analysis indicates that most of the independent variables are significantly related to the dependent variable. Reporting of presence of TB and the direction of the relationship with the variables is also as expected. In order to avoid the effects of confounding on the dependent variable and to understand more about the causal factors, logistic regression analysis is used for the present study. In the study the input value for the category suffering from TB is 1 and for the category - not suffering from TB it is given the value '0'. The results are presented in Table 2.

Looking at the analysis regarding TB prevalence, gender works as one of the significant variables.

Prevalence of TB is seen highest among population with a low standard of living (odds ratio =1.881 and p=0.000), followed by those who cannot read and write (odds ratio =1.804 and p=0.000), belonging to scheduled tribe (odds ratio =1.676 and p=0.000) and staying in *kachha* houses (odds ratio =1.264 and p=0.000). It highlights the fact that the most vulnerable population in India should be covered through existing programme or monitored carefully in future. **Although living conditions, type of house and poverty are known factors, education and belonging to scheduled tribes have emerged as the most significant factor, calling for identification as areas of concern for effective implementation of TB programme.** Vulnerability of the scheduled tribe is independent on the living conditions. This once again reflects the limitation in programme implementation. At the same time, ability to read and write can reduce one's vulnerability.

Other significant variables include sex of the person, place of residence, standard of living and the factor of belonging to scheduled caste.

Treatment seeking behaviour

Infection with TB bacilli is a biological occurrence and to some extent, it depends on economic status of the family. However, seeking treatment is more of a social, economic nature. It also can be regarded as behavioural.

Information on treatment seeking is collected only from those who have reported TB. Among 3001 reported persons 2385 (i.e. approximately 80 per cent) have sought some treatment for TB. It includes 59 percent males and 41 percent females. Overall treatment seeking ranged from 73 to 87 per cent. Females above 60 years are the least likely (73.45 per cent) to seek treatment

Table 4: Results of logistic regression for treatment for TB with selected background indicators, India, 1998-99

Predictor variables	Odds ratio	Signi-ficance
Male	0.835	0.117
Ref – Female		
Rural	0.943	0693
Ref – Urban		
Low SLI	2.011	0.003
Medium SLI	1.164	0.488
Ref – High SLI		
Scheduled caste	1.249	0.191
Scheduled trite	2.229	0.000
Other backward classes	1.040	0.807
Ref – Other advanced castes		
Read and write	1.049	0.692
Ref – Cannot read and write		
Kachha house	1.001	0.995
Semi-*pucca* house	0.707	0.046
Ref – *Pucca* house		
Constant	0.120	0.000

N=2576

while males above 45 years are the most likely (more than 86 per cent) treatment seekers. There is a significant (p=0.002) difference observed between urban and rural female's treatement seeking but not among the males. It is also seen that among females as age increases treatment seeking significantly decreases (p=0.007). The male-female gap is observed at all three levels of standard of living (p=0.000). Females from low standard of living seek treatment less frequently.

Marital status has made significant difference for females. Treatment seeking of females never married or those separated is significantly higher (about 80 per cent) than currently married (about 50 per cent) females. This is because of the gender difference prevailing in the society. Married females are more likely to have restrictions from other household members, especially husband and in-laws. They are also dependent on male counterparts for economic support or physical help in terms of accompanying them to the doctor. Other domestic duties and responsibilities restrict them at home or on farm and delay in treatment is seen. It is also likely that they neglect themselves, may not understand the gravity of the problem and have less preference for treatment, while never married or separated are likely to stay with parents or are independent enough to make decision in their favour.

Significant difference in treatment seeking was observed among scheduled tribe and scheduled caste females and males (p=0.000). Females are unable to reach the health facility, or, due to some other reasons lack in treatment compliance. Once again women's position in the household, their economic dependence, illiteracy would be restricting factors.

Multivariate analysis for treatment of TB

To explore the variables that explain the treatment seeking behaviour of TB infected persons, multivariate analysis -logistic regression analysis - has been carried out with dependent variable, 'whether some treatment is sought for TB or not'. The results are summarized in Table 4.

Logistic regression result shows that the most significant variable in the analysis is a person belonging to scheduled tribe (Odds ratio=2.229 and p=0.000). Other significant variables are low standard of living and *semi-pucca* housing. It is indicative of the fact that for a person to seek treatment, he / she should have better standard of living. Similarly lesser treatment for tribal population indicates that they have difficult access to health centre or appropriate facility. Gender does not appear to be significant variable in the multivariate analysis for treatment seeking. However, figures for currently married females, treatment seeking cannot be neglected.

CONCLUSIONS

Present paper brings out certain facts that are known as well as certain key issues for further research and investigation.

Apart from economic conditions and living conditions, place of residence (rural/urban) and ethnic identity made people more vulnerable in terms of prevalence of disease and access to treatment, in case of disease. Age and gender are both traditionally known variables acting in favour of adult males making females more prone to suffer due to lack of treatment and not because of rate of infection diseases.

The existing gender disparity in the society is manifested in different ways. Pattern of gender specific treatment seeking is one of such indicators which calls for more attention to rural females, tribal women and other caste groups.

Literacy plays significant role in reporting the disease. Illiterate or less educated are less likely to report it. However, the interesting fact that has come out of this analysis is that though literacy/ educational level show significant difference in reporting the disease, it is not reflected in treatment seeking behaviour.

Improving economy and reducing poverty are rather long term goals, it may take several years to bring down level of poverty and increase in standard of living or provide better housing. However,

education is key towards better future, especially for females who are traditionally prohibited to take independent decision in their favour.

REFERENCES

1. World Health Organization Department of Gender and Women's Health, Gender and Tuberculosis, Geneva, Switzerland, January 2002.
2. Nair D.M., George A., Chacko K. T. Tuberculosis in Bombay: new insights from poor urban patients. *Health Policy and Planning*, 1997; **12 (1)**: 77-85
3. McKay H.A., Editorial. Tuberculosis and the poverty-disease cycle. *Journal of* the *Royal Society of Medicine* 1999; **92(3)**.
4. Rajeswari R., Balasubramanian., Muniyandi M, Geetharamani S, Thresa X, and Venkatesan P., Socio-economic impact of tuberculosis on patients and family in India. *Int J Tuberc Lung Dis*, 1999; **3(10)**: 869-877
5. Diamond I., Matthews Z., Stephenson R., Assessing the health of the poor: towards a pro-poor measurement strategy. Health Systems Resource Centre, Department for International Development, London, 2001.
6. Diwan V.K. and Thomson A., Sex, gender and tuberculosis, *Lancet*, 1999; **353(9157)**: 1000-1001.
7. Thomson A and Diwan V.K., Gender inequalities in tuberculosis: aspects of infection, notification rates, and compliance. *Curr Opin Pulm Med*, May 2001;**7(3)**: 165-169.
8. Bashour H., and Mamaree F. Gender differences and tuberculosis in the Syrian Arab Republic: patients' attitudes, compliance and outcomes, *East Mediterr Health Journal*, July 2003; **9(4):** 757-768.
9. Ashan G., Ahmed J., Singhasivanon P., Kaewkungwal J., Okanurak K. Suwannapong N., Akarasewi P., Majid M.A., Begum V., Belayetali K., Gender difference in treatment seeking behaviours of tuberculosis cases in rural communities of Bangladesh, *Southeast Asian Journal of Tropical Medicine Public Health, March 2004*; **35(1)**: 126-35.
10. Hamid Salim M.A., Delercq E., Van Deun A., Saki K.A. Gender differences in tuberculosis: a prevalence survey done in Bangladesh, *Int J Tuberc Lung Dis. August 2004*, **8(8)**, 952-957. UN Chronicle TB: the leading infectious killer of adults…. and the single biggest killer of young women -tuberculosis. Spring, 1999
11. UN Chronicle TB: the leading infectious killer of adults ..... and the single biggest killer of young women ..... tuberculosis spring 1999.
12. Thomas B.E. Tuberculosis in women Status of tuberculosis in India -2000 published by Society for innovation and Development, Indian Institute of Science, Bangalore, Editors; R. Nayak,M.S.Shaila,G.Ramanda Rao, 2000, 25-32.
13. National Tuberculosis Institute, Sociological aspects of Tuberculosis: Summaries of published literature (1939 - 2000), Bangalore, 2002
14. Chakraborty A.K. Epidemiology of tuberculosis: Current status in India. *Indian J Med Res* 120, October 2004, pp 248-276.
15. Holmes C.B., Hausler H., Nunn P. A review of sex differences in the epidemiology of tuberculosis, *Int J Tuberc'Lung Dis*, 1998; **2:** 96-104.
16. Fine, P.E.M. Immunities in and to tuberculosis: implications for pathogenesis and vaccination. Tuberculosis: back to the future, 1983
17. Murray C.J.L., Social, economic and operational research on tuberculosis: recent studies and some priority questions. *Bull Int Union Tuberc Lung Dis* 1991; **66**:149-156. [PubMed]
18. Kumaresan. I.A; Raviglione, M.C.; Mun-ay, C.I.L. Tuberculosis. The global burden of disease and risk factors in 1990: World Health Organization Press. 1996.

The Editor-in-Chief and the members of the Editorial Board of Indian Journal of Tuberculosis wish you all a Very Happy & Prosperous New Year 2007.

Original Article

IMMUNOHISTOCHEMICAL LOCALIZATION OF *MYCOBACTERIUM TUBERCULOSIS* COMPLEX ANTIGEN WITH ANTIBODY TO 38 KDA ANTIGEN VERSUS ZIEHL NEELSEN STAINING IN TISSUE GRANULOMAS OF EXTRA-PULMONARY TUBERCULOSIS

Madhu Mati Goel[1] and Puja Budhwar[2]

(Original received on 9.5.2006. Revised Version received on 5.10.2006. Accepted on 17.10.2006)

Summary

Background: Extra-pulmonary tuberculosis often presents a diagnostic challenge because of its diverse clinical manifestations and low yield of acid fast bacilli in tissue sections.

Aim: The aim of the present study is immuno-histochemical localization of tuberculous bacilli or their components that persist in the granulomas but have lost the property of staining with acid fast stains and to assess the advantage of immuno-staining over conventional Ziehl Neelsen staining.

Material and Methods: Immuno-histochemical staining using species-specific monoclonal anti-body to 38 kDa protein of *Mycobacterium tuberculosis* complex and Ziehl-Neelsen staining for acid fast bacilli (AFB) was done on 69; 36 cases of confirmed extra-pulmonary tuberculosis and 33 non-tuberculous cases, in archival formalin fixed paraffin embedded tissue sections

Obervations: AFB positivity was observed in only 36.1% of tuberculous granulomas while immuno-histochemical staining was positive in 100% of tuberculous granulomata with zero false positivity and negativity.

Conclusions: The immuno-hiostochemical localization of tuberculous bacilli and their components in tissue sections may be an efficient diagnostic adjunct to conventional ZN staining for the diagnosis of granulomas of extra-pulmonary tuberculosis. The technique is simple, sensitive and specific. It can be standardized and performed by trained technicians in routine laboratory. This will also help in clinical decision-making and in reducing the usual practice of prescribing empirical anti-tubercular treatment based on clinical suspicion alone in the absence of demonstrable evidence of tuberculous infection. **[*Indian J Tuberc 2007; 54: 24-29*]**

Key words: Immuno-histochemistry, Extra-pulmonary tuberculosis, Monoclonal antibody, *Mycobacterium tuberculosis*, Tuberculous Granuloma, Granulomatous Inflammation

INTRODUCTION

The diagnosis of extra-pulmonary tuberculosis (EPTB) is often difficult because of its diverse clinical manifestations and low positivity of Ziehl Neelsen's staining in tissue granulomas[1,2]. Various nucleic acid amplification assays have been developed that have shown excellent sensitivity and specificity in respiratory specimens. However, use of these tests in clinical settings for non-respiratory specimens still requires validation[3] and are still out of reach in routine diagnosis in developing countries. Few immuno-histochemical (IHC) studies have suggested the probable role of immuno-histochemical staining in establishing mycobacterial etiology of caseating granulomas of lung, lymph nodes and tissue specimens with tuberculosis[4-10,12,14] but most of these have used polyclonal antibodies resulting in false positive reactions due to antigenic cross reactivity with other bacteria and fungi. The present study was undertaken to assess the utility of immuno-histochemical staining with species specific monoclonal antibody to 38 kDa antigen of *Mycobacterium tuberculosis* complex in archival formalin-fixed, paraffin-embedded tissue sections of extra-pulmonary tuberculosis and was compared with conventional Ziehl Neelsen (ZN) staining. So far to the best of our knowledge no published study has shown use of this antibody for the diagnosis of extra-pulmonary tuberculosis.

1. Professor 2. Ph.D. Scholar, Department of Pathology, King George's Medical University, Lucknow (U.P.)
Correspondence: Dr. Madhu Mati Goel, Professor, Department of Pathology, King George's Medical University, Lucknow (U.P.);
Telephone: 91-522-2257580(Off), 2334265(Res); E-mail : madhukgmc@rediffmail.com,

MATERIAL AND METHODS

CASES: A total of 69 cases were taken for detailed study, of which 36 were histologically diagnosed and successfully treated cases of extra-pulmonary tuberculosis of various sites, 8 cases of non-tuberculous granulomatous lesions (which included 3 cases of fungal infection, 3 cases of leprosy and 2 cases of sarcoidosis), 5 cases of follicular hyperplasia of lymph node, 15 cases of malignant tumours (this included 7 cases of metastatic carcinoma and 8 cases of Hodgkin's lymphoma) and 5 cases of pulmonary tuberculosis. Relevant clinical details regarding age, sex, site, signs and symptoms, family history , X-ray, PPD and previous history of anti-tubercular treatment of each case was retrieved from the case files of Department of Pathology, King Georges Medical University, Lucknow. All samples and clinical details were collected after approval by Institutional Ethics Committee.

The histological diagnosis of extra-pulmonary tuberculosis of various sites was based on the classical caseous granulomas observed on the histopathological examination of hematoxylin and eosin stained formalin fixed paraffin embedded tissue sections. All the sections were subjected to Immuno-histochemical and Ziehl Neelsen staining.

CONTROLS

Positive control: Formalin-fixed, paraffin-embedded tissue sections of cases of pulmonary tuberculosis, positive for AFB by Ziehl Neelsen staining were used as positive control for all the staining batches.

Reagent control: The sections from the same cases of pulmonary tuberculosis used as positive control

Table 1: Details of Ziehl Neelsen and Immuno-histochemical staining on 69 formalin-fixed, paraffin-embedded tissue sections of extra-pulmonary tuberculosis : cases and controls

Study Samples	No. of Cases	Ziehl Neelsen Staining for AFB*		Immuno-staining (IHC**)	
		Positive	Negative	Positive	Negative
CASES (36); Extrapulmonary Tuberculosis of various sites					
Lymph node	16	4	12	16	0
Gastrointestinal tract	2	1	1	2	0
Male Genitourinary tract	1	1	0	1	0
Female Genitourinary tract	7	3	4	7	0
Bone and joints	6	3	3	6	0
Head and neck	4	1	3	4	0
Total	**36**	**13 (36.11%)**	**23**	**36 (100%)**	**0**
CONTROLS (33) ; 5 Positive and 28 Negative Controls					
Pulmonary Tuberculosis	5	5	0	5	0
Follicular Hyperplasia of lymph node	5	0	0	0	0
Fungal granuloma	3	0	0	0	0
Leprosy	3	0	0	0	0
Sarcoidosis	2	0	0	0	0
Metastatic Carcinoma	7	0	0	0	0
Hodgkin's Lymphoma	8	0	0	0	0

*AFB - Acid Fast Bacilli
**IHC – Immuno-histochemistry

Table 2: Review of Published literature on IHC studies with polyclonal and monoclonal antibodies for *Mycobacterium tuberculosis*

S. No.	Author & Year	Staining used for acid fast bacilli	Method of IHC & Antibodies used	Nature of Work (No. of Cases)	Results/ Remarks
1	Higuchi et al 1981	NA	Immunoperoxidase; Polyclonal anti-BCG Ab	Clinical (NA)	Positive IHC and negative AFB at BCG inoculation site
2	Humphrey et al 1987	NA	Indirect Peroxidase – Antiperoxidase; Polyclonal Ab	Clinical (59)	IHC positivity = 7/9 (77.7%) No FP*
3	Barbolini et al 1989	ZN*	Indirect Avidin-Biotin complex; MoAbs 60.15, 61.3, 105.10 & 2.16 (raised in mice)	Clinical (23)	IHC positivity (100%)
4	Luo D 1990	Acid-fast-IGSS double staining	Horse Radish Peroxidase – Streptavidin Peroxidase Antiperoxidase ; NA	Both Clinical & Experimental (137)	ZN positivity = 47/137 (34.3%) IHC positivity = 95/137 (69.34%)
5	Wiley et al 1990	Kinyoun – Fite	Peroxidase – Antiperoxidase; anti-MD, anti-BCG, anti-MP (used commercially available antibodies)	Clinical (34)	Kinyoun & Fite positivity = 24/34 (70.5%) IHC positivity = 32/34 (94.1%)
6	Orrell et al 1991	ZN	Avidin –Biotin complex; Polyclonal anti- BCG Ab (used commercially available antibody)	Experimental (30 mice)	Quantitative assessment of bacillary load experimentally induced in granulomas at various time intervals by image analysis measuring amount of immunostaining per acid fast bacilli
7	Radhakrish-nan et al 1991	ZN	Peroxidase – Antiperoxidase; IgG (raised in rabbit)	Clinical (10)	AFB positivity = 0/10 (0%) Culture positivity = 2/10 (20%)IHC positivity = 10/10 (100%)
8	Kutzner et al 1998	ZN, Kinyoun, Gram	Streptavidin - Biotin; Polyclonal anti- BCG Ab (used commercially available antibody)	Clinical (254)	Anti-BCG labeled bacteria and fungi with high sensitivity and minimal background staining, but did not react with spirochetes, viruses, or protozoa (Leishmania).
9	Mukherjee et al 2002	ZN	Avidin – Biotin Complex; Polyclonal anti- BCG Ab (used commercially available antibody)	Clinical (50)	AFB positivity = 22/50 (44%) IHC positivity = 37/50 (87%)
10	Oliveira et al 2004	ZN	Immunoperoxidase	Clinical (3 case reports)	IHC positivity = 3/3 (100%)
11	Ulrichs et al 2005	ZN	Immunoperoxidase; Polyclonal anti –BCG Ab (pAbBCG)	Clinical (NA)	Positive immunostaining of *mycobacterium tuberculosis* bacilli and their products both intra and extracellular.
12	Padma-vathy et al 2005	ZN	Indirect Immunoperoxidase; Polyclonal anti-BCG (raised in rabbit)	Clinical (50)	AFB positivity = 0/50 (0%) Culture positivity = 0/50 (0%)IHC positivity = 34/50 (68%)

NA : Data not available
*ZN – Ziehl Neelsen Method
*FP – False Positive

were stained in similar manner except the primary anti-body was replaced with 1% non-immune serum (reagent control anti-body). No brown coloured staining product was produced in any of the slides.

Immuno-histochemical Staining (IHC) Procedures

Immuno-histochemical staining was performed by Streptavidin-Biotin method as per the manufacture's instructions described in the leaflet supplied with the anti-body and standardized in our laboratory. The staining was carried out with *Mycobacterium tuberculosis* species-specific (MTSS) mouse monoclonal antibody raised to 38kDa antigen of *Mycobacterium tuberculosis* complex and Universal B-Sap secondary anti-body.

Three to five microns thick tissue sections were cut from paraffin blocks. These sections were deparaffinized in fresh xylene (5 minutes) and then rehydrated with decreasing order of ethanol (5 minutes each). Alcohol was removed by distilled water (3 washings). Sections were kept in citrate buffer (pH 6.0) and antigen retrieval was followed by microwave heating (5-10 minutes). Sections were brought to room temperature and washed in tris buffer (pH 7.6) for three times (5 minutes each). The endogenous peroxidase activity of the tissue was blocked by incubating the tissue in 3% hydrogen peroxidase for 10 minutes. Again sections were washed in tris buffer for three times (5 minutes each). Sections were treated with non-immune goat serum (10 minutes) to block the non-specific binding sites followed by washing with tris buffer. Excess buffer was drained . Sections were then covered with primary antibody diluted in tris buffer solution (primary antibody : tris buffer solution = 1:30) and incubated for overnight in moist chamber at 4-7°C. Next day the sections were brought to room temperature and rinsed in tris buffer three times (5 minutes each). Link antibody was added and sections were incubated for 90 minutes and this was again followed by tris washing. Subsequently Streptavidin peroxidase conjugate was added to the sections for 45 minutes. Finally, the chromogen diluted in the substrate buffer (substrate : chromogen = 50 : 1)

Figure 1: Photomicrograph of immuno-histochemical staining in the area of caseous necrosis in histological section of tuberculous lymphadenitis showing extracellular and intracellular antigenic dust as well as individual bacillary fragments (Immuno-peroxidase 1000 oil immersion)

Figure 2: Photomicrograph of immuno-histochemical staining in histological section of tuberculous endometritis showing intensely stained extracellular/intracellular antigenic dust (Immuno-peroxidase 1000 oil immersion)

was added to section and left for 35-45 minutes. Finally, the sections were counterstained with 10% Hematoxylin (2-3 minutes) followed by washing in running tap water, dried and mounted with DPX mountant. Brown colour reaction products indicated positive staining.

RESULTS

Ziehl Neelsen staining showed acid fast bacilli in 13/36 (36.1%) cases and 36/36 showed positivity with IHC staining (Table 1). Positive staining deposits of brown coloured product were seen in and around the areas of granulomas throughout the histological sections. Areas of caseous necrosis showed staining of extra-cellular bacilli, large and small fragments of bacilli and antigenic dust (Figure 1) whereas non-caseating areas, showed extra-cellular and intra-cellular bacilli as well as the mycobacterial antigens characteristically located as diffusely staining granular brownish material within the cytoplasm of macrophages and Langhan's giant cells (Figure 2).

All five sections of pulmonary tuberculosis (positive controls) showed positive results both with Ziehl Neelsen staining and immuno-histochemical staining. None of the non-tuberculous cases showed positivity with either of the stains.

DISCUSSION

In the present study, the immuno-staining showed positive staining with *Mycobacterium tuberculosis* antigens that included whole organisms, their fragments and debris in histological sections of all the 36 cases (100%) of extra-pulmonary tuberculosis whereas Ziehl Neelsen stain for acid fast bacilli was positive in only in 13/36 cases (36.1%) . Low AFB positivity could be due to the fact that only the intact bacilli take up the stain. The positive immnuno-staining in areas where acid fast bacilli were absent or scarce, indicated that concentrated debris derived from mycobacteria apparently retained its antigenic property although it had lost its AFB staining property. The details of published literature reviewed are shown in Table 2. There are only sporadic reports on comparison of Ziehl Neelsen's staining with immuno-histochemical staining[7,10,11,13,15] in experimental and clinical granulomata with ZN positivity ranging from zero per cent to 44% and immuno-histochemistry positivity from 69% to 100%. All the non-tuberculous cases including fungal and sarcoid granulomas did not show staining with IHC.

As evident from Table 2, majority of the workers have used anti-BCG polyclonal antibodies either raised in house or commercially available for immuno-staining. Barbolini et al 1989 experimented with four types of monoclonal antibodies raised in mice against different proteins of *Mycobacterium tuberculosis* and observed that antibody 61.3 to 35 kDa protein of *Mycobacterium tuberculosis* was species specific for *Mycobacterium tuberculosis* complex and was not reactive to *Mycobacterium kansasii*[5]. In our study, species specific monoclonal antibody was directed towards a 38 kDa protein of *Mycobacterium tuberculosis* complex showing 100% positivity with no false positives or false negatives.

The classical histological picture of tuberculous granulomatous inflammation is not a diagnostic problem in a tissue biopsy. However, when the sections show non-caseous epithelioid granulomas mimicking tuberculosis, which is a common occurrence in our biopsies, it poses a diagnostic dilemma. The positive immuno-staining with species specific antibodies in these cases will rule out the differential diagnosis of sarcoidosis or other non-specific tuberculoid granulomas. Further, the technique may also help in assessing the extent of bacillary load and also provide an evidence of tuberculous infection from residual antigen deposits in the tissues, when whole bacilli have been successfully cleared[9].

To conclude, the immuno-staining with species specific monoclonal antibodies to 38 kDa protein of *Mycobacterium tuberculosis* complex may be an efficient diagnostic adjunct to conventional ZN staining for the diagnosis of tissue granuloma of extra-pulmonary tuberculosis. The technique is simple, sensitive and specific. It can be standardized and performed in routine laboratories by trained technicians.

This will also help in clinical decision-making and in reducing the usual practice of prescribing empirical anti-tubercular treatment based on clinical suspicion in the absence of demonstrable evidence of tuberculous infection .

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the financial assistance provided by the Council of Science & Technology, Government of Uttar Pradesh, for this work.

REFERENCES

1. Kamboj SS, Goel MM, Tandon P, Natu SM, Nath P. Correlation study of histopathology and bacteriology in patients of tubercular lymphadenitis. *Ind J Chest Dis Allied Sci* 1994; **36**: 187-191.
2. Goel MM, Ranjan V, Tapan DN, Srivastava AN, Mehrotra A, Khushwaha MRS, Jain A. Polymerase Chain Reaction vs conventional diagnosis in fine needle aspirates of tuberculous lymph nodes. *Acta Cytol* 2001; **45**: 333-340.
3. Hazbon MH. Recent advances in molecular methods for early diagnosis of tuberculosis and drug-resistant tuberculosis. *Biomedica* 2004; **24(Suppl.):** 149-62.
4. Humphrey DM, Weiner MH. Mycobacterial antigen detection by immuno-histochemistry in pulmonary tuberculosis. *Hum Pathol* 1987; **18(7):** 701-8.
5. Barbolini G, Bisetti A, Colizzi V, Dalmiani G, Migaldi M, Vismara D. Immuno-histologic analysis of mycobacterial antigens by monoclonal antibodies in tuberculosis and mycobacteriosis. *Hum Pathol* 1989; **20(11):** 1078-83.
6. Wiley EL, Mulhollan TJ, Beck, Tyndall JA Freeman RG. Polyclonal antibodies raised against Bacillus Calmette-Guerin, *Mycobacterium duvalii*, and *Mycobacterium paratuberculosis* used to detect mycobacteria in tissue with the use of immuno-histochemical techniques. *Am J Clin Pathol* 1990; **94(3):** 307-12.
7. Mukherjee A, Kalra N, Beena KR. Immuno-histochemical detection of mycobacterial antigen in tuberculous lymphadenitis. *Indian J Tuberc* 2002; **49**: 213-216.
8. Padmavathy L, Rao LL, Ramanadhan, Shakila. Mycobacterial antigen in tissues in diagnosis of cutaneous tuberculosis. *Indian J Tuberc* 2005; **52**:31-35.
9. Higuchi S, Moritaka S, Dannenberg AM Jr. et al. Persistence of protein, carbohydrate and wax components of tubercle bacilli in dermal BCG lesions. *Am Rev Respir Dis* 1981; **123**: 397-401.
10. Orelle JM, Brett SJ, Ivanyi J, Coghill G, Grant A, Beck JS. Measurement of the tissue distribution of immunoperoxidase staining with polyclonal anti-BCG serum in lung granulomata of mice infected with mycobacterium tuberculosis. *J Pathol* 1991; **164**: 41-45.
11. Radhakrishnan VV, Mathai A, Radhakrishnan NS, Rout D, Sehgal S. Immunohistochemical demonstration of mycobacterial antigens in intracranial tuberculoma. *Indian J Exp Biol* 1991; **29**: 641-644.
12. Kutzner H, Argenyi ZB, Requena L, Rutten A, Hugel H. A new application of BCG antibody for rapid screening of various tissue microorganisms. *J Am Acad Dermatol* 1998; **38(1):** 56-60.
13. Oliveira BFT, Takay FC, Shida TM, Santo RM, Souza AC, Matayoshi S. Orbital tuberculosis diagnosed by immunohistochemistry : Case Reports. *Rev Inst Med Trop S Paulo* 2004; **46(5):** 291-294.
14. Ulrichs T, Lefmann M, Reich M, Lars M, Roth A, Brinkmann V, Kosmiadi GA, Seiler P, Aichele P, Hahn H, Krenn V, Gobel UB, Kaufmann SHE. Modified immunohistological staining allows detection of ziehl-neelsen negative *mycobacterium tuberculosis* organisms and their precise localization in human tissues. *J Pathol* 2005; **205(5):** 633-640.
15. Luo D. Immunohistochemical demonstration of mycobacterial antigen. *Zhonghua Jie He He Hu Xi Za Zhi* 1990; **13(6):** 360-2,381-2.

Original Article

EFFECTIVE PARTICIPATION OF TB SANATORIA IN REVISED NATIONAL TB CONTROL PROGRAMME (DOTS) IN A METROPOLITAN CITY

K.R. Govinda[1], P. Vijayakumaran[2], P. Krishnamurthy[3] and M.S. Bevanur[4]

(Original received on 8.6.2006; Revised version received on 14.11.2006; Accepted on 16.11.2006)

Summary

***Background*:** Revised National TB Control Programme has been implemented since 1998 in Bangalore metropolitan city which has several big general hospitals, including two TB sanatoria which attract a large number of respiratory symptomatic and TB patients. Till recently there was significant loss of patients for follow up because of lack of mechanism to reach the patients, good recording practices and linkage with district TB control office.

***Objective*:** To establish an effective referral mechanism between TB sanatoria and peripheral health institutions of the government for providing un-interrupted supervised treatment (DOT) to all newly detected TB patients.

***Method*:** TB sanatoria remain as "islands" when Revised National TB Control Programme (RNTCP) with DOTS strategy is implemented. Damien Foundation India Trust (DFIT) provided a Technical Support Team (one medical consultant and three supervisors) to assist in implementation of RNTCP in the district. DFIT liaised with both partners and established procedures for recording correct address, informing health institutions and Senior TB Supervisors (STS) and monitoring referrals. Referral slip and a copy of treatment card were given to patients. One copy of treatment card was sent to respective health facility. Initially the number of STS was not adequate to follow up the patients. The supervisors of Technical Support Team ensured that they were treated in peripheral health institutions or near patients' residence. All STS were in position one year after initiation of this effort.

***Results*:** The referral system is functional. Case holding improved from about 50% to 85% during 2002-2005 by effective transfer through referrals.

Conclusion: Interfacing of NGO between district TB control office and TB sanatoria enabled the establishment of effective collaboration. Initial reluctance was replaced by complete participation in the TB control programme.

[Indian J Tuberc 2007; 54: 30-35]

Key words: TB control, DOTS, Sanatoria

INTRODUCTION

TB sanatoria were established in India in several places, mainly in major cities, as special centres for care of persons with tuberculosis. These centres provide TB diagnostic and treatment services in hospital-based set up. Their specialised nature attracts a large number of respiratory symptomatics. TB patients reporting to these centres are not only from the districts in which they are located but also from neighbouring districts and states even. These persons often do not provide correct address. The sanatoria do not have resources to verify the address of these patients. They do not have field workers to follow up the patients. Less said about the patients from other districts and states the better because of lack of collaboration between TB sanatoria and TB control programme. The difficulties became very much obvious when Revised National TB Control Programme (RNTCP) was implemented with supervised short course therapy. Survey of 94 TB hospitals[1] in India revealed that there were sub-optimal practices. In view of large number of TB patients utilising the services at such health facilities urgent steps were recommended to review the policies for hospitals with beds for TB patients.

There was an acute need for establishing effective referral mechanism between TB sanatoria and peripheral health institutions of the government for providing un-interrupted supervised treatment (DOT) to all newly detected TB patients. TB sanatoria are not under administrative control of General Health System (GHS). Can interfacing of NGO between

1. District Medical Advisor, Technical Support Team – RNTCP, Bangalore Urban district.
2. Chief Medical Advisor, Damien Foundation India Trust, Chennai
3 Secretary, Damien Foundation India Trust, Chennai.
4 District Medical Advisor (retired), Technical Support Team – RNTCP, Bangalore Urban district.

Correspondence: Dr. P. Vijayakumaran, Chief Medical Advisor, Damien Foundation India Trust, 14, Venugopal Avenue, Spurtank Road, Chetpet, Chennai 600031 (Tamil Nadu).E-mail: damienin@airtelbroadband.in

Table 1: Major Health Institutions and infrastructure of RNTCP in Bangalore urban district

1	District TB Centre	1
2	TB Units	5
3	Microscopy Centres	24
4	Primary Health Centres (PHC)	35
5	Primary Health Unit (PHU)	17
6	India Population Project (IPP) Centres	14
7	Large Hospitals	11
8	TB Sanatoria	2
9	Non-Government Organizations (NGO)	9
10	Medical Colleges	2
11	Multi Purpose Health Workers (MPHW)* – male	266
12	Multi Purpose Health Workers (MPHW)* – female	302
13	Anganwadi workers	578

**MPHW = Multi Purpose Health Worker*

district TB control office and TB sanatoria bring about an effective collaboration?

BACKGROUND

Bangalore urban district has covered a population of 1.65 million when RNTCP was introduced in 1998. Population (0.85 million) covered by India Population Project (IPP) is added in late 2003. Though Bangalore is a metropolitan city, it has a combination of urban and rural type of health institutions (Table 1). They are assisted by 578 Anganwadi workers. These health institutions have good number of Multi Purpose Health Workers (MPHW); male 266 and female 302. There are many hospitals (Government and others) in addition to a large group of Private Medical Practitioners.

Through Revised National TB Control Programme (RNTCP), five TB units have been formed in this district. District TB Officer (DTO) is the programme manager for RNTCP in the district. DTO is assisted by one Medical Officer in each TB unit. One STS in each TB unit was planned to coordinate registration and supervision of DOT provider and TB patients on DOT. There were only two STS in position against the requirement of five in the district and they could not cover all the health facilities and patients.

When Revised National TB Control Programme (RNTCP) was introduced in 1993 in India and in 1998 in Bangalore urban, role of TB sanatoria was not clear. The TB sanatoria in Bangalore urban did not follow the guidelines of TB control programme. All newly diagnosed TB patients at TB sanatoria were hospitalised for about a month. Treatment regimens were different from those of TB control programme. When the patient was discharged, there was no way of knowing whether the patients continued the treatment or not. Staff of TB sanatoria were not trained in RNTCP even 5 years after implementation of RNTCP in the district. These centres remained as "islands" amidst National TB Control Programme.

METHOD

Damien Foundation India Trust placed Technical Support Team consisting of a Medical Advisor and three supervisors for assisting District TB Officer (DTO) in implementing RNTCP in the district. Baseline data on the situation in the TB sanatoria and the consequent impact on the programme were collected from the records at the sanatoria and peripheral health institutions and visiting patients. A series of discussions were initiated between authorities at TB sanatoria and district TB office.

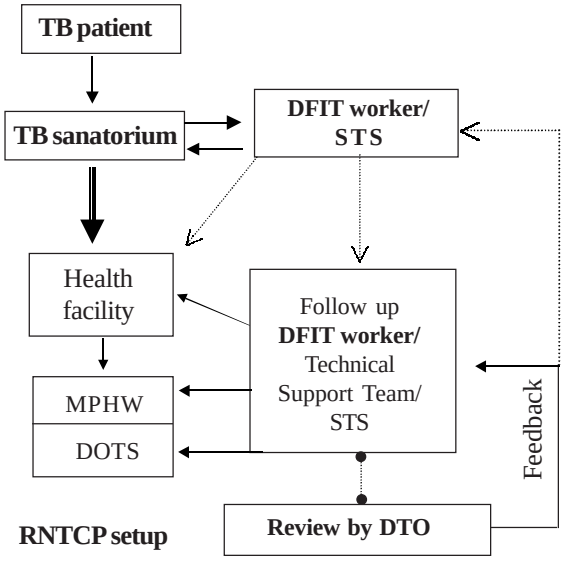

Fig.1: Referral system from TB sanatoria in case detection and treatment delivery in RNTCP.

Procedures for referral were formulated (Fig.1). Formats for referral were developed.

Damien Foundation India Trust placed one supervisor (trained in implementation of RNTCP) in the TB sanatorium. He was responsible for recording of correct address, verification of address, counselling of patients for DOTS and handing over referral slips to health facilities in Bangalore urban. Three copies of referral slips were prepared - one to patient, one to STS and one to health facility. He collected list of patients every week and verified whether they had been started on treatment by visiting health facility and patients' residence. If DOT provider was found to be not appropriate alternate DOT provider from the community was identified and trained on the spot. Majority of the patients were re-visited by the supervisor two or three times during the treatment.

Later, one of the nurses at the out patient clinic of TB sanatorium was identified and trained where upon she took over the responsibility of initial counselling and recording of correct address. Common practice was that all newly diagnosed TB patients were hospitalised for a month and treated with conventional TB treatment regimen. RNTCP drug packs were not supplied to TB sanatorium. This led to difficulties in categorisation of TB patients for treatment in RNTCP. Changes were brought in the admission policy. Patients from TB sanatoria were assured that they would get the treatment if correct address was provided.

Patients without complications were hospitalised for a short stay and those with complications for a longer period. The hospital authorities minimised hospitalisation to a few days for investigation only. These patients were referred to peripheral health institutions for initiation of anti-TB treatment. Patients with complications were hospitalised longer. All eligible in-patients were started on appropriate RNTCP regimen using extension pouch. Patient treatment cards for hospitalised TB patients were maintained as per RNTCP guidelines. The same cards were transferred to health facility when they were discharged from the hospital and treatment continued at the peripheral health institutions.

DFIT worker (later STS) contacted the absentees and ensured that majority of patients continued the treatment. In the meantime all the vacant STS positions were filled and they started involving in the follow up procedures. List of patients was given to STS who would ensure follow up of these patients at health facility (in addition to visits by TST). Progress of the activities was discussed at the weekly review meeting by DTO.

RESULTS

TB sanatorium-I was taken up in end of 2002. Less than 30% of the TB patients referred by TB sanatorium reached health facilities as per preliminary assessments done during last quarter of 2002. The progress was slow (44 – 50% of effective transfer) during the initial period - 2003 (Table 2) as one DFIT worker was involved in referral and follow up procedures. The results improved to 85% and more during 2003, as there was active involvement of STS from RNTCP and Technical Support Team. The number of referrals had doubled after

Table 2: Status of TB patients referred from TB sanatoria in Bangalore urban district in 2002-05 (registration for treatment at health facility)

	TB sanatorium I			TB sanatorium II		
Year / Quarter	**TB Cases Referred**	**TB Cases Registered**	**%**	**TB Cases Referred**	**TB Cases Registered**	**%**
2002						
I Quarter	63	28	44.4	-	-	-
II Quarter	93	47	50.5	-	-	-
III Quarter	97	45	46.4	-	-	-
IV Quarter	134	65	48.5	-	-	-
Total:	**387**	**185**	**47.8**	-	-	-
2003						
I Quarter	169	153	90.5	77	42	54.5
II Quarter	220	189	85.9	73	50	68.5
III Quarter	196	178	90.8	73	61	83.6
IV Quarter	170	144	84.7	93	78	83.9
Total:	**755**	**664**	**87.9**	**316**	**231**	**73.1**
2004						
I Quarter	149	121	81.2	58	36	62.1
II Quarter	182	160	87.9	40	37	92.5
III Quarter	150	136	90.7	89	76	85.4
IV Quarter	131	121	92.4	82	72	87.8
Total:	**612**	**538**	**87.9**	**269**	**221**	**82.2**
2005						
I Quarter	152	135	88.8	102	90	88.2
II Quarter	128	102	79.7	128	102	79.7
III Quarter	140	119	85.0	110	87	79.1
IV Quarter	151	118	78.1	99	78	78.8
Total:	**571**	**474**	**83.0**	**439**	**357**	**81.3**

introduction of referral system. The achievement was maintained through 2004-2005.

TB sanatorium-II was taken up gradually in 2003. There was significant improvement (54% to 83%) in 2003. The achievement was maintained through 2005. In this district TB patients referred from TB sanatoria constitute 12% of annual case detection in 2002 (Table.3). It improved to 25 to 41% during subsequent period. The referral system has become a routine practice in both sanatoria. Progress of effective referrals is reviewed during weekly review meetings at District TB Office. Migration of TB patients with in the district is intimated to the concerned STS for effective follow up.

After witnessing the success of this procedure following arrangements have been done at TB sanatorium.

Table 3: Case notification by TB sanatoria and Bangalore urban district (2002-05)

Year	Total TB patients registered in the district	Total TB patients effectively transferred from TB sanatoria	
		Number	%
2002	1516	185	12.2
2003	2165	895	41.3
2004	2859	759	26.5
2005	3142	831	26.4

- RNTCP section has been established at out patient clinic.
- Microscopy centre as per RNTCP guideline has been established.
- Lab Technician is trained in RNTCP procedures.
- TB sanatorium has provided a Medical Officer trained in RNTCP.

As per recent guidelines from Central TB Division DOT centre with one Health Visitor has been established at TB sanatorium to coordinate the activities.

DISCUSSION

Even though the role of TB sanatoria in TB control is doubtful in the present context one cannot take away their importance from the point of view of large number of TB patients diagnosed and managed there. Our experience though limited to two sanatoria makes us wonder if the problems observed are not more widespread.

Wherever TB sanatoria exist they attract a large number of patients from within the district as well as outside. Since they are not under the administrative span of general health system it is often difficult to introduce changes in the diagnosis, registration and treatment practices. Initial assessment in TB sanatoria in Bangalore indicates that about 70% of newly diagnosed TB patients were not registered for treatment. This means that a large number of TB patients are lost for treatment after diagnosis. It requires a lot of effort and goodwill on the part of all major players to bring them into the mainstream of TB control. The transformation in the functioning of two sanatoria in Bangalore urban with respect to management of persons with tuberculosis is noteworthy.

TB patients used to receive one-month supply of TB drugs for self administration. There was no mechanism to ensure regular drug intake. It was also observed that TB patients tended to hop from one service provider to another for various reasons. They might receive different treatment regimen at different periods. There was no mechanism to ensure regular drug intake. There was risk of loss of patients for follow up and irregular treatment resulting in risk of developing drug resistance. TB sanatoria and big hospitals do not have field staff and hence these health facilities can offer Directly Observed Treatment (DOT) to a limited number of patients detected in their out patient clinics.

Remarkable contribution in terms of case notification (18% to 85%) and sputum positive case notification had been reported[2-6] by involvement of private service providers. The outcome would be more if hospitals treating TB patients are also brought into the network of RNTCP. As per the observation in this study about 25 to 41% of annual case detection is from TB sanatoria (Table 3) in Bangalore district alone. It means that 25 to 41% of TB patients would not have been benefited with adequate and

appropriate treatment if this referral system had not been in place. It may be appropriate to assert that this referral system prevented considerable number of TB patients from getting lost for follow up and at least some of them deteriorating to drug resistant TB. Revised RNTCP guidelines[7] 2005 includes the similar procedures.

CONCLUSION

TB sanatoria and RNTCP infrastructure are under different administrative heads. Collaboration is not an easy task. An intermediate agency can play an important role in bringing them together to establish a sustainable referral system. **Persistent efforts by DFIT staff and STS have resulted in complete acceptance of the change and effective participation. This technical support improved the quality of TB treatment services in the district. Similar referral system may be applicable to major hospitals in urban settings.**

ACKNOWLEDGEMENTS

We thank the State TB Officer, District TB Officer and Superintendents of TB sanatoria for their co-operation. All peripheral health institutions in Bangalore urban need special mention for the active participation in RNTCP. The STS of RNTCP have taken up the challenge and proved their efficiency. Mr. Madavareddy, Mr. Ramanjeneyalu and Mr. Venugopal (Supervisors of Technical Support Team) have done a remarkable achievement.

REFERENCES

1. A.A. Singh, T.R. Frieden, G.R. Khatri, R. Garg. A survey of tuberculosis hospitals in India. *The Int J Tuberc Lung Disease* 2004; **Volume 8, Number 10, October**: 1255-1259(5)
2. Quy H.T, Lan N.T.N, Lönnroth K, Buu T.N, Dieu T.T.N., Hai L.T. Public-private mix for improved TB control in Ho Chi Minh City, Vietnam: an assessment of its impact on case detection. *The Int J Tuberc Lung Disease* 2003; **Volume 7, Number 5, May**: 464-471(8)
3. Arora V.K, Sarin R, Lönnroth K. Feasibility and effectiveness of a public-private mix project for improved TB control in Delhi, India. *The Int J Tuberc Lung Disease* 2003; **Volume 7, Number 12, December**: 1131-1138(8)
4. Ambe.G, Lönnroth.K, Dholakia.Y, Copreaux.J, Zignol.M, Borremans.N, Uplekar.M. Every provider counts: effect of a comprehensive public-private mix approach for TB control in a large metropolitan area in India. *The Int J Tuberc Lung Disease* 2005; **9(5):** 562-568.
5. Kumar.M.K.A, Dewan.P.K, Nair.P.K.J, Frieden.T.R, Sahu.S, Wares.F, Laserson.K, Wells.C, Granich.R, Chauhan.L.S. Improved tuberculosis case detection through public-private partnership and laboratory-based surveillance, Kannur district, Kerala, India, 2001-2002 *Int J Tuberc Lung Dis* 2005;**9(8):**870-876.
6. Maung, M, Kluge, H, Aye, T, Maung, W, Noe, P, Zaw, M, Jost, S.P, Uplekar, M, Lönnroth, K. Private GPs contribute to TB control in Myanmar: evaluation of a PPM initiative in Mandalay Division. *The Intl J Tuberc Lung Disease* 2006; **Volume 10, Number 9, September:** 982-987(6)
7. Technical and Operational Guidelines for Tuberculosis Control (Oct 2005) Annex 6A and 6B, Central TB Division, Directorate General of Health Services, Ministry of Health and Family Welfare, Nirman Bhavan, New Delhi 110011

BOARD OF DIRECTORS OF UNION

Dr. M.M. Singh, Vice-Chairman (OR), TAI was elected as the Chairman of the South East Asia Region of The Union and he was also elected as one of the Directors on the Board of Directors of The Union.

Original Article

A STUDY OF PHAGE BASED DIAGNOSTIC TECHNIQUE FOR TUBERCULOSIS

Purabi Barman and Deepa Gadre

(Original received on 26.6.2006. Revised version received on 9.11.2006. Accepted on 16.11.2006)

Summary

Background: Tuberculosis (TB) is a leading infectious disease in India.Diagnosis of TB has always been a problem due to slow rate of growth of *Mycobacterium tuberculosis*. In this study we have compared the conventional tools for diagnosis of TB with the new Fast Plaque TB ™.

Material and Methods: Two hundred and twelve clinically suspected cases of TB were enrolled for the study. Three consecutive early morning sputum samples were collected from each patient. Sputum smears were examined after staining with ZN stain and the sputum samples were later subjected to culture and phage assay.

Results: It was seen from the study that out of the total 212 cases, 106 were phage positive and 106 were phage negative. Sensitivity of the phage test with respect to AFB smear is 94.34% and specificity of the phage test is 93.88%. A total of 120 specimens grew on LJ media, of which 112 were *Mycobacterium tuberculosis*, 2 were *Mycobacterium Kansasii*, 4 were *Mycobacterium avium complex* and 2 grew *Mycobacterium fortuitum* group. Out of these 120 specimens which grew on LJ, 104 were also positive for phage assay. All the 8 Non-Tubercular Mycobacteria were negative by the Fast Plaque Assay. Out of the 92 which were negative on LJ, 2 were positive by phage assay. Sensitivity and specificity of phage assay with respect to growth on LJ was 92.86% and 97.83% respectively.

Conclusion: The study showed that phage assay is a rapid, reliable and cost effective method in diagnosing pulmonary tuberculosis from clinical samples. **[*Indian J Tuberc 2007; 54:36-40*]**

Key words: Tuberculosis, *Mycobacterium tuberculosis*, Bacteriophage assay

INTRODUCTION

Tuberculosis (TB) is a leading health problem worldwide and remains one of the leading causes of death from infectious diseases. An estimated 2 billion people (i.e., one third of the world's population) are infected with *M. tuberculosis*. Each year, approximately 9 million people suffer from the disease, and approximately 2 million die as a result.[1] Tuberculosis kills more adults in India than any other infectious disease. More than 1,000 people a day or one in every minute die of TB in our country.[2]

The prevalence of all forms of TB in India is estimated to be 5.05 per thousand, prevalence of smear positive cases 2.27 per thousand and average incidence of smear positive cases is 84 per 100,000 annually.[3] The incidence of TB is expected to increase substantially worldwide because of the interaction between TB and human immunodeficiency virus (HIV) /AIDS epidemic.

Nearly 1.8 million Indians get infection every year. Everyday, about 5000 people develop the disease and around 1000 die.[4] In India TB kills more in the younger age group thus compounding to the economic loss of the country. The direct cost of the disease in India annually is estimated at US$300 million; the annual indirect cost is US$3 billion.[4]

In our country, with a high prevalence of Tuberculosis, diagnosis is mainly based on the conventional methods like clinical assessment, radiology, sputum microscopy and culture in Lowenstein Jensen (LJ) media. New diagnostics approaches, including nucleic acid amplification, antibody detection, liquid culture, cellular immune response, antigen capture, and chemical and physical

Department of Microbiology, University College of Medical Sciences and Guru Teg Bahadur Hospital, Delhi-110095.
Correspondence: Dr. Purabi Barman, Department of Microbiology, University College of Medical Sciences and Guru Teg Bahadur Hospital, Delhi-110095; Email: purabiduorah@sify.com; Phone: 09899094295 (m)

detection tests have been developed.[5] Many molecular methods have been developed for direct detection, species identification, and drug susceptibility testing of mycobacteria.[6] These require expertise and finance, and are not easily affordable in low income countries. The sensitivity of smear microscopy has been between 20%-80% in culture confirmed TB cases.[7] Though smear microscopy can detect positive cases if properly performed, it can miss quite a number of paucibacillary cases. The quality of results with smear microscopy is heavily dependant on the workload, skill and motivation of the technician reading the slides.[8] Culture techniques are available but the time required and negative results in paucibacillary cases are important limitations.[9] Chest X-ray is commonly used to aid the diagnosis of TB. However, since radiological changes are not specific for TB and do not always reflect active disease, over-reliance on chest X-ray can lead to misdiagnosis.[8] Therefore, there is need for a rapid, reliable and sensitive method for diagnosis of pulmonary tuberculosis so that early treatment can be started and the disease can be contained.

Fast Plaque TB ™ is a rapid manual bacteriophage based test to detect viable *Mycobacterium tuberculosis* (*M.tb*) in clinical specimens. The technique uses a mycobacteriophage which is able to infect and replicate in slow growing pathogenic strains e.g *M.tb*, *M. ulcerance* and also in some rapidly growing strains as *M. smegmatis*. Mycobacteriophages have the potential to become useful tools in the diagnosis of TB, as they are specific for mycobacteria and only replicate in, and hence detect, viable cells. Phage-based techniques involve simple manual manipulations and yield results rapidly.

In this technique, phages are added to the decontaminated sputum samples so that viable target bacilli are infected. A potent virucide is added which rapidly destroys any bacteriophage outside the target cells, without affecting phages inside the bacilli. These surviving bacteriophages replicate inside the tuberculer bacilli and lyse the bacteria in order to release the progeny bacteriophage. Virucide added earlier is neutralized and non-pathogenic rapidly growing mycobacterium which is also susceptible to the bacteriophage is then added. This is then plated on agar mixture as a lawn.The rapidly growing mycobacterium grows overnight and if it is infected with the phage, plaques are formed which indicate that viable tuberculer bacilli were present in the original specimen.

In this study, we have tried to compare the new Fast Plaque TB ™ with the conventional methods for diagnosing pulmonary tuberculosis i.e. direct microscopy as per RNTCP and culture on Lowenstein-Jensen (LJ) media.

MATERIAL AND METHODS

Two hundred and twelve suspected cases of tuberculosis attending DOTS Centre at UCMS & GTB Hospital, Delhi, were enrolled for the study. Under DOTS, tuberculosis "Suspect" patients are those who present with symptoms and signs suggestive of TB, in particular cough of long duration.

Three consecutive early morning sputum samples from all 212 patients were examined. These were collected in a clean leak proof labelled sterile container. First, smear was prepared and stained by Ziehl-Nielsen (ZN) stain. These stained smears were examined for the presence of acid fast bacilli (AFB) and graded as per RNTCP recommendations. These samples were then decontaminated and concentrated as per manufacturer's specifications. The assay was carried out by using Fast Plaque TB™ kit. For the assay, decontaminated and concentrated sediment was mixed with FPTB Medium Plus and incubated at 37^0C overnight to enrich viable TB bacilli present in the sample. After enrichment, Actiphage™ solution was added and incubated for further one hour to allow infection to take place. Then Virusol™ solution was added for destruction of all bacteriophages, which have not infected host cells and then incubated at room temperature for 15 minutes. FPTB Medium Plus was again added to neutralize excess of virucide, followed by Sensor™ cells. 5 mL of FPTB molten agar was poured to pre-labelled petridish and to it was added the above reaction mixture. The plates were mixed well and allowed to set at room temperature. Then they were incubated at 37^0C

overnight. Next day results were recorded as plaque formation. Plaque formation indicated presence of viable bacilli in the original sample. Results were interpreted as positive if ≥ 20 plaques were present and 0-19 plaques signified negative results.

.

The deposits formed after concentration of the sputum samples were also inoculated on LJ media slope in duplicate. These were examined weekly for growth. Any growth was checked by ZN staining. Identification was done on the basis of rate of growth, colony morphology, pigment production, biochemical tests like niacin production, aryl sulphatase, catalase production, nitrate reduction, growth on PNB, tween hydrolysis and TCH susceptibility test as per CDC manual ,1985.

RESULTS

Out of the total 212 tuberculosis "Suspect" patients, 110 were male and 102 were female (male: female ratio – 1:0.92). The age of the patients ranged from 10-60 years with maximum patients in age group 20-39 years (84, 39.62%) followed by 40-59 years (78, 36.80%).

Table 1 represents distribution of tuberculosis patients as per DOTS programme. Accordingly there were 124 "Cases" of tuberculosis. 112 of these were "Definite" cases and rest 12 were not as 2 were diagnosed by radiology and 10 were only one smear and radiologically positive.

Definite TB cases under DOTS are those with positive culture for *Mycobacterium tuberculosis* and in countries where culture is not routinely available, two sputum smears positive for AFB is also considered a "Definite" case.

In all, 104 of these "Definite" TB cases were both smear and culture positive and 8 were only culture positive but smear negative.

Out of 212 Tuberculosis "Suspect", 114 were found to be positive by sputum smear examination.10 out of these were one smear positive and supported by radiology. 98 were sputum smear negative. Out of these, 8 grew on culture and 2 were supported by radiology, raised ESR, etc.

Table 2 shows results of Fast Plaque Assay with respect to AFB smear positivity. Of 212, 106 were phage positive and rest 106 were phage negative. Out of 114 smear positive cases, 100 were phage positive and 14 were phage negative; 8 of which were Non Tubercular Mycobacteria (NTM).

Table 1: Case distribution as per DOTS case definition.

No. of cases of tuberculosis (124)		Definite cases (112)		Smear positive Pulmonary Cases (114)		Smear negative Pulmonary Cases (10)	
New cases	Relapse cases	Culture +ve & 2 smear +ve	Culture +ve & smear -ve	Culture+ve & 2 Smear+ve	1 smear +ve & X-ray +ve	Culture+ve & Smear-ve	Only X-ray+ve
108	16	104	8	104	10	8	2

Table 2: Results of Fast Plaque Assay with respect to AFB smear positivity

	Phage positive	Phage negative	Sensitivity (%)	Specificity (%)
AFB smear positive (114)	100	6+8(NTM)	94.34	93.88
AFB smear negative (98)	6	92		
Total (212)	106	106		

Table 3: Comparison of growth on LJ with phage assay.

	Phage positive	Phage negative	Sensitivity (%)	Specificity (%)
LJ positive (120)	104	8+8(NTM)	92.86	97.83
LJ negative (92)	2	90		

Amongst the 98 smear negative samples, 6 samples were positive by phage assay.

The sensitivity of the phage test with respect to AFB smear positivity was 94.34% and specificity was 93.88%. The positive predictive value was 94.33% and negative predictive value was 93.88%.

All 212 samples were cultured on LJ media and 120 grew acid fast bacilli (56.60%) which were confirmed by ZN smear and biochemical tests. These included 112 *M.tuberculosis* and 8 NTM isolates. These were 2 *M.Kansasii*, 4 *M.avium complex* and 2 *M.fortuitum* group. There were 10 sputum negative pulmonary TB cases, 8 of which grew *M.tuberculosis* on LJ media. They also had raised ESR.

Table 3 compares result of culture positivity with phage assay. It was seen that of a total 120 culture positive samples, 104 were also phage positive. Rest 16 were phage negative of which 8 were NTM. 92 samples did not grow on LJ media and out of these 2 were positive by Phage assay. Thus the sensitivity and specificity of phage assay with respect to growth on LJ media was 92.86% and 97.83% respectively. The positive predictive value was 98.11% and negative predictive value was calculated at 91.84%.

DISCUSSION

Diagnosis of TB has been a problem due to slow growth of the organism. This hampers in treatment of cases, thus increasing the mortality and morbidity of the disease. Smear microscopy is simple and most rapid procedure currently available to detect AFB in clinical specimens. The limit of detection with this method is that it requires at least $5x10^3$ bacilli per ml of sputum.[10] The major drawback of growing mycobacteria in conventional media is its slow growth which requires an incubation period of at least 4 weeks. Thus there is need for a rapid, reliable and sensitive method for the diagnosis and treatment of the disease. Phage assay is a simple technique which does not require any expensive instrumentation and can be used in most routine mycobacteriology laboratory.

Phage assay has short detection time of 24-48 hours compared to conventional growth on Lowenstein Jensen media. Results are easily available in terms of plaques and easy to interpret. In our study, plaques varied in number from 25 to more than 300. In majority of highly positive cases by smear, more than 300 plaques were seen as confluent lysis on agar plate. Variation in plaque number can be attributed to number of viable bacilli present in sputum samples.

In this study, samples were taken from DOTS centre in our hospital. In DOTS, AFB smear is taken as diagnostic test and AFB positive patient is started on anti-tubercular treatment (ATT) with assumption that patient has *M.tuberculosis* infection. On comparison of AFB smear with Fast Plaque Test, (table 2) it was found that out of 212 samples, 100 were both AFB smear and phage positive. 14 were AFB smear positive and phage test negative; 8 of them later on grew NTM. Phage test is highly specific for *M. tuberculosis* complex and so could not detect NTM. Two samples not detected on phage test were re-treatment case, already on ATT. Either ATT drugs interfered with phage test or else bacilli were non-viable and phage test detects only live bacilli.

There were 6 cases which were phage test

positive and smear negative. These 6 phage positive cases also later grew *M.tuberculosis* on culture. Phage assay has analytical sensitivity of 100 bacilli, so it detected paucibacillary specimens, which were missed on AFB smear alone.

The sensitivity of phage test with respect to AFB smear positivity was 94.34% and specificity was 93.88% in our study. S. Shenai et al[11] recorded a similar result with a sensitivity of 90.6% and specificity of 100%. Phulputo et al[12] reported sensitivity and specificity at a low of 54.16% and 83.33% when Fast Plaque was compared with smear positivity. Muzarraf et al[13] recorded sensitivity and specificity of 87.4% and 88.2% respectively while Albert et al[14] recorded sensitivity and specificity of 86.8% and 83.8% respectively.

In our study the sensitivity and specificity of phage assay when compared to growth on LJ media (Table 3) were 92.86% and 97.83% respectively. Muzarraf et al[13] showed sensitivity and specificity of 81.6% and 97.7% respectively, which is comparable to our study. Phulputo et al.[12] recorded sensitivity and specificity of 86.23% and 96.42% respectively while Shennai et al[11] recorded 93.1% and 88.2% in their study. A sensitivity and specificity of 58.3% and 99.1% was stated by Alcaide et al.[15]

Our study showed high sensitivity and specificity, making it useful as a good tool for diagnosing tuberculosis. The assay could be useful in a country like ours where the disease is highly prevalent and a prompt diagnosis is important from both health and economic points of view. Phage assay is a rapid, reliable and cost-effective method. It does not require specialised techniques and is easy to perform. The test is sensitive enough to detect and confirm clinically suspected smear negative cases. Moreover, since it gives result within 2 days, it hastens the diagnosis of the disease, thereby helping in the treatment of the same. It can also be recommended as an additional diagnostic test in the health centres. However, more research needs to be conducted to determine its usefulness at the peripheral level.

The cost of the test though a little more than sputum microscopy, can still be included as a routine diagnostic procedure since it will cut down hospital visits, hospital stay, morbidity and mortality resulting from Tuberculosis which in turn will further the economic growth of the country.

REFERENCES

1. Cdc.gov [homepage on the internet]. Atlanta, Centers for Disease Control and Prevention, World TB Day — March 24, 2006 MMWR 2006; 55 (No. 11) (cited March 23, 2006) available from http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5511a1.htm
2. Tuberculosis- a guide for practicing Physicians. Available at http://www.trc-chennai.org/Rntcp/GuideforPrPhysicians.pdf
3. Chakraborty AK. Epidemiology of Tuberculosis: Current status in India. *Indian J Med Res.* 2004 Oct; **120(4):** 248-76.
4. TB India 2006. RNTCP Status Report (cited 24th march 2006). Available from http//www.tbcindia.org.
5. Perkins MD.New diagnostic tools for detection of tuberculosis. *Int J tuberc Lung Dis*. 2000; **4**: s182-s188
6. Hanna Soini, James M. Musser. Molecular diagnosis of Mycobacteria. *Clinical Chemistry* 2001; **47(5):** 809-814.
7. Colebunders R, Bastian I. A review of the diagnosis and treatment of smear negative pulmonary tuberculosis. *Int J Tuberc lung Dis* 2000; **4**: 97-107.
8. Tracy Seaman, Andre Trollip, Richard Mole, Heidi Albert. The use of a novel phage- based technology as a practical tool for the diagnosis of tuberculosis in Africa. *African journal of Biotechnology* 2003 Feb; **Vol(2):** 40-45.
9. Katoch V.M.Newer diagnostic techniques for tuberculosis. *Indian J Med Res* 2004; **120:** 418-428.
10. What is new in the diagnosis of Tuberculosis? Part 1: Techniques for diagnosis of Tuberculosis. *ICMR Bulletin* 2002, August.**Vol 32:** No. 8.
11. Shenai S, Rodriques C, Mehta AP. Evaluation of a new phage amplification technology for rapid diagnosis of tuberculosis. *Ind J Med Microbiol* 2002; **20(4):** 194-199.
12. Phulpoto MA, Qayyum S, Rizvi N, Khuhawar SM. Diagnostic yield of fast plaque TB test for rapid detection of Mycobacterium in tuberculosis suspects. *J Pak Med Assoc* 2005 Feb; **55(2):** 57-60.
13. Muzaffar R, Batool S, Aziz F, Naqvi A, Rizvi A. Evaluation of Fastplaque TB™ assay for the direct detection of Mycobacterium tuberculosis in sputum specimens in Pakistan. *Int J Tuberc lung Dis* 2002; **6**: 635-640.
14. Albert h, Heydenrych A, Brookes R, Mole RJ, Harley B, Subotskye, Henry R, Azevedo v. Performance of a rapid phage- based test, Fastplaque TB™ to diagnose pulmonary tuberculosis from sputum specimens in South Africa. *Int J Tuberc lung Dis* 2002; **6**: 529-537.
15. Alcaide F, Gali N, Dominguez J, Berlanga P, Blanco S, Orus P, Martin R. usefulness of a new mycobacteriophage-based technique for the rapid diagnosis of pulmonary tuberculosis. *Journal of Clinical Microbiology* 2003 **July, p**: 2867- 2871.

Original Article

REVERSE PASSIVE HAEMAGGLUTINATION (RPHA) TEST FOR DETECTION OF MYCOBACTERIAL ANTIGEN IN THE CEREBROSPINAL FLUID FOR DIAGNOSIS OF TUBERCULAR MENINGITIS

K. Venkatesh, S. C. Parija, S. Mahadevan* and V. S. Negi**

(Original received on 27.12.2005. Revised version on 16.11.2006. Accepted on 21.11.2006)

Summary

Background: Various serological techniques have been developed to detect antibodies and antigens in the cerebrospinal fluid (CSF) for diagnosis of tubercular meningitis. Most of the serological assays are ELISA based. Attempts have been made to use much simpler antigen detection techniques like the reverse passive haemagglutination (RPHA) which is simple and cost-effective.

Aims: To evaluate the reverse passive haemagglutination (RPHA) test for detection of mycobacterial antigens in the CSF for diagnosis of tubercular meningitis.

Methods: In the present study, we have made the use of polyclonal antiserum against heat killed whole *Mycobacterium tuberculosis* bacilli to sensitize the RBCs in RPHA to detect antigens in clinically suspected cases. A total of 46 cases (clinically suspected TBM 24, culture proven TBM 2, non- TBM cases 20) were included in the present study for detecting *M.tuberculosis* antigen in the CSF specimens.

Results: Of the 26 test CSF specimens, 13 CSF specimens were positive by RPHA while 4 of the 20 control CSF specimens were also reactive. Two culture positive specimens included in the study were positive by RPHA. Of the 4 control CSF specimens positive by RPHA, 3 were culture proven cases of pneumococcal meningitis and 1 was a case of cryptococcal meningitis. The RPHA is found to be 50% sensitive and 80% specific; and showed a 76.4 % positive predictive value and a 55.2 % negative predictive value.

Conclusion: The RPHA is a simple test that could be used as an adjunct in diagnosing TBM. It does not require any special equipment or technically trained or skilled manpower. It is economical and can be afforded for use in community where TBM is more prevalent. Even though the present study showed a poor sensitivity and specificity, further identification, characterization and evaluation of better immuno-dominant and specific antigens or epitopes, and the usage of antibodies developed against such mycobacterial antigens might improve the sensitivity and specificity of this test. ***[Indian J Tuberc 2007; 54: 41-48]***

Key words: Tubercular Meningitis (TBM), Reverse Passive Haemagglutination (RPHA), Antigen Detection, CSF, *Mycobacterium Tuberculosis.*

INTRODUCTION

The incidence of tuberculous meningitis (TBM), the most serious form of extra-pulmonary manifestation, parallels the prevalence of pulmonary tuberculosis in the community. Despite the availability of effective anti-tubercular agents, the mortality and morbidity due to TBM remains high. This is mainly due to the delay in the diagnosis, as early TBM can present with non-specific signs and symptoms[1, 2]. Clinically, early TBM can mimic many other central nervous system pathologies. It is most often confused with partially treated pyogenic meningitis. Conventional methods like demonstration of acid fast bacilli in Ziehl-Neelsen smear and by culture though considered as the gold standard are less sensitive[3]. As a result, not only is there an under-diagnosis of early TBM but also an unjustified use of empirical ATT for non-tuberculous diseases[4].

Various serological techniques have been developed to detect antibodies and antigens in the cerebrospinal fluid (CSF) [5-7]. Most of the serological assays are ELISA based[5,7,8]. Other methods like radio-immunoassay (RIA) and electo-immunotransfer blot (EITB) were also used by some workers[9-13]. Attempts have been made to use much simpler antigen detection techniques like latex agglutination (LAT)

Departments of Microbiology, Paediatrics* and Medicine**; Jawaharlal Institute of Postgraduate Medical Education & Research, Pondicherry
Correspondence: Professor Subhash Chandra Parija, Director-Professor & Head, Department of Microbiology, Jawaharlal Institute of Postgraduate Medical Education & Research, Pondicherry 605006; E mail: parijasc@vsnl.com

and reverse passive haemagglutination (RPHA)[6,13,14]. These are simple and cost-effective. But to increase the sensitivity and specificity of these serological tests, there is a need for improvisation in these techniques[15].

In our present study, we have evaluated the reverse passive haemagglutination (RPHA) test for detection of mycobacterial antigens in CSF for diagnosis of tubercular meningitis.

MATERIAL AND METHODS

Patients and controls

CSF specimens were collected from clinically suspected cases of tuberculous meningitis (TBM) and the control CSF specimens were collected from non-TBM cases admitted to Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER) hospital, Pondicherry, during the study period from 2002 to 2004.

Groups included:

Group I (Clinically suspected cases of TBM): This group included 26 clinically diagnosed cases of TBM, taking into consideration the criteria proposed by Ahuja et al.[16] of which 2 were culture positive confirmed cases of TBM.

Group II (non-TBM control cases): This group included 20 patients. These 20 non-TBM cases consisted of 7 culture proven bacterial meningitis, 5 of cryptococcal meningitis, 3 of neonatal meningitis, 2 of neurocysticercosis and 3 with non-infectious CNS disorders.

Cerebrospinal fluid

One ml to two ml of the cerebrospinal fluid was collected from the patients by doing a lumbar puncture under aseptic precautions. The CSF specimens were collected from both the clinically suspected cases of TBM and non-TBM cases. The CSF specimens were stored at -20ºC till use.

Preparation of Mycobacterial antigen

H37Rv *Mycobacterium tuberculosis* strain obtained from Tuberculosis Research Centre, Chetpet, Chennai, was used. Antigen was prepared from this strain according to the method described by Radhakrishnan et al with modifications[17]. Briefly, this strain was grown in Sauton's culture medium (250-300ml) in 1000ml Roux culture bottle. 1ml suspension of viable bacilli from 10-14 day old 'seed' culture was inoculated into the Roux culture bottle. After incubation for 8-10 weeks, the culture was autoclaved and the bacillary sediment was obtained after centrifugation at 10,000 rpm X 30 min at 4ºC. The sediment was washed with distilled water, dried at 60ºC and retained for immunization.

Preparation of hyperimmune antimycobacterial antiserum

Hyperimmune antiserum was raised in rabbits as per the procedure described by Radhakrishnan et al with modifications[17]. In the primary immunization, each rabbit received 2mg of dried autoclaved bacillary sediment mixed thoroughly in 2ml of incomplete Freund's adjuvant. The 2ml suspension was injected in divided doses into two gluteal intramuscular sites and two flank subcutaneous sites. The immunization schedule was repeated on 14th, 21st, 28th, 35th day following primary immunization.

The antiserum collected by bleeding from the ear vein was purified as per the method described by Gottstein (1984)[18]. Briefly, 1 ml of the cold serum was mixed with 1ml of cold saline at pH 7. The serum-saline mixture (2 ml) was added drop wise to 2 ml of cold saturated ammonium sulphate (pH 7) by stirring for 30 minutes in ice and then centrifuged at 3000Xg at 0ºC. The supernatant was then discarded and the precipitate was suspended in 2ml of saline and the procedure was repeated until the supernatant was colourless. The final precipitate was suspended to 1ml and dialyzed against PBS (pH 7.2) to remove all the residual ammonium sulphate. Titre of the purified antiserum was 1 in 1024 by IHA test done by coating Tuberculin-PPD to double aldehyde stabilized chick RBCs[19]. The purified antiserum was

stored in aliquots at -20º C.

Reverse passive hemagglutination (RPHA)

The mycobacterial antigens were detected in CSF by reverse passive hemagglutination (RPHA) as described below:

The procedure consists of the following steps:

(i) *Collection of chick red blood cells:* Under aseptic conditions, 10 ml of chick blood was collected in a flask containing 30 ml of Alsever's solution. The cells were kept at 4ºC for 24 hours.

(ii) *Sensitisation of chick RBCs:* The cells were washed 3 times thoroughly with PBS 7.2 followed by 3 times in 0.9% NaCl and then packed to 0.1ml. The optimum sensitizing dose (OSD) of the antisera was obtained by chequer board method using RBCs sensitized with different dilutions of antisera. The coupling of antibodies to RBCs was done as described by Goding (1976)[20] with a few modifications. Chromium chloride solution was prepared by adding 30 mg of chromium chloride in 10 ml of 0.85% sodium chloride. 0.2M sodium hydroxide solution was prepared by adding 800mg of sodium hydroxide to 10 ml of 0.85% sodium chloride. 300ìl of the sodium chloride solution was added drop-by-drop with stirring to the chromium chloride solution. 100ìl of the resultant solution was added to 0.1ml packed RBCs followed by 100ìl of purified antimycobacterial antiserum. After 90 min of incubation at room temperature, a suspension of 4% RBCs was made in PBS 7.2 and used for the test. RBCs coated with normal rabbit serum were

Table 1: Evaluation of reverse passive haemagglutination (RPHA) for detection of mycobacterial antigens in the CSF for diagnosis of TBM

Subject Group	***No. of subjects***	***No.(%) of samples positive for CSF antigen by RPHA***	***No.(%) of samples negative for CSF antigen by RPHA***
Clinically suspected cases of TBM	26	13 (50%)	13 (50%)
Non TBM control cases			
a) Bacterial meningitis	7	3	4
b) Cryptococcal meningitis	5	1	4
c) Neonatal meningitis	3	0	3
d) Neurocysticercosis	2	0	2
e) Non-infectious CNS disorders	3	0	3
Total	20	4 (20%)	16 (80%)

Table 2: Statistical analysis of RPHA in the diagnosis of TBM

Statistical analysis	***CSF antigen detection by RPHA***
Sensitivity	50%
Specificity	80%
Positive predictive value	76.4%
Negative predictive value	55.2%

Table 3: Detection of mycobacterial antigens in the cerebrospinal fluid

Study group	*Technique*	*Sensitivity (%)*	*Specificity (%)*	*Remarks*
Bal et al [1983][36]	Competitive inhibition ELISA	100	96.6	Irradiated *M.tuberculosis* cells used as solid phase immunosorbents
Krambovitis et al[1984][14]	Latex particle agglutination	94.4	99.4	Plasma membrane antigen of *M.tuberculosis* detected
Chandramukhi et al [1985][6]	Reverse passive haemagglutination test	88 (culture +ve) 73% (Culture – ve)	21% +ve in pyogenic meningitis 8% +ve in head trauma and mental retardation	LAM antigen detected by murine monoclonal antibody (IgM)
Katti [2001][13]	Reverse passive haemagglutination test	94	99	Polyclonal antibody against sonicated MTB extract
Kadival et al [1986][37]	Sandwich ELISA	75	100	Polyclonal antibody against *M.tuberculosis* raised in rabbits and burrows used
Tripathi [1988][38]	Stick sandwich ELISA	76.5	17.7	Rabbit anti PPD-RT 23 Ig used
Wagale et al [1990][39]	Double antibody ELISA	73	100	---
Radhakrishnan & Mathai [1991][40]	Dot immunobinding assay	62.5 (Bacteriological +ve) 46.4 (Clinically suspected)	100	Nitrocellulose paper used to detect antigen 5 and specific IgG to antigen 5 in CSF
Sood [1991][41]	Stick sandwich ELISA	76.4	80.6-83.4	Rabbit anti PPD-RT 23 Ig used
Ashtekar et al [1987][42]	RIA	79%	---	---
Miorner et al [1995][43]	ELISA for detection of immune complex	64%	91%	Detection of mycobacterial IgG immune complex
Radhakrishnan et al [1990][44]	Inhibition ELISA	67%	100%	Culture filtrate antigen
Sada et al [1983][45]	ELISA	81%	95%	Using rabbit anti-BCG IgG
Sumi et al [2002][46]	Dot immunobinding assay	75.67%	100%	Using rabbit anti-14kDa antibody

used as control.

(iii) *RPHA test procedure:* Serial two-fold dilution of the CSF was made in 25 ìl of PBS (diluent) containing 1% BSA in U-bottom microtiter plates. 25 ìl of 4% v/v coated cells were dispensed per well and the hemagglutination pattern noted after 30 minutes of incubation at room temperature. The RBCs coated with normal rabbit serum showed button formation which is considered as negative. In a positive test, hemagglutination in the form of mat formation was observed.

RPHA test was performed with CSF specimens from both TBM and non-TBM cases.

Statistical analysis of the immunoassays

The sensitivity, specificity, positive predictive value and negative predictive value of the tests were calculated according to the method described by Galen and Gambino (1975)[21].

Statistical analysis of the immunoassays

Table 2 shows the sensitivity, specificity, positive predictive value and negative predictive value of RPHA for diagnosis of tuberculous meningitis.

RESULTS

Total of 46 cases (clinically suspected TBM 24, culture proven TBM 2, non- TBM cases 20) were included in the present study for detecting *M.tuberculosis* antigen in CSF specimens.

Reverse Passive Haemagglutination test (RPHA)

In a positive RPHA test, addition of sensitized chick RBCs to the test CSF resulted in haemagglutination leading to mat formation. Unsensitized control RBCs did not show any agglutination. In a negative test, RBCs settled down in the microtitre plates in the form of a button.

Of the 26 test CSF specimens, 13 CSF specimens were positive by RPHA while 4 of the 20 control CSF specimens were also reactive. Two culture positive specimens included in the study were positive by RPHA.

Of the 4 control CSF specimens positive by RPHA, 3 were culture proven cases of pneumococcal meningitis and one was a case of cryptococcal meningitis.

The results of RPHA from test and control subjects are summarized in Table 1.

DISCUSSION

Antibody detection is likely to be less sensitive in immuno-compromised patients. In TBM, it has been shown that there occurs intrathecal synthesis of *M.tuberculosis* specific IgG[22]. But in areas where PTB is endemic, CSF levels of antimycobacterial antibodies may be high in other conditions where the blood brain barrier is breached. Improperly collected traumatic CSF specimens mixed with blood can also lead to false positive antibody detection assays in endemic areas. Since the serum antibodies can get into CSF in such conditions, detection of antibodies in CSF for diagnosing TBM is less specific. Methods to directly detect the antigens in CSF have been developed to overcome these inadequacies. The reported sensitivity and specificity of various antigen detection methods in CSF for the diagnosis of TBM is summarized in Table 3.This includes only two reports of RPHA 6 ,used for detection of mycobacterial antigen in the CSF for diagnosis of tubercular menigitis .

Antigen detection assays have been developed for many bacterial causes of meningitis including TBM[23]. Most of the antigen detection assays are ELISA based or RIA based. There is a need for a sensitive and specific, simple and rapid, antigen detection immunodiagnostic test for TBM.

In the present study, therefore, attempt was made to evaluate the RPHA test for detection of *M.tuberculosis* antigens in the CSF. The RPHA as a rapid, simple and sensitive immunological method using polyclonal as well as monoclonal antibodies has been evaluated for many infectious conditions for antigen detection in serum and other body fluids[24-28].

The RPHA has been shown to be very sensitive for diagnosis of TBM[13]. Here again, the sensitivity and specificity is largely dependant upon the nature of the antiserum used to coat the RBCs [13]. Immunoassay systems based on red-cell labelled antibodies shows a remarkable sensitivity of the order 1 mg/ml for macromolecular analytes, which matches that of radioimmunometric and ELISA methods[29]. Although other alternative particles such as latex, sepharose and poly-urethane microcapsules can be used, they are not as sensitive as erythrocyte based assays[29].

Various methods are described for coupling of RBCs to antibody[30, 31]. The chromic chloride reaction for coupling is found to be the method of choice as it is a one step procedure performed in isotonic conditions[29,32,33]. Therefore, in the present study, we have used the chromic chloride for coupling of RBCs to the antibody. Although red cell-labelled antibodies do not offer fully quantitative analysis due to subjectivity of accurate end point determination, their ease of execution, together with their sensitivity and safety makes them ideally suited for large-scale screening assays or for use where more complex procedures and equipment are not available or cost-effective[29]. A major limitation of the general applicability of red cell-labelled antibodies has been their relatively short shelf-life[34]. Fresh RBC-Ab conjugates can be stored upto 3 weeks at 4ºC and non-enzyme treated RBCs can be stored for 2-3 months[35]. The RBC-Ab conjugates can be stabilized using low concentration of glutaraldehyde following antibody coupling[35].

In the present study we have made the use of polyclonal antiserum against heat killed whole bacilli to sensitize the RBCs in RPHA to detect antigens in clinically suspected cases. RPHA is found to be 50% sensitive and 50% specific. The sensitivity of 50% by RPHA in our study seems to correlate well with the published reports. As the study using monoclonal antibody to liproarabinomannan (LAM) was found to be less specific, the specificity of 50% obtained by our method is reasonable.In the present study, 4 of the control CSF specimens showed false positive reactions .This could be explained by the use of polyclonal antibodies prepared against heat inactivated mycobacterial antigens to coat the RBCs, hence the possibilitiy of cross-reaction with other infectious agents may not be ruled out . Similar observations are reported by a study conducted by Chandramuki et al [6] using RPHA for detection of mycobacterial antigen in the CSF for diagnosis of tubercular menigitis .

In a study using polyclonal antiserum against *M. tuberculosis* sonicate extract, the sensitivity and specificity of RPHA were found to be 94% and 99% respectively[13]. In another RPHA based study using monoclonal anti-liproarabinomannan antibody, the sensitivity was found to be 88% in culture positive CSF and 73% in culture negative CSF specimens[6]. In the same study, 21% of pyogenic meningitis cases were also shown to be positive and 8% of patients with non-infectious neurologic disease were also positive for antigen in CSF.

Sensitivity and specificity of 94% and 99% respectively for antigen detection in TBM have been reported when RPHA was performed using antimycobacterial IgG raised against *mycobacterium tuberculosis* sonicate extract (MSE) [13]. The sensitivity limit of RPHA for detecting tuberculous antigen was found to be 400 mg/ml. Another RPHA based study for diagnosing TBM using monoclonal antibody against the Lipoarabinomannan (LAM) polysaccharide antigen was found to be positive in 72.6% of culture negative cases[6].

In conclusion, results of the present study showed that RPHA is a simple test that could be used as an adjunct in diagnosing TBM. It does not require any special equipment or technically trained or skilled manpower. It is economical and can be afforded for use in community where TBM is more prevalent. The present study is a preliminary work carried out to evaluate the usefulness of the RPHA employing polyclonal mycobacterial antibodies to detect specific mycobacterial antigen in the CSF for diagnosis of tubercular menigitis. Further study is being planned to fractionate and purify the crude mycobacterial antigen ; and to purify the antigen and to identify the antigen of diagnostic importance for use in the

RPHA to make the test more sensitive and specific .

REFERENCES

1. Marton KI and Gean AD. The spinal tap: a new look at an old test. *Ann Med* 1986; **104**: 840-848.
2. Roberts FJ. Problems in the diagnosis of tuberculous meningitis. *Arch neural* 1981; **38**: 319-320.
3. Daniel TM. New approaches to the rapid diagnosis of tuberculous meningitis. *J Infect Dis* 1987; **155**: 599-603.
4. Mahadevan S. Clinical utility of serodiagnosis of tuberculosis. *Indian J Paediatr* 1997; 64: 97-103.
5. Hernandez R, Munoz O and Guiscafre H. Sensitive immunoassay for early diagnosis of tuberculous meningitis. *J Clin Microbiol* 1984; **20**: 533-535.
6. Chandramuki A, Allen PR, Keen M and Ivanyi J. Detection of mycobacterial antigen and antibodies in the cerebrospinal fluid of patients with tuberculous meningitis. *J Med Microbiol* 1985; **20(2):** 239-247.
7. Prabhakar S and Oommen A. ELISA using mycobacterial antigens as a diagnostic aid for tuberculous meningitis. *J Neurol Sci* 1987; **78(2):** 203-211.
8. Kalish SB, Radin RC, Levitz D, Zeiss CR and Phair JP. The enzyme-linked immunosorbent assay method for IgG antibody to purified protein derivative in cerebrospinal fluid of patients with tuberculous meningitis. *Ann Intern Med* 1983; **99(5):** 630-633.
9. Nassau E and parsons ER. Detection of antibodies to *Mycobacterium tuberculosis* by solid phase radioimmunoassay. *J Immunol Methods* 1975; **6**: 261-271.
10. Kadival GV, Samuel AM, Mazarelo TBMS and Chaparas SD. Radio immunoassay or detecting *Mycobacterium tuberculosis* antigen in cerebrospinal fluids of patients with tuberculous meningitis. *J Infect Dis* 1987; **155**: 608-611.
11. Patil SA, Gourie-Devi M, Ray Chanduri J and Chandramuki A. Identification of antibody responses to *Mycobacterium tuberculosis* antigens in the CSF of tuberculous meningitis patients by Western blotting. *Clin Immunol Immunopathol* 1996; **81**: 35-40.
12. Mathai A, Radhakrishnan VV, George SM and Sarada C. A newer approach for the laboratory diagnosis of tuberculous meningitis. *Diag Microbiol Infect Dis* 2001; **39**: 225-228.
13. Katti MK. Immunodiagnosis of tuberculous meningitis: rapid detection of mycobacterial antigens in cerebrospinal fluid by reverse passive hemagglutination assay and their characterization by Western blotting. *FEMS Immunol Med Microbiol* 2001; **31**: 59-64.
14. Krambovitis E, Lock PE, Mc Illmurray MB, Hendrickse W and Holzel H. Rapid diagnosis of tuberculous meningitis by latex particle agglutination. *Lancet* 1984; **2**: 1229-1231.
15. Watterson SA and Drobniewski FA. Modern laboratory diagnosis of mycobacterial infections. *J Clin Pathol* 2000; **53**: 727-732.
16. Ahuja GK, Mohan KK, Prasad K and Behari M. Diagnostic criteria for tuberculous meningitis and their validation. *Tuber Lung Dis* 1994; **75(2):** 149-152.
17. Radhakrishnan VV, Mathai A, Rao SB and Sehgal S. Immunoelectrophoresis of mycobacterial antigens. *Indian J Exp Biol* 1990; **28(9):** 812-815.
18. Gottstein B. An immunoassay for the detection of circulating antigens in human echinococcosis. *Am J Trop Med Hyg* 1984; **33(6):** 1185-1191.
19. Parija SC, Mishra SR and Rao RS. Sensitized chick cells in the indirect haemagglutination test for echinococcosis. *J Med Microbiol* 1986; **22(3):** 237-239.
20. Goding JW. The chromic chloride method of coupling antigens to erythrocytes: definition of some important parameters. *J Immunol Methods* 1976; **10(1):** 61-66.
21. Galen RS and Gambino SR. 1975. Beyond normality: the predictive value and efficiency of medical diagnoses. pp. 30-40. New York, John Wiley and sons.
22. Cho TY, Park SC, Cho SN, Lee HR, Kim SK and Lee BI. Intrathecal synthesis of immunoglobulin G and *Mycobacterium tuberculosis*-specific humoral immune response in tuberculous meningitis. *Clin Diagn Lab Immunol* 1995; **2(3):** 361-364.
23. Das BK, Gurubacharya RL, Mohapatra TM and Mishra OP. Bacterial antigen detection test in meningitis. *Indian J Pediatr* 2003; **70(10):** 799-801.
24. Cayzer I, Dane DS, Cameron GH and Denning JV. A rapid haemagglutination test for hepatitis-B antigen. *Lancet* 1974; **1(7864):** 947-949.
25. Kunitomo K, Yahara S, Saji H and Hosoi T. Monoclonal antibodies to hepatitis B surface antigen (HBsAg) as a tool for the automated HBsAg screening. *Vox Sang* 1983; **45(2):** 104-111.
26. Cranage MP, McLean CS, Buckmaster EA, Minson AC, Wildy P and Coombs RR. The use of monoclonal antibodies in (reverse) passive haemagglutination tests for herpes simplex virus antigens and antibodies. *J Med Virol* 1983; **11(4):** 295-306.
27. Cranage MP, Stott EJ, Nagington J and Coombs RR. A reverse passive haemagglutination test for the detection of respiratory syncytial virus in nasal secretions from infants. *J Med Virol* 1981; **8(3):** 153-160.
28. Cranage MP, Campbell AD, Venters JL, Mawson S, Coombs RR and Flewett TH. Detection and quantitation of rotavirus using monoclonal antibody coupled red blood cells: comparison with ELISA. *J Virol Methods* 1985; **11(4):** 273-287.
29. Coombs RR, Scott ML and Cranage MP. Assays using red cell-labelled antibodies. *J Immunol Methods* 1987; **101(1):** 1-14.
30. Steinitz M and Tamir S. Solubilization of cell-membrane-antigen and its coupling to fixed and non-fixed erythrocytes. *J Clin Lab Immunol* 1985; **18(4):** 195-198.
31. Furfang J and Thierfelder S. Effective avidin coating of erythrocytes by disulfide bond formation. Production of a stable resetting agent for multiple applications. *J Immunol Methods* 1986; **91(1):** 123-128.
32. Jandl JH and Simmons RL. The agglutination and sensitization of red cells by metallic cations: interactions between multivalent metals and the red-cell-membrane.

Br J Haematol 1957; **3(1):** 19-38.
33. Gold ER and Fudenberg HH. Chromic chloride: a coupling reagent for passive haemagglutination reactions. *J Immunol* 1967; **99(5):** 859-866.
34. Parija SC, Kasinathan S and Rao RS. Long shelf-life of antigen-sensitized erythrocytes by double aldehyde stabilization for the serodiagnosis of amoebiasis by indirect haemagglutination. *J Diarrhoeal Dis Res* 1988; **6(1):** 29-34.
35. Cranage MP, Gurner BW and Coombs RR. Glutaraldehyde stabilization of antibody-linked erythrocytes for use in reverse passive and related haemagglutination assays. *J Immunol Methods* 1983; **64(1-2):** 7-16.
36. Bal V, Kamat RS, Kamat J and Kandoth P. Enzyme-linked immunosorbent assay for mycobacterial antigens. *Indian J Med Res* 1983; **78**: 477-483.
37. Kadival GV, Mazarelo TB and Chaparas SD. Sensitivity and specificity of enzyme-linked immunosorbent assay in the detection of antigen in tuberculous meningitis cerebrospinal fluids. *J Clin Microbiol* 1986; **23(5):** 901-904.
38. Tripathi P. 1988. Diagnostic importance of stick sandwich ELISA in smear negative cases of pulmonary tuberculosis. Thesis submitted to Nagpur University, India, for MD Microbiology.
39. Wagle N, Vaidya A, Joshi S and Merchant SM. Detection of tubercle antigen in cerebrospinal fluids by ELISA for diagnosis of tuberculous meningitis. *Indian J Pediatr* 1990; **57(5):** 679-683.
40. Radhakrishnan VV and Mathai A. A dot-immunobinding assay for the laboratory diagnosis of tuberculous meningitis and its comparison with enzyme-linked immunosorbent assay. *J Appl Bacteriol* 1991; **71(5):** 428-433.
41. Sood J, Gupta OP, Narang P, Cheirmaraj K, Reddy MV and Harinath BC. Penicillinase ELISA for detection of tubercular antigen in tuberculosis. *J Commun Dis* 1991; **23(3):** 173-177.
42. Ashtekar MD, Dhalla AS, Mazarello TB and Samuel AM. A study of *Mycobacterium tuberculosis* antigen and antibody in cerebrospinal fluid and blood in tuberculous meningitis. *Clin Immunol Immunopathol* 1987; **45(1):** 29-34.
43. Miorner H, Sjobring U, Nayak P and Chandramuki A. Diagnosis of tuberculous meningitis: a comparative analysis of 3 immunoassays, an immune complex assay and the polymerase chain reaction. *Tuber Lung Dis* 1995; **76(5):** 381-386.
44. Radhakrishnan VV, Sehgal S and Mathai A. Correlation between culture of *Mycobacterium tuberculosis* and detection of mycobacterial antigens in cerebrospinal fluid of patients with tuberculous meningitis. *J Med Microbiol* 1990; **33(4):** 223-226.
45. Sada E, Ruiz-Palacios GM, Lopez-Vidal Y and Ponce de Leon S. Detection of mycobacterial antigens in cerebrospinal fluid of patients with tuberculous meningitis by enzyme-linked immunosorbent assay. *Lancet* 1983; **2(8351):** 651-652.
46. Sumi MG, Mathai A, Reuben S, Sarada C, Radhakrishnan VV, Indulakshmi R, Sathish M, Ajaykumar R and Manju YK. A comparative evaluation of dot immunobinding assay (Dot-Iba) and polymerase chain reaction (PCR) for the laboratory diagnosis of tuberculous meningitis. *Diagn Microbiol Infect Dis* 2002; **42(1):** 35-38.

TUBERCULOSIS HEALTH VISITORS' COURSE

The 2007-2008 Tuberculosis Health Visitors' Course of 9 months' duration will be conducted at the New Delhi Tuberculosis Centre. The minimum qualification for admission to this course is 10 + 2 with science and/or hygiene. Science education up to class 10 is essential. Application forms for admission to the course can be obtained from the Secretary General, Tuberculosis Association of India, 3, Red Cross Road, New Delhi-110 001. The last date for receipt of applications is 30th April, 2007.

Indian J Tuberc 2007; 54: 49-51

STATUS REPORT ON RNTCP- 3Q 2006*

Pursuing high quality DOTS services

Revised National TB Control programme is focusing on consolidation of the progress made till now and to pursue high quality DOTS services through out India.

Performance

The over all performance of RNTCP at the national level has been satisfactory. The case detection rate for the 3rd quarter 2006 was 69% with a total of 361,954 patients placed on treatment. The success rate amongst the new smear positive PTB cases registered in the 3rd quarter 2005 was 85%. The sputum conversion rate and cure rate among the new sputum positive cases was 88% and 83% respectively.

In this quarter, more than 1,600,000 suspects were examined and 215,809 sputum positive cases were diagnosed. In addition to the 143,349 new smear positive cases, 103,716 new smear negative cases, 46,358 extra pulmonary cases, 50,585 smear positive re-treatment cases and 19,469 'Others' were also initiated on treatment in this quarter.

Analysis of state-wise performance, indicates that 24 states have recorded an improvement in their new smear positive case detection in the current quarter compared to their performance in 3rd quarter of 2005. Significant improvements in NSP case detection rates have been noted in states of Jharkhand (22%), Assam (11%), Bihar (6%), Chhatisgarh (4%), Goa (11%), Manipur (18%), Nagaland (18%), Punjab (9%), Tripura (19%), and Uttar Pradesh (5%). This improvement in performance could be attributed to the regular monitoring by the centre and improved field supervision by the State and District programme officers.

States/UTs like Andhra Pradesh, Gujarat, Delhi, Chandigarh, Himachal Pradesh, Pondicherry, Sikkim, Rajasthan and West Bengal have already achieved the benchmark and consistently performing over and above the global targets of CDR and treatment success rate. Efforts should be made to try and identify underserved geographical areas and vulnerable groups like tribal areas/ desert areas/ urban poor/ and address gender differentials in seeking health care. Efforts should be directed to improve access to each and every TB patient in the community.

Other initiatives in this quarter

1. Biannual meeting of the State TB Officers and WHO-RNTCP Consultants was organized in July (South zone- Chennai) and August (North zone - Surajkund) to review the performance and programme-related issues in the states.

2. National level RNTCP sensitization workshop for IMA was organized on 13th-14th Aug 2006 at Surajkund. All state IMA Presidents had attended the meeting and provided commitment for extending support to RNTCP activities in their respective States

3. Meeting of the Lab Committee was convened to review the ongoing activities in the states for establishment of Intermediate Reference laboratories and their accreditation. The process for accreditation of IRLs and rolling out of DOTS plus in the States of Gujarat and Maharashtra were also discussed.

4. Training on drugs and logistic management was organized in Lucknow on 20th-21st July 2006 to build the capacity of the district staff in efficient drug and logistic management.

5. Meeting of the expert committee to plan for the upcoming National Annual Risk of Tuberculous Infection (ARTI) survey was organized at NTI

* Dr. L. S Chauhan, DDG (TB), Directorate General of Health Services, Ministry of Health and Family Welfare, Government of India, New Delhi

Table: Performance of RNTCP Case Detection (2006 third quarter), Smear Conversion (2006, second quarter), and Treatment Outcome (2005, third quarter)

State	Population (in lakh) covered by RNTCP[1]	Suspects examined per lakh population	No of Smear positive patients diagnosed[2]	Total patients registered for treatment[3]	Annualized total case detection rate	New smear positive patients registered for treatment	Annualized new smear positive case detection rate (%)		3 month conversion rate of new smear positive patients	Cure rate of new smear positive patients [4]	Success rate of new smear positive patients [4]	No of new smear negative cases registered for treatment	No of new EP cases registered for treament	No of smear positive retreatment cases registered for treatment
Andaman & Nicobar	4	284	98	209	212	58	59	79%	94%	77%	79%	72	43	22
Andhra Pradesh	804	137	17006	26717	133	11519	57	76%	89%	83%	86%	8386	2192	3758
Arunachal Pradesh	12	244	361	687	235	244	83	111%	88%	85%	87%	192	77	111
Assam	290	111	5360	9203	127	4036	56	74%	88%	79%	83%	2751	790	1005
Bihar	908	71	8305	18014	79	5798	26	34%	79%	72%	82%	7804	1017	2002
Chandigarh	10	362	444	576	227	204	81	85%	92%	85%	85%	113	164	61
Chhatisgarh	229	132	3375	7319	128	2740	48	60%	89%	84%	87%	3134	691	479
D & N Haveli	2	168	47	98	158	33	53	67%	86%	65%	65%	21	20	14
Daman & Diu	2	459	59	86	193	26	58	73%	87%	86%	93%	23	10	11
Delhi	161	246	6507	12262	305	3559	89	93%	88%	86%	87%	2485	3378	1791
Goa	15	178	292	522	136	168	44	55%	81%	62%	62%	120	129	68
Gujarat	548	165	15083	19654	143	8390	61	77%	90%	86%	87%	3017	2111	4579
Haryana	230	176	6037	9209	160	3523	61	64%	88%	83%	84%	1928	1334	1910
Himachal Pradesh	64	264	2078	3409	212	1222	76	80%	91%	86%	88%	640	729	697
Jammu & Kashmir	116	158	1334	2520	87	892	31	32%	85%	82%	83%	694	575	270
Jharkhand	292	115	5295	9142	125	4059	56	74%	90%	83%	86%	3213	545	831
Karnataka	561	156	10617	16686	119	6530	47	62%	84%	76%	78%	3874	2746	2627
Kerala	336	154	3442	6417	76	2720	32	43%	84%	80%	83%	1371	1537	603
Lakshadweep	1	68	2	3	18	2	12	16%	0%	NE	NE	1	0	0
Madhya Pradesh	668	106	11511	19072	114	7290	44	55%	86%	79%	83%	6196	1813	2895
Maharashtra	1041	145	19291	33780	130	13294	51	64%	90%	85%	87%	9409	4934	3884
Manipur	26	144	434	1355	212	340	53	71%	86%	87%	89%	473	253	112
Meghalaya	25	128	461	1068	173	309	50	67%	81%	77%	81%	230	245	142
Mizoram	10	226	218	502	210	139	58	78%	94%	92%	94%	127	147	51
Nagaland	21	124	371	800	150	298	56	75%	92%	87%	87%	187	109	118
Orissa	391	124	6542	11700	120	5077	52	61%	86%	79%	86%	3337	1712	980
Pondicherry	10	364	363	350	134	152	58	78%	87%	75%	82%	69	77	47
Punjab	260	158	5497	9430	145	3722	57	60%	87%	83%	85%	2092	1795	1428
Rajasthan	624	156	18929	29040	186	10740	69	86%	90%	87%	88%	8573	3318	5496
Sikkim	6	360	197	397	274	125	86	115%	82%	88%	88%	73	108	56
Tamil Nadu	653	236	12374	20911	128	8096	50	66%	88%	82%	84%	5733	4178	2376
Tripura	34	85	430	607	71	336	39	52%	84%	80%	86%	124	50	80
Uttar Pradesh	1839	131	34854	59606	130	23833	52	55%	87%	83%	87%	20068	5542	8501
Uttaranchal	92	172	2103	3136	136	1176	51	54%	93%	85%	85%	893	382	577
West Bengal	858	160	16492	27467	128	12699	59	79%	89%	86%	87%	6293	3607	3003
Grand Total	**11142**	**144**	**215809**	**361954**	**130**	**143349**	**51**	**69%**	**88%**	**83%**	**85%**	**103716**	**46358**	**50585**

1. Projected population based on census population of 2001 is used for calculation of case-detection rate. 1 lakh = 100,000 population
2. Smear positive patients diagnosed include new smear positive cases and smear positive retreatment cases
3. Total patients registered for treatment includes new sputum smear positive cases, new smear negative cases, new extra-pulmonary cases, smear positive retreatment cases and 'Others'
4. Cure rate and success rate are not expected for states that began implementing RNTCP after 2nd quarter 2005

on the 28th-29th July 2006. The survey is planned between 2007-09.

6. TB- HIV Review meeting was held in Chennai, to review TB-HIV coordination activities in the six high prevalence states. The meeting was chaired by Additional Secretary, GoI and AS & DG (NACO).

7. Sensitization workshop of all CGHS (Central Government Health Services) providers was completed during the quarter.

The programme is making all efforts at the central level to support the states to improve the quality of services in the field. The states and districts are encouraged to identify and reach the underserved geographical areas and vulnerable groups like tribal areas/ desert areas/ urban poor. Efforts are also being made to improve access to each and every TB patient in the community by improving the patient provider interaction and creating patient friendly environment. We are confident that sincere and committed RNTCP team in the field is fully equipped to take up the responsibility of working towards TB free India.

Case Report

ULCERATIVE LUPUS VULGARIS OF FACE: AN UNCOMMON PRESENTATION IN INDIA

L. Padmavathy[1], L. Lakshmana Rao[2], N. Ethirajan[3] and B. Krishnaswami[4]

(Original received on 3.4.2006. Revised version received on 7.8.2006. Accepted on 8.8.2006)

***Summary*:** Tuberculosis affects the population world wide, more among those living in developing countries. The incidence of tuberculosis registered an upward trend even in developed countries, with the advent of HIV infection. Cutaneous tuberculosis accounts for about 1% of cases of extra-pulmonary tuberculosis. Cutaneous tuberculosis presents with various lesions ranging from ulcerative to proliferative or hyperkeratotic lesions. The lesions may sometimes be associated with marked destruction of the tissues resulting in marked disfigurement, especially when it involves face as seen in cases of Lupus Vulgaris. A case of Lupus Vulgaris in a young woman with extensive ulceration of face which responded to ATT resulting in scarring of the face is reported for its rarity amongst Indian population as against western population.
[Indian J Tuberc 2007; 54:52-54]

***Key words*:** Lupus Vulgaris, Cutaneous Tuberculosis**.**

INTRODUCTION

Tuberculosis is a very common disease among the population world wide, especially those living in developing countries. With the advent of HIV infection the incidence of tuberculosis has registered an upward trend in developed countries. The disease is caused by *Mycobacterium tuberculosis*, *M. bovis* and under certain conditions the Bacillus Calmette-Guerin (BCG), the attenuated strain of *M. bovis*[1]**.** Extra pulmonary tuberculosis is yet another manifestation of tuberculosis the disease quite common among the people of low socio-economic strata in the developing countries, affecting, among other sites, skin.

Cutaneous tuberculosis, especially Lupus Vulgaris, was described repeatedly in seventeenth and eighteenth centuries. The word 'Lupus' was described by earlier authors to describe lesions of Cutaneous Tuberculosis. The word 'Lupus' meaning wolf was given to the lesion because of the ulcerating and devouring character of the lesion[2]. Lupus Vulgaris is a chronic and progressive form of Cutaneous Tuberculosis occurring in individuals with moderate immunity and high degree of tuberculin sensitivity[3]. Lupus Vulgaris most commonly affects the trunk and lower extremities[4] while face is most common site of involvement among Western population[2,5]. A case of ulcerative Lupus Vulgaris on the face of a young woman is reported.

CASE REPORT

A 25 year-old woman presented with a single ulcerative lesion on the face of two-year duration. The lesion initially started as a small papule, which broke down resulting in an ulcer on the left cheek. The ulcer gradually extended to the other cheek. Patient had dry cough for more than six months but gave no history of fever. Both parents of the patient died of pulmonary tuberculosis.

On examination, a 15 x 8 cms ulcerated plaque on the left side of the face, extending over the nasal bridge to the right malar region was observed. Ala nasi was destroyed, more so on the left side (Fig.1). Periphery of the lesion showed '*apple-jelly*' nodules. Patient did not have any nasal symptoms.

Hematological and biochemical investigations were within normal limits. X-ray chest showed minimal basal consolidation on the left lung.

1. Dermatologist, Urban Health Centre 2. Professor of Pathology & HOD 3. Professor & HOD, Dept. of Community Medicine 4. Professor of Pathology
Rajah Muthiah Medical College, Annamalai University, Annamalai Nagar, Chidambaram (Tamil Nadu)
Correspondence: Dr. L. Padmavathy, B3, RSA Complex, Annamalai University, Annamalai Nagar - 608 002, (Tamil Nadu)
Phone: 04144-238066(R); E-mail: padmavathy.lanka@gmail.com

Fig. 1. Clinical photograph showing the ulcerative lesion extending over the malar regions and causing destruction of the ala nasi.

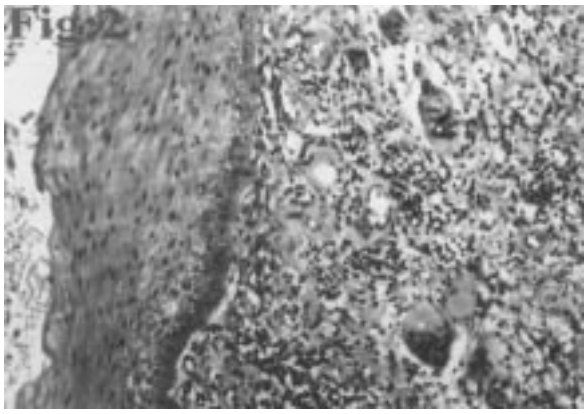

Fig. 2: Biopsy from the ulcerated lesion, showing epithelioid cell granuloma and Langhan's giant cells along with mononuclear infiltrate in dermis. X20 H&E.

Right lung was clear. VDRL and ELISA tests for HIV were negative. Sputum smears were negative for AFB by Z.N. stain.

Biopsy from the edge of the skin lesion showed a typical tuberculous lesion comprising epithelioid cell granulomas with Langhan's giant cells. (Fig.2).

Patient was put on standard ATT and the ulcer healed completely in six months. However, gross deformity of nose with destruction of both alae nasi persisted. (Fig.3)

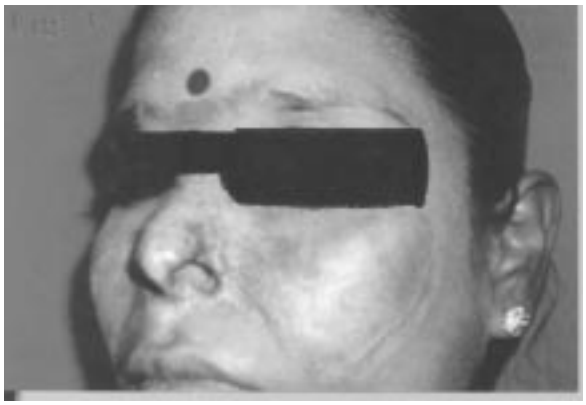

Fig. 3: Clinical photograph showing the healed lesion with scar after ATT.

DISCUSSION

The earliest description of Lupus Vulgaris was by Erasmus Wilson in 1865, which compared the lesions to ravages of a wolf and used the word 'lupus' (Lupus=Wolf). The adjective - vulgaris, in Lupus Vulgaris reflects the commonness of this condition in earlier times[2]. The synonyms for this condition are Tuberculosis Luposa Cutis and Tuberculosis Luposa[6].

Lupus Vulgaris occurs in individuals with immunity produced by previous tuberculous infection. The morphological manifestations are closely related to the state of allergy and degree of immunity[2]. Hence, Lupus Vulgaris in most instances is a chronic progressive disease, beginning as a small nodule, growing peripherally and forming an ever enlarging plaque, character of which is altered by noticeable changes in epidermis or cutis brought about by inflammation. If the epidermis becomes hyperkeratotic, the lesion assumes a verrucous character, or it becomes tumefied if lymph stasis predominates in the corium. Irrespective of the type of the lesion, the basic process remains the same, the tuberculous inflammation beginning and extending in the cutis exhibiting characteristics unique to Lupus Vulgaris[7].

The bacilli from another reactive focus of tuberculosis reach the skin by hematogenous route

or by contiguity. Frequent localization of the lesions around the nose and on cheeks is attributed to the rich and porous venous plexuses with stasis, cold and hypoxia, impaired fibrinolysis and host defense at a lower temperature, as prevailing in western countries[2]. Thus face is most commonly affected site in European countries[3,5]. Higher temperatures prevailing in most parts of India may be responsible for relatively low frequency of the facial lesions in comparison to the Western world.

A case of a 40 year-old woman who presented with erythematous infiltrating plaque on the face involving both the ears with destruction of nasal cartilage and satellite lesions on the right shoulder was reported. A clinical diagnosis of Hansen's disease was made. Later this proved to be a case of Lupus Vulgaris[8]. Another case of a 55 year-old woman with gradually progressive erythematous scaly plaques, simulating psoriasis, on extremities and face for last 20 years which on histology was diagnosed as Lupus Vulgaris and also reported from Chennai for its notorious presentations[9]. A case of lupus vulgaris affecting the face and resulting in destruction of ala nasi, nasal septum and columella, called 'lupus vorax' was reported[10].

Generally, Lupus Vulgaris is asymptomatic. However, the lesions can be extensive with marked disfigurement due to destruction of nasal or auricular cartilage with atrophic scarring[1]. This could probably be the reason for the patient not seeking treatment in early stage of the disease and presented with disfigurement of the face. Occurrence of pulmonary tuberculosis or tuberculosis of bones and joints is reported in 10-20% of patients with Lupus Vulgaris. The morbidity is reported to be 4 to 10 times higher in patients of Lupus Vulgaris with pulmonary tuberculosis than in the general population[1]. No osseous or joint tuberculosis was present in the present case though she had presented with basal consolidation of the left lung.

The present case is reported as the involvement of the face in Lupus Vulgaris amongst the Indians is relatively uncommon. This case also highlights the disfigurement which could have been prevented, had the patient had recourse to anti-tuberculous treatment earlier. The importance of spreading awareness among general public about tuberculosis cannot be overemphasized.

REFERENCES

1. Tappeiner G, Wolff K. Tuberculosis and other mycobacterial infections. In Fitzpatrick's dermatology in general medicine, V edition. Eds. Freedberg IM, Eissen A.Z., Wolff k, Frank Austin K, Goldsmith LA, Katz SI, and Fitzpatrick TB, Vol II, McGraw-hill, Newyork 1999: 2274-2279.
2. Findlay GH. Bacterial infections. In: The dermatology of Bacterial infections. 1st ed. Ed. Findlay GH, Blackwell Scientific, London, 1987, 71-83.
3. Marcoval J, Srvitje O, Moreno A, Jucgla A, Pyri J: Lupus vulgaris: clinical, histopathologic and bacteriologic study of 10 cases. *J Am Acad Dermatol* 1992; **26**: 404.
4. Pandhi RK, Bedi TR, Kanwar AJ, Bhutani LK. Cutaneous tuberculosis- A clinical and investigative study. *Indian J Dermatol* 1977; **22**; 99-107.
5. Wolff K, Tappeiner G. Mycobacterial diseases: tuberculosis and atypical mycobacterial infections. In Dermatology in general medicine, Eds. Fitzpatrick Tb, Eisen AZ, Wolff K., McGraw-Hill, Newyork 1987; 2151-2170.
6. Pomeranz M Ketz, Philip Orbuch, Jerome Shupack, Rena Brand. Mycobacteria and skin. In: Tuberculosis 1996, 1st Ed. Eds. William M Rom, Stuart Garay. Little Brown company, London, 51: 657-667
7. Michelson EH, Criteria for the diagnosis of certain tuberculomas. The Am J Med Assoc. 1948:138:10:721-25.
8. Bhargava P, Kumar K, Kuldeep CM, Mathur NK. Lupus vulgaris mimicking Hansen's disease. *Indian J Dermatol* 1997; **42(2)** : 91-92
9. Srikumar G, Hameedullah A, Arunadevi R, Somasundaram V, Parijatham BO, Meenakshi, Ilangovan G. Ochronosis. *Indian J Dermatol* 2000 : **45(4)**: 208-210
10. Thomas. S, Suhas, K.M. Pai and A. R. Raghu. Lupus vulgaris-Report of a case with facial involvement. *Br Dent J* 2005;**198(3)**:135-7

Indian J Tuberc 2007; 54:55-57

37TH UNION WORLD CONFERENCE ON LUNG HEALTH, PARIS - A REPORT

M. M. Singh*

The International Union Against Tuberculosis and Lung Disease (The Union) organized the 37th World Conference from 31st October to 4th November, 2006 at Palais Des Congres, Paris. More than 2500 delegates from all over the world participated in the Congress. It was a mega affair organized successfully by the Union. Following delegation from India attended the Conference and other meetings:

1. Dr. M.M. Singh, Chairman, SEAR(Union) and Vice-Chairman (OR), TB Association of India.
2. Dr. V.K. Arora, Vice-Chairman, TAI
3. Dr. L.S. Chauhan, DDG (TB), DGHS
4. Brig (Dr.) S.L. Chadha, Honorary General Secretary, Delhi TB Association

The theme of the Conference was "**Strengthening Human Resources for better Lung Health**". The focus of the Conference was also towards "confronting the challenges of HIV and MDR in TB prevention and Care".

The 31st October, 2006 was full of administrative meetings, including the regional and inter-regional meetings of all five regions of the Union. Meeting of the South East Asia Region (SEAR) was held under the chairmanship of Dr. M.M. Singh followed by inter-regional council meeting along with Asia Pacific Region of the Eastern Region.

It was decided that the first Regional Conference of the Eastern Region will be held at Kuala Lumpur in August 2007 to be organized by **Asia Pacific Region and Malaysian TB Association**.

The next Regional Conference of Tuberculosis and Chest Diseases will be organized by South East Asia Region (SEAR) in September/October, 2008. It will be organized along with our National Conference organized by TB Association of India and SEAR. All countries of Eastern Region starting from Australia, Japan, Korea, Hong Kong, China, South East Asia, India, Pakistan, Bangladesh, Nepal, Afghanistan, etc., are expected to participate. It will be a mega affair for the country and the Ministry of Health, TB Division and National Institutes of TB and Chest in India are expected to support the TB Association and SEAR in this event.

The scientific conference was organized by the Organizing Committee under the following sections:

(1) Bacteriology and Immunology
(2) Nursing and Allied Professions
(3) Respiratory Diseases
(4) Tobacco Prevention
(5) Tuberculosis
(6) Tuberculosis in Animals

The Chairmen and Working Groups of all the sections worked hard to give best of academic, scientific and research papers to the delegates.

Besides this, there were several special and plenary sessions on important issues e.g. **XDR-TB (Extremely Drug Resistant Tuberculosis)**

XDR TB

The session discussed about, how did we get here and what are we doing about it. It was a very lively session and gave the update

*Chairman, SEAR & Vice-Chairman (OR), Tuberculosis Association of India

on the problems and challenges in detecting XDR TB patients, the limitation in availability of all types of drugs, and their management was discussed. The proposals for development of policy and relevant issues concerning XDR were discussed. Some countries gave their experience in the management of XDR TB. The proposals for development of policy and role of rapid diagnosis were discussed.

DOTS TO STOP TB STRATEGY

A special session was organized to highlight the building up and concept on achievements for future planning, implementation and scale up of the Stop TB Strategy. Making the advocacy, communication and social mobilization essential and appropriate for the purpose.

Plenary Sessions (2nd November, 2006)

(1) Special guest lecture on "Global responsibilities in investing in the health work force for sustainable health systems" was delivered by the **United Nations Special Envoy to stop TB – Jorge Sampaio.** This was followed by **Awards Ceremony,** under the chairmanship of the **President of the Union**, Doctor Asma el Sony (Sudan).

(1) Special medal of the Union awarded to Prof. Margaret R Becklake of Canada

(2) Stop TB Partnership Kochan Prize awarded to:

(a) Dr. **L.S. Chauhan**, DDG (TB) in the Ministry of Health and the Programme Manager of RNTCP in India

(b) WINSTON ZULU (Zambia)

For being the leading advocate for those affected by TB and HIV/AIDS

(3) Scientific Prize awarded to

Dr. Stephen D Lawn (South Africa)

For his research on TB and HIV in the era of anti-retroviral treatment

(4) Princess Chichibu – Global TB Memorial Award

Awarded to **Thomas R Frieden (USA)** who helped India in 1996 to develop one of the most effective tuberculosis control programmes.

Brig (Dr.) S.L. Chadha presented a paper poster presentation on innovative DOTS Therapy for reaching the unreached – Shelterless and homeless, pavement dwellers of Delhi . This presentation was highly appreciated.

Plenary Session (2nd November, 2006)

Consequences of **Smoking and Tobacco on Lung Diseases** in developing countries by Dr. Garith Thomas (U.K.). Smoking is a preventable risk factor. Smoking exacerbates the chronic diseases. Strategies adopted to quit smoking to improve the quality of life were discussed.

Plenary Session (3rd November, 2006)

Clinical Trials on ethical issues in high burden countries were discussed by **S Olly** Benetar(South Africa).

Plenary Session (4th November, 2006)

Avian Influenza - Detection and management of Pandemic (Enis Baris) (World Bank)

Besides the plenary sessions, there were several post-graduate courses and workshops on

varied subjects for the benefit of young graduates and delegates.

On 4th November, 2006, meetings of the region and inter-regional meetings were held.

The General Assembly meeting was held under the chairmanship of Dr. Asma El Sony from 16.30 P.M. to 18.30 P.M. Various resolutions pertaining to regions of Union were passed. In the election for Board of Directors, Dr.M.M. Singh (Chairman, SEAR) was elected as one of the Directors of the Union. India was awarded the First Prize for TB Seals of the year 2006. The prize was received by Dr.M.M. Singh and Dr. V.K. Arora from the President of the Union Dr. Asma El Sony.

This was followed by the meeting of the Union's Board of Directors, wherein policy matters and other details for working of the Union were discussed and decided.

This marked the conclusion of the Conference after the thanks-giving note from the President of the Union.

TB Anywhere is TB everywhere

This is the theme for the World TB Day on 24th March, 2007. This will mark 125 years since Robert Koch discovered tubercle bacillus that causes the misery. The theme was announced at the World Conference by the **United Nations Special Envoy to stop TB Dr. Jorbe Sampio.** The World TB Day is an important opportunity to create awareness of the TB epidemic and strengthens support for the global plan to stop TB 2006-2015. All Associations are requested to organize special awareness functions in their States on 24th March, 2007.

ABSTRACTS

A novel method of staining Acid-Fast Bacilli in Sputum Containers

N. Selvakumar, D. Ravikumar, S. Sivagamasundari, P.G. Gopi and P.R. Narayanan. *Indian J Med Res* 2006; **123**: 776-780.

They carried out this study to stain the centrifuged deposits with carbol fuchsin in sputum containers and to decolourize and counter-stain their smears made on glass slides. The centrifuged deposits of 180 sputum samples from Pulmonary Tuberculosis patients were used for making smears (initial deposit smears) and staining by Ziehl-Neelsen (ZN) method for the detection of AFB. Each of the sputum deposit was then treated with one ml of 11 per cent carbol-fuchsin and a smear made between 2 to 3 hrs was then decolourized and counterstained by the same procedures followed in ZN method (2 h stained deposit smear). The coded initial deposit smears and the corresponding 2 h stained deposit smears were read by the same readers and the results compared. One hundred and fifty (70 positive and 80 negative) 2 h stained deposit smears were compared with initial deposit smears and the difference was not statistically significant. Centrifuged deposits of sputum in sputum containers can be stained by carbol-fuchsin within 2-3 h and their smears made subsequently on glass slides can then be decolourized and counter-stained by the procedures followed in ZN method for detection of AFB by light microscopy.

Seasonality in various forms of Tuberculosis

N. Nagayama and M. Ohmori. *Int J Tuberc Lung Dis* 2006; **10**: 1117-1123.

The objective was to study the seasonality of notification for various forms of TB. Descriptive analyses of newly notified cases by month of the year were done. Seasonal variations were compared among various forms of TB. In almost all the forms of TB cases examined, the number of newly notified TB cases were the lowest from November to January. For childhood and pleural TB, the number of cases of TB were highest from March to May (spring) and from April to June (spring to early summer) respectively. For sputum smear-positive pulmonary tuberculosis (PTB) in the young, the number of cases was also highest from March to May. For lymph node TB (except mediastinal) and sputum smear-positive PTB in the elderly (aged $\geq$ 50 years), the numbers of cases were highest from May to July (late spring to summer) and from June to August (summer) respectively. The seasonality of TB notification differs for the various forms of TB, which may reflect differences in the seasonality of clinical development of different types of TB disease such as primary, early and late post-primary TB.

Association of Conversion and Cure with initial smear grading among New Smear Positive Pulmonary Tuberculosis patients treated with Category I regimen.

P.G. Gopi et al. *Indian J Med Res* 2006; **123**: 807-814.

The present investigation was undertaken to study the association of conversion and cure with initial smear grading among pulmonary tuberculosis (TB) patients registered in a directly observed treatment - short course (DOTS) programme in Tiruvallur district, south India. All new smear positive cases registered from May, 1999 to December, 2002 were analysed for conversion and cure related to initial smear grading. Of the 1463 patients, 1206(82.4%) were converted at the end of the intensive phase and 1109 (75.8%) were declared 'cured' after the completion of treatment. The cure rate decreased as the initial smear grading increased and the decrease in trend was statistically significant (P=0.01). Similarly, a significant decrease in conversion rate was also observed with increase in initial smear grading (P<0.001). In multivariate analysis, lower cure rate was significantly associated with patient's age (AOR=1.5, 95% CI=1.1-2.1), alcoholism (AOR=1.7, 95% CI 1.2-2.4) and conversion at the end of intensive phase (AOR=3.5, 95% CI= 2.6-4.8). Cure and conversion rates were linearly associated with initial smear grading. High default and death rates were responsible for low cure

and conversion. The proportion of patients who required extension of treatment and those who had an unfavourable treatment outcome were significantly higher among patients with a 3+ initial smear grading. This reiterates the need to pay more attention in motivating these patients to return to regular treatment and sustained commitment in the control of tuberculosis. There is a need to extend the treatment for one more month in the intensive phase of treatment.

Intra-observer and overall agreement in the radiological assessment of tuberculosis.

J-P. Zellweger et al. *Int J Tuberc Lung Dis* 2006; **10**: 1123-1126.

The objective was to assess the intra-observer and overall agreement in the interpretation of chest X-rays (CXRs) performed for detecting tuberculosis (TB). Four hundred digitalised CXRs from the files of immigrant registration centres were selected and read twice in random order by three readers. The readers had to assess 1) if the picture was normal or abnormal; 2) if an abnormality was suggestive of TB; and 3) if the suspicion of TB needed an immediate examination (potentially smear-positive TB). The intra-observer and overall agreements were expressed as kappa with standard error. Due to losses for technical reasons, 377 of the 400 pictures were analysed. The intra-observer agreement was 0.39-0.90 for any abnormality, and 0.60-0.82 for TB needing an urgent examination. The overall agreements were: 0.55 (all three readers) and 0.84 (two best readers) for any abnormality, and 0.64 (all three readers) and 0.80 (two best readers) for active TB. The intra-observer and overall agreements for the detection of abnormalities on digitalised CXRs and for the presence of possible active TB depend on the reader's experience. It was good between experienced readers and fair between and within the inexperienced reader.

Impact of BCG vaccination on tuberculin surveys to estimate the Annual Risk of Tuberculosis Infection in south India

P.G. Gopi, R. Subramani, T. Nataraj and P.R. Narayanan. *Indian J Med Res* 2006; **124**: 71-76

Annual risk of tuberculosis infection (ARTI) computed from prevalence of infection measures the extent of tuberculosis transmission in the community and it is used to monitor the tuberculosis control programme. This is usually derived from tuberculin surveys among children not vaccinated with BCG. This study explores whether the estimated ARTI among BCG vaccinated children is comparable to that of unvaccinated children. Three tuberculin surveys were undertaken among children aged <10 year as part of assessing the impact of DOTS implemented in Tiruvallur district, south India. The prevalence of infection was estimated using the anti-mode method among vaccinated and unvaccinated children. The ARTI was computed separately and compared in all the three surveys. The prevalence of infection among unvaccinated and vaccinated children in the first survey were 7.8 per cent (95% CI: 7.1-8.6) and 7.9 per cent (95% CI: 7.1-8.8) respectively (ARTI was estimated to be 1.6 per cent in both groups) and the difference was not statistically significant. The corresponding figures for children test read in the second and third surveys were 6.9 per cent (95% CI: 6.2-7.6) and 6.8 per cent (6.0-7.5) and; 6.0 per cent (5.2-6.7) and 6.0 per cent (5.5-6.5) respectively. The computed ARTI was respectively 1.4 and 1.2 per cent among unvaccinated children in the second and third surveys; and 1.4 and 1.2 per cent among vaccinated children in the second and third rounds. There was no difference in the infection with *Mycobacterium tuberculosis* among vaccinated and unvaccinated children. BCG vaccinated children may thus be included for estimation of infection to assess the extent of transmission in the community as well as for monitoring purpose.

Protein antigen b (Pab) based on PCR test in diagnosis of Pulmonary and Extra-pulmonary Tuberculosis

S.S. Negi et al. *Indian J Med Res* 2006; **124**: 81-88.

Diagnosis of tuberculosis (TB) is largely based on microscopy and culture examination which are either less sensitive, or time consuming. In the present study a PCR (polymerase chain reaction) test based on DNA sequence coding for a 38-kilodalton protein antigen b (Pab) ,specific for *Mycobacterium tuberculosis* was compared with Ziehl-Neelsen (ZN) stained AFB (acid fast bacilli) smear examination, culture based on conventional Lowenstein-Jensen (LJ) medium and radiometric BACTEC 460 system for the diagnosis of TB using

clinical samples obtained from pulmonary and extra-pulmonary cases of TB. Clinical samples obtained from 168 patients of suspected TB (pulmonary and extra-pulmonary) were subjected to ZN smear examination, LJ culture, radiometric BACTEC culture and a PCR test by amplifying 419bp sequence coding for Pab, a glycoprotein of molecular weight 38 kDa. A significant difference was seen in the sensitivity of different tests, the figures being 74.2 per cent for PCR test, 53.4 per cent for BACTEC culture, 47.1 per cent for LJ medium based culture and 35.2 per cent for ZN smear examination ($P<0.05$). However, there was no significant difference between different tests as far as specificity was concerned. PCR test sensitivity in pulmonary and extra-pulmonary clinical samples were 74.3 and 71.5 per cent respectively, being significantly higher ($P<0.05$) when compared with sensitivity of other tests. The mean detection time for *M. tuberculosis* was 4.0 days by LJ media culture, 12.8 days by BACTEC culture and less than 1 day by smear examination and PCR test. PCR test is more sensitive than ZN smear examination, LJ medium culture and BACTEC culture for diagnosing TB in pulmonary and extra-pulmonary clinical samples.

Decentralized DOTS shortens delay to TB treatment significantly.

Saint Saly et al. *Kekkaku* 2006; **81**: 467-473

The objective was to compare delays to treatment and behaviour of patients up to diagnosis, between the pilot districts where DOTS is decentralized through the health centers, and the control districts where DOTS is provided through hospitals. It was a cross sectional study with structured questionnaire interviews to all new smear-positive TB patients aged 15 years or older who were registered in the study sites from May 1st to July 31st in 2002. The total delay in the pilot districts was significantly shorter than that in the control districts (median 58 days vs. 232 days, $p< 0.01$). The median doctors' delay within TB service in the pilot districts was 10 days and that in the control was 6 days. The period between first consultation to any health care provider and first visit to a TB service centre, subsequent contact delay, was longer than any other type of delay and significantly different (24 days in pilot vs. 185 days in control, $p<0.0l$). The distance and travel costs to a TB service centre were the factors associated with delay in seeking diagnosis of tuberculosis. No other variables had any significant association with the delay. Decentralizing DOTS to primary care health centers is highly effective in reducing the delay to TB treatment in Cambodia.

An evaluation of Symptom and Chest Radiographic screening in Tuberculosis Prevalence Surveys.

S. den Boon et al. *Int J Tuberc Lung Dis* 2006; **19(8):** 876-882

A tuberculosis (TB) prevalence survey was performed in 2002 in two urban communities in Cape Town, South Africa. The population was 36334 in 2001, and the TB notification rate was 341 per 100000 population for new smear-positive TB in 2002. Objective of the study was to evaluate the relative contributions of symptom and chest radiographic (CXR) screening in the detection of subjects with smear- and/or culture-positive TB in prevalence surveys. Information on symptoms, CXR abnormalities, sputum smear and culture was gathered from a random cluster sample of 1170 adults (aged $\geq$15 years). Smear and/or culture-positive TB was used as the gold standard. Of 1,170 adults, 29 had bacteriologically positive TB (smear- and/or culture-positive). The presence of any abnormalities on CXR had the highest sensitivity for detecting subjects with bacteriologically positive TB (0.97, 95%CI 0.90-1.00). Specificity for any abnormalities on CXR was 0.67 (95%CI 0.64-0.70). The specificity of any of five TB-related symptoms was 0.68 (95% CI 0.65-0.71). Individual symptoms had low sensitivities, ranging from 0.10 for fever to 0.54 for cough of $\geq$2 weeks. In this TB prevalence survey, CXR screening, but not symptom screening, was a sensitive alternative to sputum examination of all participants.

Number of sputum cultures by MGIT system needed to diagnose pulmonary tuberculosis

Kunihiko ITO et al. *Kekkaku* 2006; **81**: 511-518.

Purpose was to study the number of sputum cultures by MGIT system (Becton-Dickinson) needed to diagnose pulmonary tuberculosis. It was a prospective study of all patients who visited our

hospital, and were strongly suspected of pulmonary tuberculosis during the period from Jan. 2002 to Sept. 2003. In these patients, 3 consecutive sputum cultures were done by both MGIT - system and egg-based Ogawa medium (1 slant). Altogether 290 cases of sputum-culture positive pulmonary tuberculosis were available for analysis. In 210 first-sputum-smear positive cases, incremental yield of 3rd sputum culture in 3 consecutive MGIT cultures was equal to or less than 1.0% and 98.1 % of culture positive cases were detected by 2 consecutive MGIT cultures. In 80 first-sputum-smear negative cases, incremental yield of 3rd sputum culture in 3 consecutive MGIT cultures was equal to or more than 5.0 %, and 90.0% of culture positive cases were detected by 2 consecutive MGIT cultures. This detection rate was almost the same as the calculated detection rate (91.4 %) by 3 consecutive Ogawa (2 slant) cultures (previous standard method). It was suggested that 2 consecutive sputum cultures by MGIT were sufficient to detect *M. tuberculosis* in first-sputum-smear positive cases, but 3 consecutive sputum cultures by MGIT were relatively useful in first-sputum-smear negative cases.

K.K. Chopra

FIRST PRIZE FOR TB SEALS ON "CULTURAL HERITAGE OF INDIA"

Dr. M.M. Singh and Dr. V.K. Arora, Vice Chairmen, TAI receiving the award from Dr. Asma El Sony, President of the Union Bureau in the closing ceremony of the 37th Union World Conference on Lung Health

International Union Against Tuberculosis and Lung Disease (The Union)

SEAL CONTEST

At its General Assembly held in Paris 2006 The Union granted

the 1st PRIZE

to The Tuberculosis Association of India

The President of The Union

Indian Journal of Tuberculosis

Registered with the Registrar of Newspapers of India under No. 655/57

Indian Journal of Tuberculosis

Published quarterly by the Tuberculosis Association of India

Vol. 54: No. 1 **January 2007**

Subscription

Inland	
Annual	Rs. 600
Single Copy	Rs. 150
Foreign	
For SAARC countries	US $ 25
For South East Asian and Eastern countries	US $ 30
For other countries	US $ 35

Cheques/D.Ds. should be drawn in favour of "Tuberculosis Association of India, New Delhi"
The views expressed in the articles are those of the authors and not necessarily of the Tuberculosis Association of India or the Editors of the Journal.

Contents

Bibliographic details of the journal available in ICMR-NIC Centre's IndMED data base (http://indmed.nic.in). Full-text of articles from 2000 onwards are available online in medIND data base (http://medind.nic.in).

Published and Printed by Shri S.C. Goyal, on behalf of the Tuberculosis Association of India, 3, Red Cross Road, New Delhi-110001, and Printed at Cambridge Printing Works, B-85, Naraina Industrial Area-II, New Delhi-110 028

THE INDIAN JOURNAL of TUBERCULOSIS

INDEX
to
VOLUME 53

THE TUBERCULOSIS ASSOCIATION OF INDIA
3 Red Cross Road, New Delhi-110001

Supplement to Volume 54, No.1 January 2007

THE INDIAN JOURNAL OF TUBERCULOSIS

Key to Volume 53

No.	*Date of Issue*	*Text Pages*
1.	January, 2006	1-60
2.	April, 2006	61-120
3.	July, 2006	121-184
4.	October, 2006	185-235

Volume 53
No.1-4

CONTENTS

CONTENTS

CONTENTS

CONTENTS

INDEX

INDEX

INDEX

INDEX

INDEX

INDEX