Indian Journal of Tuberculosis

Vol. 56 **New Delhi, January 2009** **No. 1**

Editorial

GRADUAL FILLING UP OF TB DRUG PIPELINE: HOW CAN WE PLAY OUR ROLE BETTER ?

[*Indian J Tuberc 2009; 56:1-4*]

With the introduction of streptomycin (1944), p-aminosalicylic acid (1949), isoniazid (1952), pyrazinamide (1954), cycloserine (1955), ethambutol (1962) and rifamycin (1963), as anti-tuberculosis agents, hope dawned on the gloomy world of patients of tuberculosis. By the end of the 20th century, the changing disease and patient profiles had necessitated to step-up the global hunt for better and more effective drugs in the changed scenario.

The drugs currently in use, given in combination regimens, revolutionized the management and prognosis of TB patients. The situation further brightened with introduction and expansion of the directly observed therapy short-course (DOTS) strategy, but not before years of meticulously planned and painstaking research generated the evidence for change of policy and practice. For example, for over forty years the British Medical Research Council evaluated all the drugs currently in use for treatment. It tested roughly 250 different regimens, enrolling 25 000 patients in trial. Unfortunately, as DOTS coverage expanded, it became apparent that performance of existing drugs limited more efficient implementation of strategy. The duration of treatment required for curing patients could not be reduced to less than six months. In some situations, the regimens are associated with high rates of patients' non-adherence with consequences of increased mortality and creation of chronic infectious drug resistance strains. The infrastructure required for DOTS is cumbersome, labour intensive, and expensive which some resource constrained countries find difficult to sustain. The co-infection of TB-HIV, and emergence of MDR and XDR strains have added further complexities. The tuberculosis treatment regimens in use, although highly successful, needed to be augmented.

The TB drugs in vogue, target bacterial functions vital for survival of replicating *M tuberculosis* cell wall (e.g. isoniazid, ethambutol), transcription (e.g. rifamycins), and energy metabolism (e.g. pyrazinamide). To be effective against circulating MDR and XDR-TB strains newer drugs using alternate and novel approaches and new mechanisms of action will be needed. Moreover, to contribute substantially to the efficacy of the frontline combination drugs by shortening treatment duration, drugs that target populations of slowly multiplying or non-replicating bacilli would also be required.

New tuberculosis drugs are needed to improve current treatment by shortening the total duration of therapy and/or by providing for more widely spaced intermittent treatment, be effective against susceptible and resistant strains, to be compatible with anti-retroviral therapies for the HIV-TB patients, and provide more effective treatment of latent tuberculosis infection in programmes that are able to implement them.

The initiatives of setting up of bodies like Global Alliance for TB Drug Development (2000) have started to populate the discovery of new drugs more robustly. Till very recently seven candidate TB drugs from five different clinical classes were undergoing clinical trials, i.e. fluoroquinolones (gatifloxacin and moxifloxacin), diarylquinolone (LTMC207), nitroimidazoles (PA-824 and OPC-67693), ethylenadiamine

(SQ 109), and pyrrole (LL-3858)[1]. Research groups from academia, large and small pharmaceuticals, government, and not-for-profit public private partnerships are exploring several dozen targets and chemical classes for their potential to contribute to improving tuberculosis therapy. Promising leads are starting to emerge and are being watched with great interest and expectations.

Recently, a team from Howard Hughes Medical Institute, Rutgers University has unlocked the workings of a crucial bacterial enzyme - RNA polymerase (RNAP), which is the linchpin in the process that helps in the assembly of proteins. The team has identified a region in RNAP that can be blocked by a novel antibiotic molecule they have developed called myxopronin. They have also found that other molecules like corallopyronin, and ripostatin can also do the same job. It is believed that the bacteria will not turn resistant to it. This may even be successful in reducing the treatment time. And since bacteria depends upon RNA polymerase to survive, blocking it might be a plausible strategy to deal with emerging multi-drug resistant forms of TB[2].

Researches have identified a key membrane protein that is essential to the defences that *M. tuberculosis* mounts against acidic environment of macrophages and escapes being phagocytosed. Without these proteins (Rv3671c) the bacterium becomes vulnerable to acidification and is killed. A strain of *M tuberculosis* disrupted in Rv3671c, a previously uncharacterized gene encoding a membrane-associated protein became sensitive to acid environment. Disruption of *M tuberculosis'* acid resistance properties is an attractive target for chemotherapy[3]. This is another example of a new class of potential targets being examined.

The investigators have begun to focus on identifying essential targets within non-replicating mycobacteria with the goal of killing the population of bacilli that persist in host even when efficacious drug treatment is given. It is now known that even the dormant bacilli need to produce small quantities of ATP to survive. A new compound R207910 has been found to successfully target the proton pump and block ATP synthesis. Mutant strains produced by treatment with R207910 remained sensitive to major clinical drugs. In studies against established infections in mice, it was at least as active as the triple combination of rifampin, isoniazid and pyrazinamide[4]. A synergistic interaction between R207910 and pyrazinamide has also been described. Three drug combinations containing these two agents have the potential to significantly shorten the treatment duration in patients[5]. It is claimed to be more effective in killing dormant or physiologically 'turned off' bacteria as compared to actively replicating ones.

In India too, a boost to drug development activities is being given.

In September 2008, the CSIR has launched an 'open source drug discovery' (OSDD) aimed to build a consortium of voluntary researchers from across the world to work around the patent regime that makes drugs expensive . It is a concept to aggregate the biological and genetic data available to scientists in order for them to use it for the discovery of drugs. This will provide an opportunity for scientists, doctors and technocrats with diverse expertise to work for a common cause. Open source is a development methodology that harnesses the power of distributed peer-review and transparency of the project. Open source is expected to provide better quality, higher reliability, more flexibility, lower cost, and an end to close-door activities which increases the drug discovery cost to a great extent. To begin with, OSDD has taken up research on discovering new drugs for treatment of tuberculosis. Laboratory experiments during the process would be carried out at CSIR sponsored centers, and an amount of Rs.150 cr. has been earmarked in the 11th Plan period and an equivalent amount is expected to be raised from external agencies.

Again, Council of Scientific and Industrial Research's New Millennium Indian Technology Leadership Initiative (NMITLI) has encouraged *inter alia* development of an investigational new drug for tuberculosis coming from a pharma company, Lupin. The Company has successfully completed Phase I studies for its lead TB molecule (LL 3858) alone and in combination with standard anti-TB drugs (LL 4858). It has submitted Phase I report to the DCGI in March 2008, the Company is awaiting permission for conducting Phase II studies.

Basic science research in India is also providing vital insights into *Mtb* survival strategies within the host. For example, collaborative research between various institutes is contributing to the understanding of mycobacterial adaptability and survival mechanisms in highly intricate and fiercely competitive host environments and the role of iron regulatory networks therein. Abrogation of such iron sequestering pathways could then form the basis of an effective intervention against this human pathogen[6]. This has opened potential avenues of developing treatment drugs.

At the global front, it is now hoped that at least one new drug would be ready for registration by 2010. Of the others, which are in various stages of development at least some are expected to move forward in the pipeline. Collectively all these add up to a promising pipeline of new anti-tuberculosis drugs.

What should these positive actions and promising results mean especially to India and other tuberculosis endemic countries?

India has the highest TB burden in the world. About 5000 persons developing the disease per day and two deaths occur every three minutes due to tuberculosis. We have a worrying prevalence of HIV-TB co-infection and rising concern over MDR and XDR-TB. In addition to the direct cost of the control programme, the indirect cost to the society is more than US$ 3 billion/year. India stands to gain the most with introduction of new drugs.

The promising early findings and some known and yet unknown issues would need further investigation which would need high quality clinical trial capacity. If we want to be part of this global effort and contribute more meaningfully India must surge its clinical trial capacity.

Sample sizes for tuberculosis trials, more so those in Phase-III are quite large. Based on analysis of previous trials conducted internationally, capacity must exist to enroll 3000-5000 patients for each of the seven to ten compounds currently in clinical development. Do we have the infrastructure and financial support on the scale needed? Assistance would be needed in study design, data analysis, drug procurement, and training of research personnel. Capacity for ethical and regulatory review would be required. There is limited capacity for conducting clinical trials, which are GCP compliant. The Global Alliance for TB Drug Development recently completed a survey of 51 potential sites where clinical trials for TB might be conducted. Preliminary results indicate that only a few sites around the world notably Rio de Janeiro, (Brazil), and Durban (South Africa) have the needed components and experience to begin enrolling patients in sufficient numbers.

Experiences of groups currently conducting trials indicate that the estimated costs of TB trials needed to create new regimens and regimens useful against MDR and XDR-TB are considerable. Based on the actual costs of trials conducted in the past few years by TB Trials Consortium, the European Union funded consortium and African institutions, and the Johns Hopkins TB Research Centre, establishing infrastructure for 25 sites with appropriate clinical, laboratory, and regulatory expertise will be on the

order of US $1 to US $2m per site per year, while costs for actual trials will range between US $4000-US$12,000 per patient according to the location. Granted that in India the cost of clinical trials may be much less, but still the amounts would be substantial.

Now that the Ministry of Health, Government of India, has created a new Development of Health Research within the Ministry of Health & Family Welfare, some innovative approaches and strategies are feasible to seize the opportunity. Development of a structure on the pattern similar to the TB trials consortium of the US's CDC or the British Medical Council's TB Research Units can be thought of with the Tuberculosis Research Centre taking the lead. The Department could take up time-bound skills development programme in identified clinical trial centers, including those for members of Data and Safety Monitoring Board, members of ethics review committees at institutional levels. Support would also be crucial for monitoring, training and protocol development. Laboratory support to measure the trial end-point, GCP, GLP and good project management practices need to be strengthened. To move forward towards realizing our role in this global effort we need a strong political support, technically sound blue-print, adequate funds, and determined leadership.

Can we achieve what appears to be quite doable?

Lalit Kant
Scientist 'G' and Head(Epidemiology and Communicable Diseases)
Indian Council of Medical Research
New Delhi

REFERENCES

1. Ginsberg AM. Emerging Drugs for Active Tuberculosis. *Seminars in Respiratory and Critical Care Medicine* 2008; **29**:552-559.
2. Mukhopadhyay J, Das K; Ismail S, Koppstein D; Jang M, Hudson B, Sarafianos S, Tuske S, Patel J, Jansen R, Irschik H, Arnold E and Ebright RH. The RNA Polymerase "Switch Region" is a Target for Inhibitors *Cell* 2008; **135**: 295-307.
3. Vandal OH, Pierini LM, Schnappinger D, Nathan CF and Ehrt S. A membrane protein preserves intrabacterial pH in intraphagosomal mycobacterium tuberculosis 2008; *Nature Med* **14**, 849-854.
4. Andries K, Verhasselt P, Guillemont J, Goehlmann HWh, Neefs JM, Winkler H, Van Gestel J, Timmerman P, Zhu M, Lee E, Williams P, de Chaffoy D, Huitric E, Hoffner S, Cambau E, Truffot-Pernot C, Lounis N, Jarlier V. A diarylquinoline drug active on the ATP synthase of Mycobacterium tuberculosis. *Science* 2005; **307**: 223-227.
5. Ibrahim M, Andries K, Lounis N, Chauffour A, Truffot-Pernot C, Jarlier V, Veziris N. Synergistic activity of R207910 combined with pyrazinamide against murine tuberculosis. *Antimicrob Agents Chemother* 2007; **51**:1011-1015.
6. Farhana A, Kumar S, Rathore SS, Ghosh PC, Ehtesham NZ, et al.(2008) Mechanistic Insights into a Novel Exporter-Importer System of Mycobacterium tuberculosis Unravel its Role in Trafficking of Iron. *PloS ONE* 2008; 3(5): e2087. dol:10.1371/journal.pone.0002087

Original Article

WEIGHT GAIN IN PATIENTS WITH TUBERCULOSIS TREATED UNDER DIRECTLY OBSERVED TREATMENT SHORT-COURSE (DOTS)

M. Vasantha, P.G. Gopi and R. Subramani

(Received on 3.1.2008; Accepted after revision on 14.8.2008)

Summary

***Set up*:** One Tuberculosis Unit (TU) in Tiruvallur district, Tamil Nadu, India where Tuberculosis (TB) patients treated under Directly Observed Treatment Short Course (DOTS) programme.

***Objective*:** To identify the effects of weight gain among TB patients at the end of treatment on different factors such as socio-economic and demographic characteristics, smoking and drinking habits, treatment under supervision, the type of DOTS centres and problems in taking drugs.

***Methods*:** TB patients registered between May 1999 and December 2004 formed the study population. Multiple regression method was used for the analysis.

Results: Among 1557 smear-positive TB patients registered under DOTS programme, the changes in weight ranged from a loss of 4 kgs to a gain of 20 kgs at the end of TB treatment; the average change in weight was 3.22 kgs. The gain in weight at the end of treatment was associated with age (<45 years), DOT at government centres, no problems in taking drugs as reported by patients and cure rate.

Conclusion*:** The findings showed that there is an association between gain in weight with DOT at government centres and cure of patients. ***[Indian J Tubrc 2009; 56: 5-9]

***Key words*:** Tuberculosis, Weight gain after treatment, Multiple regression.

INTRODUCTION

Patients with Tuberculosis (TB) often suffer from severe weight loss, a symptom that is considered immuno-suppressive and a major determinant of severity and disease outcome[1]. Malnutrition is an important risk factor for TB, because cell-mediated immunity (CMI) is the key host defense against TB. The association between body weight, TB mortality and morbidity has been studied extensively since 1986[2-4]. Directly Observed Treatment Short-course (DOTS) is the internationally recommended strategy for TB control, adopted as the Revised National TB Control Programme (RNTCP) in India since 1997. The country was covered under the programme by March 2006 and has almost achieved the global target of 85% cure and 70% case detection. There are about 8.9 million patients with TB in India, of whom half are infectious (sputum smear-positive pulmonary TB)[5]. Currently, nation wide coverage results in a success rate of 86% and a death rate of 4%[6].

The weight of the patient taken at different time points during treatment is an important component to assess the progress of patients. The relationship between change in weight among patients during anti-TB treatment and other factors such as socio-economic demographic characteristics, smoking and drinking habits, whether the patient took treatment under supervision, the type of DOT centres and problems in taking drugs has not been well documented. The objective of the study was to identify the association of weight gain during treatment to these factors and relative importance of these in RNTCP.

METHODS

The study was conducted at a rural TB unit (TU) in Tiruvallur district, South India. The study

Tuberculosis Research Centre, Chennai.

Correspondence: The Director, Tuberculosis Research Centre (ICMR). Mayor V.R. Ramanathan Road, Chetput, Chennai-600 031. Ph: 91 044 28369600, 28369671; Fax: 91 044 28362528; E-mail: icmrtrc@vsnl.com

area included 209 villages and nine urban clusters consisting of a population of 5,80, 000 scattered across approximately 200 km^2. The DOTS strategy has been implemented in this area since May 1999[7]. Seventeen governmental health facilities (HFs) participate in the programme and, of these, seven offer diagnostic facilities for sputum examination. All the patients diagnosed with TB at one of these HFs are given DOT in accordance with RNTCP policies[7]. This study was approved by the Scientific Advisory Committee and the Institutional Ethics Committee as per the Indian Council of Medical Research (ICMR) guidelines.

Between May 1999 and December 2004, 5366 TB patients were registered for treatment under DOTS in this area. Data on socio- economic and demographic characteristics was collected within a week of starting the treatment. Trained field staff interviewed the patients at their residence and collected information on smoking and drinking habits (only from men), whether the patient took treatment under supervision, the type of DOTS centres and any problems in taking drugs using a pre-tested semi-structured questionnaire. Data regarding treatment outcome was collected at the end of the treatment from the TB register maintained at the TU. Standard international definitions were followed to classify TB patients according to outcome[8]. Weight recorded at the initial stage of treatment and at the end of anti-TB treatment was collected from the treatment cards of the patients.

Data were scrutinized and entered twice in order to ensure accuracy, corrected for discrepancy and missing information. The analysis was confined to smear-positive cases and hence extra-pulmonary cases and Category III patients were removed from final analysis. Multiple regression (SPSS version 13.0) was performed to identify the association of weight gain on different factors. The adjusted hazard ratio and 95% confidence intervals (CI) were estimated for the factors. The level of statistical significance was defined as $p<0.05$.

RESULTS

Among the 5366 TB patients, 1557 smear-positive patients whose weight was available at the initiation of treatment, and at the end of treatment were considered for the analysis. Of the 1557 TB patients registered under DOTS programme, 1175 (75%) were males, 690 (44%) were aged 45 years or more, 623 (40%) were illiterate and 478 (31%) were unemployed. Major life style indicators for the patients included the following: 638 (41%) smokers and 474 (30%) alcoholics. Of the 1557 patients, 1285 (83%) were treated under Category I, and 272 (17%) were treated under Category II (Table 1). For these patients, the treatment outcomes were as follows: 1394 (89.5%) were cured, four (0.3%) completed treatment, 52 (3.3%) defaulted, nine (0.6%) expired, 97 (6.2%) failed treatment and one (0.1%) was transferred out.

The mean weight of patients at the initial stage of treatment was 42 kgs. At the end of treatment, the change in weight for 1557 patients by category- wise is set out in Table 2. Overall, the change in weight ranged from a loss of 4 kgs to a gain of 20 kgs with an average change in weight of 3.22 kgs. Weight remained constant from initial stage to the end of treatment for 98 (6.3%) patients. Sixty-eight (4.4%) patients lost weight (mean = -1.79, Standard Deviation (SD) = 1.43, range (-9,-1) where as 1391(89.3%) attained weight (mean = 3.67, SD = 2.45, range (1, 20). Among the 1391 patients, 228 (16.4%) patients gained weight less than 2 kgs, 762 (54.8%) gained 2-4 kgs, 401 (28.8%) gained more than 4 kgs.

Among the Category I patients, the change in weight ranged from -4 to 20 kgs with an average of 3.34 kgs whereas among Category II, it ranged from -3 to 13 kgs with an average of 2.56 kgs. Weight remained constant from initial stage to end of treatment for 72 (5.6%) Category I patients compared to 26 (9.6%) Category II patients. The corresponding figures for patients who lost weight were 42 (3.3%) and 26 (9.6%); and for those gained weight were 1171(91.1%) and 220 (80.8%) respectively. The difference in weight gain between

Table 1: Characteristics of TB patients registered under DOTS in a rural district, south India

Factors	n = 1557 Total (%)
Sex	
Female	382 (25)
Male	1175 (75)
Age (Years)	
<45	867 (56)
≥45	690 (44)
Education	
Illiterate	623 (40)
Literate	828 (53)
Non-availability	106 (7)
Occupation	
Unemployed	478 (31)
Employed	974 (62)
Non-availability	105 (7)
Smoking	
No	813 (52)
Yes	638 (41)
Non-availability	106 (7)
Alcoholism	
No	978 (63)
Yes	474 (30)
Non-availability	105 (7)
DOT centre	
Government	931 (60)
Non-Government	529 (34)
Non-availability	97 (6)
Supervision under IP	
No	319 (20)
Yes	1130 (73)
Non-availability	108 (7)
Problem in taking drugs	
No	476 (30)
Yes	588 (38)
Non-availability	493 (32)
Category	
I	1285 (83)
II	272 (17)

Category 1 and Category II patients was statistically significant ($p<0.001$).

The gain in weight at the end of treatment was associated with age (<45 years) ($p<0.05$), Government DOTS centres ($p<0.05$), no problems in taking drugs ($p<0.01$) and cure rate ($p<0.05$). (Table 3).

DISCUSSION

This study showed that weight gain during the treatment was associated with age (<45 years), DOT at government centres, no problems in taking drug as reported by patients and cure of patients. TB control programme prevents infection and stops progression from infection to active disease, treats all active cases and completely cures all of them. The DOTS strategy has been found to be very effective all over the world and our country has almost achieved the global target. In the study area, the success rate of patients treated under DOTS during the study period was about 80%. Patients put on treatment gaining body weight show improvement towards the end of treatment. Our study has supported that the cure is significantly associated with patient's gain in body weight. Another factor associated with gain in weight was age of the patients; younger patients are more likely to gain body weight during treatment compared to older patients. Patients who took treatment at Government centres and those who reported that they had no problems in taking drugs were more likely to have gained body weight.

There are many studies that have shown the effect of body weight on the treatment outcome. An earlier report from our centre[7] showed that 39 (6%) of 676 TB patients died during the treatment period, and that higher death rates were independently associated with base line body weight <35 kgs and a history of previous treatment for TB. The study recommended that nutritional interventions should be considered among underweight patients to reduce mortality. Another study from our centre found that the higher death rates were independently associated with patient's age (≥45 years), previous history of treatment, alcoholism and initial body

Table 2: Category-wise distribution of change in weight at end of treatment period

Category	No. of patients	Difference in weight (kgs)			Average (Range) (kgs)
		No change No. (%)	Loss No. (%)	Gain No. (%)	
I	1285	72 (5.6)	42 (3.3)	1171 (91.1)	3.34 (-4, 20)
II	272	26 (9.6)	26 (9.6)	220 (80.8)	2.56 (-3, 13)
Total	1557	98 (6.3)	68 (4.4)	1391 (89.3)	3.22 (-4, 20)

Table 3: Results of the multiple regression analysis of factors for gain in weight among 904 TB patients at the end of treatment

Factors	**Non-standardised Co-efficient**		**Standardized Regression Co-efficient**	**95% C.I.for B**	**P**
	B	**S.E**			
Male	-0.035	0.232	-0.006	(-0.490, 0.420)	0.879
Age (<45 years)	0.349	0.173	0.071	(0.010, 0.689)	<0.05
Cure of the patient	0.350	0.135	0.086	(0.084, 0.615)	<0.05
Employed	0.059	0.181	0.012	(-0.296, 0.413)	0.745
Non-smokers	-0.075	0.219	-0.015	(-0.505,0.355)	0.731
Non-alcoholics	-0.134	0.210	-0.026	(-0.547,0.279)	0.525
DOT at Government. Centres	0.346	0.164	0.071	(0.025, 0.668)	<0.05
Treatment under supervision	0.294	0.214	0.046	(-0.125,0.714)	0.169
No problems in taking drugs	0.434	0.163	0.089	(0.115, 0.753)	<0.01

B – Regression co-efficient, S.E- Standard error, C.I – Confidence Interval
The number of patients is less than 1557 due to non-availability of patients at the time of interview, within a week after treatment started.

weight (<35 kgs)[9]. In another study, the treatment success rate was increased as the body weight increased, and the increase in trend was statistically significant (Trend Chi square 22.0; p<0.001) (unpublished). All these findings showed that patient's body weight is associated with treatment outcome. Our study has substantiated this finding of the association between weight gains of patients with cure of the patients. The proportion of Category I patients who gained weight was significantly higher compared to Category II patients. This finding emphasizes that patients under treatment have to

complete the full course of DOT without interruption. Khan et al reported that among persons who were underweight at diagnosis, weight gain of 5% or less among underweight tuberculosis patients after two months of treatment was associated with an increased risk of relapse[10].

A study from Japan reported that the ageing of the Japanese population partially accounts for the increase in the number of patients with TB. The disease is often transmitted from these aged patients to those who were uninfected. After admission, in patients showing negative conversion of bacilli, there was a positive correlation between the increase in PNI (Prognostic Nutritional Index) and the gain of body weight ($p<0.01$, $r=0.30$) [11]. Weight loss is associated with impaired physical function as well as increased mortality in patients with TB. Our study highlights the need to improve the body weight during treatment for a successful treatment outcome. A higher likelihood of weight gain was associated with patients taking treatment in DOT at government centres. Another important finding of this study was the need to have DOTS patient friendly.

In conclusion, weight gain at the end of treatment was associated with age (<45 years), DOT at government centres, no problems in taking drug as reported by patients and cure of patients. TB patients should be educated on optimizing nutritional intake as part of the routine management of TB control programme.

ACKNOWLEDGEMENTS

The authors thank the staff of Electronic Data Processing Department of Epidemiology Unit for computerization of data pertaining to this study. The authors appreciate the field staff and social workers for meticulous data collection under the guidance of Dr. C. Kolappan and Dr. K. Sadacharam. The authors are grateful to Mr. S. Radhakrishnan for maintaining the TB register and other documents. The study was supported in part by the World Health Organization (WHO) with financial assistance provided by United States Agency for International Development (USAID) under the Model DOTS project.

REFERENCES

1. Van Crevel R, Karyadi E, Netea M G, Verhoef H, Nelwan RH, West CE, Va der Meer J W. Decreased plasma leptin concentrations in Tuberculosis patients are associated with wasting and inflammation. *J Clin Endocrinol Metab* 2002; **87(2):**758-63.
2. A Tverdal. Body mass index and incidence of Tuberculosis. *Eur J respire Dis* 1986; **69**: 355-62.
3. England A, Bjorge T, Sogaard AJ, Tverdal A. Body mass index in adolescence in relation to total mortality: 32-year follow-up of 227,000 Norwegian boys and girls. *Am J Epidemiol* 2003; **157(6):** 517-23.
4. Sacks LV, S Pendle. Factors related to in-hospital deaths in patients with Tuberculosis. *Arch Intern Med* 1998; **158(17):** 1916-22.
5. TB India 2005. RNTCP status report. New Delhi: Central TB Division, Directorate General of Health Services, Ministry of Health and Family Welfare, Government of India; 2005.
6. Central TB Division. Directorate of General Health Services Ministry of Health and Family Welfare, Nirman Bhavan, New Delhi 110 011. http://www.tbcindia.org (Accessed on May April 2006).
7. Santha T, Garg R, Frieden T.R, Chandrasekaran V, Subramani R, Gopi P.G, Selvakumar N, Ganapathy S, Charles N, Rajamma J, Narayanan PR. Risk factors associated with default, failure and death among tuberculosis patients treated in a DOTS programme in Tiruvallur District, South India, 2000. *Int J Tuberc Lung Dis* 2002; **6(9):** 780-88.
8. Khatri G.R, Frieden T.R. The status and prospectus of Tuberculosis control in India, *Int J Tuberc Lung Dis* 2000; **4:** 193-200.
9. Vasantha M, Gopi P G, Subramani R. Survival of Tuberculosis patients treated under DOTS in a rural TU, South India. *Indian J Tuberc* 2008; **55**: 64-69.
10. Awal khan, Timothy R. Sterling, Randall Reves, Andrew vernon, Robert Horsburgh C, and the Tuberculosis Trails Consortium. Lack of weight gain and relapse risk in a large Tuberculosis treatment trial. *Am J Respir Crit Care Med* 2006; **174**: 344-48.
11. Handa M. A nutritional and immunological investigation of patients with Tuberculosis. *Kekkaku* 1994; **69(7):** 463-9.

Original Article

PREVALENCE OF TUBERCULOUS INFECTION AMONG SCHOOL CHILDREN IN KERALA

Sunil Kumar[1], Radhakrishna[2], V.K. Chadha[3], R. Jeetendra[4], P. Kumar[5], L.S. Chauhan[**], R. Srivastava[6], Umadevi[7] and R. Kirankumar[8]

(*Received on 20.12.2007. Accepted after revision on 30.10.2008*)

Summary

Background: There is paucity of information on epidemiological situation of Tuberculosis (TB) in the State of Kerala. The DOTS strategy under the Revised National Tuberculosis Control Programme (RNTCP) was introduced in the year 1998 to cover the entire State by 2002.

Objective: To estimate the prevalence of tuberculous infection among children attending 1-4th standard in a sample of selected schools in Kerala.

Methods: A cluster-sample school-based tuberculin survey was carried out in 70 schools selected by a two-stage sampling procedure. A total of 4821 children (including those with and without BCG scar) in the age group of 5-9 years were tuberculin tested using 1 TU PPD RT23 with Tween 80 and the maximum transverse diameter of induration was measured about 72 hours later. About 81% of the children were found to have BCG scars. Analysis was also undertaken by mixture model.

Results: While 67% of children without BCG scar and 62% with scar did not elicit any induration at the test site, the mode or anti-mode of reactions due to infection with tubercle bacilli could not be identified from the distribution graphs. Analysis by mixture model also did not provide the best fit thus precluding estimation of prevalence of infection. About 5% of children had reactions ≥10 mm, 3% had reactions ≥12 mm and 2% had reactions ≥14 mm.

Conclusion: Low proportion of reactors indicated a low level of transmission of infection in Kerala. Considering the problems in interpretation of tuberculin survey data, it may not be feasible to use ARTI as an epidemiological parameter to monitor future trends of TB situation in the state. ***[Indian J Tuberc 2009; 56:10-16]***

Key Words: Tuberculosis, Infection, Prevalence, Annual Risk, Tuberculin test, India.

BACKGROUND

The State of Kerala having a population of about 32 million is located at the extreme southern tip of the Indian sub-continent[1]. Wedged between the Arabian Sea to the west and the Western Ghats to the east, Kerala lies well within the humid equatorial tropics and has a long coastline of 580 kilometres (km) while its width varies between 35-120 km[2]. It is among the third world's longest-lived, healthiest, most gender-equitable and most literate regions[2,3].

The Revised National Tuberculosis Control Programme (RNTCP) adopting the DOTS strategy was introduced in the State in 1998 and was expanded in a phased manner to cover the entire State by 2002. Under the programme, treatment success rates in the order of 85% among new pulmonary smear-positive cases (PTB) have been accomplished consistently[4]. However, the annual case notification rate of new sputum smear positive cases is among the lowest in India. In the year 2006, it was 32 per 100,000 population compared to 50 at the all-India level[4]. It was thus planned to assess the present epidemiological situation of TB in the State. The estimation of Annual Risk of Tuberculous Infection (ARTI) was preferred in view of the relative practicability of tuberculin surveys over disease and mortality surveys. ARTI defined as the average

1. Medical Officer 2. State TB Officer-State TB Centre, Kerala 3. Senior Epidemiologist* 4.Computer Operator* 5.Director* 6. Field Investigator* 7. Chief Statistical Officer* 8. Chief Statistical Officer (Ex-since transferred*)

* National TB Institute, Bangalore

** Deputy Director General (TB), Directorate General of Health Services, New Delhi.

Correspondence: Dr. V.K. Chadha, Sr. Epidemiologist, National TB Institute, # 8, Bellary Road, Bangalore-560 003
E-mail: ntiindia@blr.vsnl.net.in; vin_chadha@yahoo.com

probability of a group of individuals acquiring new tuberculous infection in the course of one year is a sensitive indicator of the epidemiological situation of TB. It is computed from the prevalence of tuberculous infection estimated from tuberculin surveys carried out in a representative sample of children. It expresses the overall impact of various factors influencing the transmission of infection viz. incidence of infectious cases, their average duration of infectiousness, efficiency of TB control programs and the socio-environmental factors. No State level TB epidemiological study had ever been carried out in the State.

OBJECTIVE

To estimate the prevalence of tuberculous infection among children attending classes 1-4, in a sample of selected schools in Kerala and compute ARTI from the estimated prevalence.

MATERIAL AND METHODS

Choice of study population

The survey was carried out among children attending classes 1-4 of statistically valid sample of primary schools. Primary school enrollment is more than 90% in the State[5]. Since majority of the children are vaccinated with BCG (Bacille Calmette-Guérin)[6], all children, irrespective of their BCG scar status, comprised the study population since a survey restricted to BCG unvaccinated children would not be representative of the reference population besides being more expensive and cumbersome. Several studies in India have demonstrated that the influence of BCG induced tuberculin sensitivity on ARTI estimates is minimal when BCG is given at birth and the tuberculin surveys are analyzed by using mirror-image method, especially when the surveys are carried out after five years of age[7-12].

Sample size and sampling design

The sample size of 5890 was estimated to obtain the prevalence of infection within 15% of true value (relative precision) at 5% level of significance while considering the expected prevalence of infection at 8% based on the results of the south zone survey during 2000-2001[11]. Value of design effect was taken as three as generally recommended for tuberculin surveys[13].

A two-stage cluster sampling was adopted for selection of clusters. The term *cluster* denoted an elementary school for the purpose of this survey. At the first stage, four districts were selected by PPS (Population Proportional to Size) sampling method (total number of districts in Kerala=14). The districts selected were Kasaragod, Kozhikodu, Pathanamithitta and Alapuzha.

Estimated sample size was allocated equally into four districts – 1473 in each district. Second stage of sampling involved selection of primary schools within the selected districts. Lists of schools in the selected districts were obtained from the education department. For selection of schools within individual districts, all primary schools (Government as well as private) in the districts were listed in an alphabetical order. However, the information on numbers of children enrolled in each school of the selected districts was not available. To overcome this constraint, it was arbitrarily decided to select 50 schools by simple random sampling. The selected schools were listed in the order of selection. The survey was designed to start from the first selected school and proceed to the next school in the order of selection till 1473 children were satisfactorily test-read in the district. Within individual schools, all children present on the day of testing in classes 1-4 were included.

Field procedures

The field team was imparted a five-week intensive training at National Tuberculosis Institute, Bangalore, (NTI) on all tasks involved in the survey. The team members were evaluated towards the completion of three-phase training course and a preferential list was prepared to allocate the specific tasks to best performers.

Ethical clearance for the survey was obtained from ethical review committee of NTI.

The fieldwork was carried out during June to October 2006.

A planning visit was undertaken to each school two-three days prior to the testing day. Consent of school authorities was obtained after acquainting them with the purpose of survey and characteristics of tuberculin test. Printed information was sent through children to their parents about purpose of the survey, dates of testing and reading and the nature of tuberculin test two-three days before testing. The parents were given the option of refusal.

On the day of testing, all children enrolled in class one to four as per the attendance register were registered into the survey and their age, sex and presence or absence of BCG scar (pea sized hypo-pigmented shiny scar on upper third of either arm) were recorded. In case of a scar not having the typical characteristics of a BCG scar, it was recorded as doubtful.

A testing centre was set up under a shade at a suitable place in the school. Children with any of the following conditions were excluded from tuberculin testing: high fever, severe malnutrition, known history of anti-TB treatment or a known immuno-compromised condition, skin rash and in case of refusal by the parent/guardian.

The tester administered exactly 0.1 ml of tuberculin containing 1 TU of PPD RT23 with Tween 80 intra-dermally on the mid-anterior aspect of the left forearm with 1-ml disposable tuberculin syringe. The entire quantity of tuberculin was procured as a single batch procured (Batch no.643A) from Statens Serum Institute (SSI), Copenhagen. Disposable tuberculin syringes with graduations of one-tenth of an ml, fitted with a 26-gauge needle of one cm length and 20^{o} bevel were used for injections. The test was recorded as *satisfactory* if it raised a flat pale wheal with clearly visible pits and well demarcated borders. It was recorded as *unsatisfactory* in case of leakage or subcutaneous injection, as shown by a less anemic dome-shaped papule, rather than a flat pale wheal.

The reading of tuberculin reactions was undertaken at about 72 hours (3 days) after administration of the test. Only in case of exigency, the reactions were read at 48 hours. Reader identified the margins of induration by carefully palpating the edges of the reaction. The maximum transverse diameter of the induration was then measured in millimeters, using a transparent ruler calibrated in mm. Care was taken not to measure the erythema. The reader also examined the test site for presence of bulla, vesicle, necrosis or lymphangitis and dictated his observations to a secretary for recording.

The survey in the district stopped when the required numbers 1473 were satisfactorily test-read. This sample size was achieved in each of the first three districts but only 394 children were test-read in the last district due to non-availability of PPD.

The cold chain for PPD vials was maintained at all levels.

Parents of the children having reactions $\geq$ 10 mm were advised through a referral letter to approach the nearest health centre for further investigations and medical assistance.

Statistical methods

The data was entered into EPIINFO (windows version) and cross-checked for any key punch errors. It was analyzed using SPSS and Excel.

Tuberculin reaction sizes obtained among children were arranged in the form of frequency distribution table and converted to a frequency distribution graph to identify the mode and/or anti-mode of reactions attributable to infection with tubercle bacilli. To minimize the influence of digit preference if any, five-point moving averages were used for estimating proportion of reactors. Weight was assigned to each district as the ratio of the numbers originally allocated to the numbers investigated. The 95% confidence interval (CI) of the estimate was obtained using the appropriate formula[14]. Chi-square test (Yates corrected) was used to compare proportions and P value of <0.05 was considered statistically significant.

Analysis using basic mixture model was undertaken in the Bayesian way by Markov Chain Monte Carlo approach using R software (version 2.4.1, http:/lib.stat.cmu.edu/R/CRAN) and scripts available at www.tbrieder.org[15]. Two component distributions were assumed among children without BCG scar - one for infection with tubercle bacilli and one for cross-reactions to infection with environmental mycobacteriae. Among children with BCG scar, three component distributions were assumed, the third component attributable to cross-reactions due to BCG vaccination. Analysis was undertaken assuming different combinations of distributions for mixture components (Weibull, normal and log-normal).

RESULTS

A total of 5288 children were registered in class one to four in 70 schools. The age distribution of registered children is given at Table 1. Children aged 10 years were excluded from analysis owing to small numbers. Therefore, 5257 registered children between five and nine years of age comprised the study population. Of these, 5221 were tested – 5175 satisfactorily and 46 (0.9%) unsatisfactory. Of the satisfactorily tested, 4821 were test-read among whom the final analysis was carried out. Therefore, 91.7% of the registered children in five-nine years were satisfactorily test-read. About 81% of the satisfactorily test-read children were found to have BCG scar.

Table 1: Age distribution of registered children

Age in years	No. registered (%)
5	968 (18.3)
6	1181 (22.3)
7	1302 (24.6)
8	1486 (28.1)
9	320 (6.1)
10	31 (0.6)
Total	**5288**

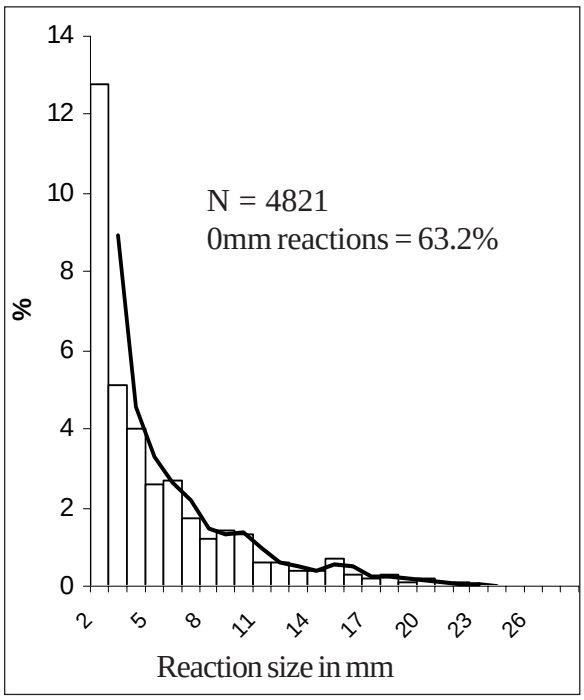


Fig. 1: Frequency distribution of reaction size among all children (including with and without BCG scar)

Table 2: Proportion of reactors at different cut-off points among children 5-9 years of age, Kerala

≥

≥

≥

*BCG scar status not available for 47 children

The frequency distribution of reaction sizes among all children (including those with and without BCG scar) and by BCG scar status is presented at figures 1 and 2. While 67% of children without BCG scar and 62% with scar did not elicit any induration at the test site, the mode or anti-mode of reactions due to infection with tubercle bacilli could not be identified from the distribution graphs due to absence of bi-modality. Analysis by mixture model also did

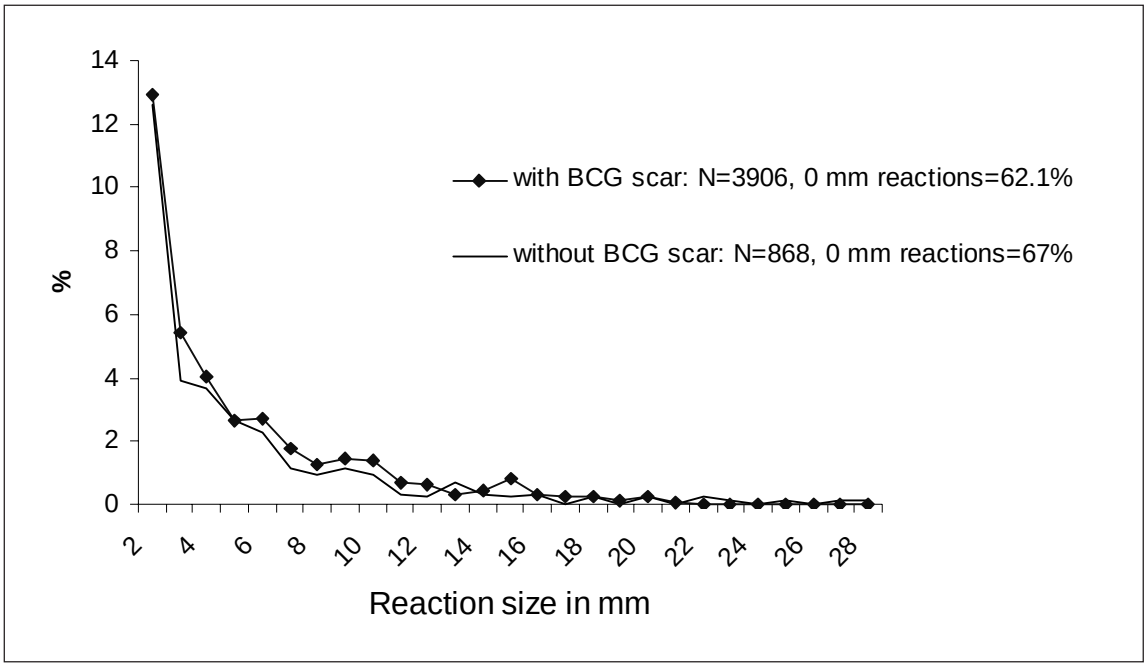


Fig. 2: Frequency distributions of tuberculin reaction sizes by BCG scar status

Table 3: Proportion of reactors at different cut-off points among children 5-9 years of age during other recently conducted tuberculin surveys in South India

Reaction size	BCG-N=868	BCG+N=3906	Southern Zone of India, 2000-01* All N=4821*		A.P., 2005*		Thiruvellur, 2004-05[20]		Bangalore 2006[19]	
10 mm	4.0	5.8	5.4							
12 mm	2.1	3.1	2.9							
14 mm	1.8	2.5	2.3							
			No.	%	No.	%	No.	%	No.	%
BCG -	≥ 10	17811		10.0	1521	15.0	8329	-	984	12.8 (9.9-15.1)
	≥ 12			8.3		12.6		6.0		12.2 (9.3-11.3)
	≥ 14			6.8		10.8		-		8.7 (5.7-7.4)
BCG +	≥ 10	32549		14.2	2098	19.0	2352	-		13.7 (11.4-13.3)
	≥ 12			11.2		15.3		-		11.6 (9.6-11.3)
	≥ 14			8.2		14.1		-		10.6 (8.9-10.5)
All	≥ 10	50533		12.7	3636	17.2	3354	-		13.5 (11.7-17.8)
	≥ 12			10.1		14.1		-		11.9 (10.2-16.8)
	≥ 14			7.7		11.6		-		8.9 (7.4-12.2)

*Unpublished data

Table 4: Prevalence of infection among children without BCG scar (1-9 years), in six surveyed districts, South Zone survey, 2000-01 (mirror-image technique)

District	No. investigated	Prevalence of infection[21]
Dashing Kannada	2,064	3.8
Belgaum	3,926	3.1
Mallapuram	3,518	2.5
Medak	2,837	11.0
Kanyakumari	873	3.6
Chingleput	4,595	9.1

not provide the best fit thus precluding estimation of prevalence of infection. Therefore, the proportions of reactors were estimated at different cut-off points and results are given at table 2. The differences between proportions by BCG scar status were statistically significant at 10 mm cut-off point but not significant at other cut-off points.

Due to lack of anti-mode and mode on the frequency distributions of reaction size, ARTI rates were not computed since these would be highly imprecise.

Thirty-six (1%) of the test-read children revealed unpleasant reactions in the form of bullae or vesicles. These proportions were similar among children with or without BCG scar.

DISCUSSION

The present tuberculin survey was the first State level Study into the epidemiological situation of TB in Kerala. However, prevalence of infection with tubercle bacilli could not be determined precisely owing to lack of anti-mode and mode of reactions due to infection with tubercle bacilli on the frequency distribution graphs of tuberculin reaction size. Such problems in interpretation of tuberculin survey results have been commonly observed in the recent times, especially in areas with low levels of transmission of infection[16-19]. Similarly, the non-interpretation of results through mixture model could be attributed to a large overlap of reactions due to infection with tubercle bacilli and cross reactions due to infection with environmental mycobacteriae and BCG vaccination. Nevertheless, the survey findings indicated a low level of transmission of infection in Kerala considering that the proportion of reactors at different cut-off points was significantly lower than those observed among the same age group of five to nine years during other recently conducted tuberculin surveys in South India (Table 3). Though the survey in southern zone of India during 2000-01 was aimed at obtaining zonal level estimates of prevalence of infection among children one to nine years of age, the district wise analysis showed the prevalence of infection to be lowest in the Mallapuram district of Kerala compared to the districts surveyed in other States of the zone (Table 4)[21]. An earlier study during 1991-92 conducted among 10 year-old school children in Trivendrum (capital district of Kerala) had also revealed a low annual risk of infection at 0.8%[22].

Comparing the proportion of reactors by BCG scar status, significant difference at 10 mm cut-off points but not at other cut-off points denotes the lack of influence of BCG vaccination at 12 mm and above.

The current scenario of lower rate of TB infection transmission in the State of Kerala may be related to better performance of the primary health care services. This is substantiated by most health indicators. The life expectancy at birth in the State is significantly higher than most other States[3]. Similarly, crude mortality rate and infant and maternal mortality rates are lower than other States[3]. Kerala is the only State in the country where sex ratio is in favour of females at 1058 females to 1000 males[2]. HIV prevalence is also lower in Kerala while all the other three south Indian States are high HIV prevalence States[23].

A possible limitation of the present survey could be that there was substantial shortfall in the numbers of children investigated in one of the districts, though the prevalence estimates were

weighted for the numbers originally allocated to each district,.

To conclude, a low level of transmission of infection was observed in Kerala State. Considering the problems in interpretation of tuberculin survey data, it may not be feasible to use ARTI as an epidemiological parameter to monitor future trends of TB situation in the state. In a similar situation in Korea where repeat tuberculin surveys carried out every five years since 1965 were abandoned after the survey in 1995 revealed very low level of transmission of infection[17].

ACKNOWLEDGEMENTS

The authors are thankful to all the field personnel for their hard work, to District TB officers of the four districts and the Education Department for their valuable support in carrying out the survey. We also thank the World Health Organization for helping to procure 1 TU PPD RT23 with Tween 80 from SSI Copenhagen, especially for this survey.

REFERENCES

1. Registrar General and Census Commissioner, India. Census of India 2001, New Delhi, India: Census India 2002. http://www.censusindia.net.in. Accessed on 8th November 06.
2. Kerala Profile. Facts and Figures. www.kerala-online.in/profile. Accessed on 15th November 2007.
3. Registrar General, India, New Delhi. SRS Bulletin, Sample Registration System. www.censusindia.gov.in/vital-statistics/SRS-Bulletins. Accessed on 15th November 2007.
4. Central TB Division, Directorate General of Health Services, Ministry of Health and Family Welfare, New Delhi. TB India 2007, RNTCP-Status Report. URL: http://www.tbcindia.org. Accessed on 3rd March 2007.
5. Personal communication, Department of Primary Education. Kerala.
6. National Family Health Survey, India. National Family Health Survey-3, National Reports, Volume 1, Childe Health, Chapter-9. URL:http://www.nfhsindia.org/NFHS/
7. Chadha VK, Jagannatha PS, Suryanarayana HV. Tuberculin sensitivity in BCG vaccinated children and its implication for ARI estimation. *Indian J Tuberc* 2000; **47**: 139-146.
8. Chadha VK, Jagannatha PS, Savanur SJ. Annual risk of tuberculosis infection in Bangalore city. *Indian J Tuberc* 2001; **48**: 63-71.
9. Chadha VK, Vaidyanathan PS, Jagannatha PS, Lakshminarayana. Annual Risk of Tuberculosis Infection in rural areas of Uttar Pradesh, India. *Int J Tuberc Lung Dis* 2003; **7**: 528-535.
10. Chadha VK, Jaganath PS., Kumar P. Can BCG vaccinated children be included for tuberculin surveys to estimate annual risk of tuberculous infection in India? *Int J Tuberc Lung Dis*. 2004; **8**: 1437-1442.
11. Gopi PG, Subramani R, Venkatesh Prasad V, Narayanan PR. Estimation of Annual Risk of Tuberculous infection among children irrespective of BCG Scar in South India. *Indian J Tuberc*; **53**:7-11.
12. Chadha VK, Kumar P, Satyanaryana AVV, Chauhan LS, Gupta J, Singh S, et al. Annual Risk of Tuberculous infection in the State of Andhra Pradesh. *Indian J Tuberc* 2007; **54**: 177-183.
13. Nagelkerke NJD, Borgdorff MW, Kalisvaart NA, Broekmans JF. The design of multi-stage tuberculin surveys: some suggestions for sampling. *Int J Tuberc Lung Dis* 2000; **4(4):** 314-20.
14. Ten Dam HG: Surveillance of tuberculosis by means of tuberculin surveys; WHO/TB/85.145, 1985.
15. Neuenschwander B E. Bayesian mixture analysis for tuberculin induration data. URL: http://www.tbrieder.org. International Union Against Tuberculosis and Lung Disease. Accessed on 27 October 2007.
16. Bosman MCJ, Swai OB, Kwamanga DO, Agwanda R, Idukitta G, Misljenovic O. National Tuberculin Survey of Kenya, 1986–1990. *Int J Tuberc Lung Dis* 1998; **2**: 272–280.
17. Hong YP, Kim SJ, Lew WJ, Lee EK, Han YC. The seventh nationwide tuberculosis prevalence survey in Korea, 1995. *Int J Tuberc Lung Dis* 1998; **2**: 27–36.
18. Singh S, Chadha V.K, Srivastava RK, Lakshminarayana, V. Magesh, Suganthi P, Umadevi G, Gupta J, Ahmed J. Prevalence and annual risk of tuberculosis infection among school children in rural Bangalore. *NTI Bulletin* 2006, **42 (3,4):** 68-73.
19. Chadha VK, Jithendra R, Kumar P, Shashidhara AN, Kirankumar R, Suganthi P, Gupta J. Change in risk of tuberculous infection over an 8-year period among school children in Bangalore city. *Int J Tuberc Lung Dis* 2008; **12 (10):**1116-1121.
20. Gopi PG, Subramani R, Narayanan PR. Trend in the prevalence of TB infection and ARTI after implementation of a DOTS programme in south India. *Int J Tuberc Lung Dis* 2006; **10:** 346–348.
21. Directorate General of Health Services, National Tuberculosis Institute, Tuberculosis Research Center. Report on "Estimation of Annual Risk of tuberculous infection in four defined zones of India, 2000-2003", 2004. Published by National Tuberculosis Institute, Bangalore.
22. Kumari Indira K S, Sivaraman S, Joshi M, Sivanandan Pillai N. Annual risk of tuberculosis infection: an estimate from ten year-old children in Trivandrum district. *Indian J Tuberc* 2000; **47:** 211–218.
23. National AIDS Control Organisation. Facts and Figures—An overview of the Spread and Prevalence of HIV/AIDS in India. New Delhi, India: Ministry of Health and Family Welfare, 2006. www.nacoonline.org/facts_overview.htm Accessed July 2005.

Original article

EFFICACY OF REPEAT SPUTUM EXAMINATION IN RNTCP

Sonia Malik[1], V.K. Dhingra[2], M. Hanif [3] and R.P. Vashist**

(Received on 25.3.2008. Accepted after revision on 19.6.2008)

Summary

***Background*:** The guidelines of repeat sputum smear examination in initial smear negative patients (ISN), who also fail the antibiotic trial of three samples have been incorporated in the RNTCP diagnostic algorithm in India in 2005. This study was conducted to assess the utility of repeat sputum smear examination in symptomatic initial smear negative patients to detect new smear positives in the state of Delhi.

***Material and Methods*:** The monthly records of the laboratory abstracts for the six quarters for all the 24 districts of Delhi were analysed w.e.f. first of January 2006 to 30th June 2007.

***Results*:** A total of 2,43,244 TB suspects were examined for diagnosis during the six quarters w.e.f. January 2006. Of these, 37,666 were found positive on sputum smear microscopy giving a positivity rate of 15.4%. During the same period, a total of 2,195 (1% of ISN) TB suspects underwent repeat sputum examination, of which 272 were found positive giving a mean positivity of 12.3%.

***Conclusion*:** A significant number of apparently smear negative TB cases may in fact be smear positive due to various reasons and can be detected by a simple repeat sputum examination. Yield of sputum positive cases in sputum re-examination is almost the same as in initial sputum examination i.e. 10-15%. Therefore, the policy of repeat sputum examination in symptomatic initial sputum negative cases failing the antibiotic trial should be meticulously followed as advocated in the RNTCP diagnostic algorithm. **[*Indian J Tuberc 2009; 56:17-21*]**

***Key words*:** Repeat sputum examination, Pulmonary, Tuberculosis, Diagnosis

INTRODUCTION

Active cases of pulmonary TB , who excrete tubercle bacilli in their sputum, are the main sources of transmission of infection . They are responsible for almost 95% of the transmission of infection in the community. They are also more likely to suffer from more extensive disease and are at higher risk of dying from it or developing drug resistance[1]. Hence, early detection and adequate management of active pulmonary tuberculosis cases is the priority of any tuberculosis control programme[2]. Wider availability of direct sputum microscopy for acid fast bacilli (AFB) in primary care permits detection of more pulmonary TB cases around the world. However, > 50% of patients with pulmonary TB may have negative smears on AFB microscopy[3]. In poorly resourced countries of the developing world, where mycobacterial cultures are not available, clinicians rely critically on chest radiography to clinch the diagnosis in TB suspects with negative smears[4].

Recently, newer WHO TB diagnostic algorithm stipulates a further set of repeat sputum smears preceding chest radiography in initial smear negative (ISN) patients, who fail the antibiotic trial[5]. These guidelines of repeat sputum smear examination of three samples have been incorporated in the RNTCP diagnostic algorithm in India in 2005[6]. This study was conducted to assess the utility of repeat sputum smear examination in symptomatic initial smear negative patients, who fail the antibiotic trial, to detect new smear positives in the state of Delhi.

---

1. Microbiologist RNTCP* 2. Director* 3. Bacteriologist*
* New Delhi Tuberculosis Centre, New Delhi.
** State TB Control Officer, Delhi
Correspondence: Dr. VK Dhingra, Director, New Delhi Tuberculosis Centre, Jawahar Lal Nehru Marg, New- Delhi 110002; Ph: 23234270; Email: ndtbc@yahoo.com

MATERIAL AND METHODS

RNTCP has been implemented in all districts of Delhi w.e.f 1997 with DOTS expansion already completed. For the management of RNTCP, the State has been divided into 24 District Tuberculosis Centres (DTCs) and 183 Designated Microscopy Centres (DMCs) catering to 160 lakh population. The External Quality Assurance (EQA) of sputum smear microscopy including

Table: Trend of repeat sputum examinations at 24 chest clinics in the State of Delhi during first quarter 2006 – IInd quarter 2007

	1st QTR 2006	2nd QTR 2006	3rd QTR 2006	4th QTR 2006	1st QTR 2007	2nd QTR 2007
% of ISN patients re-examined (range)	0.1% 0-5.7%	1% 0-7.92%	1% 0-7.05%	1.16% 0-6.8%	1.14% 0-6.4%	1.3% 0-6.42%
% of DTCs with > 1% sputum ISN Re-examined	20%	35%	30%	45%	40%	55%
% of DTCs with no sputum REs	20%	25%	20%	10%	10%	5%

ISN: Initial smear negatives
REs: Repeat examinations

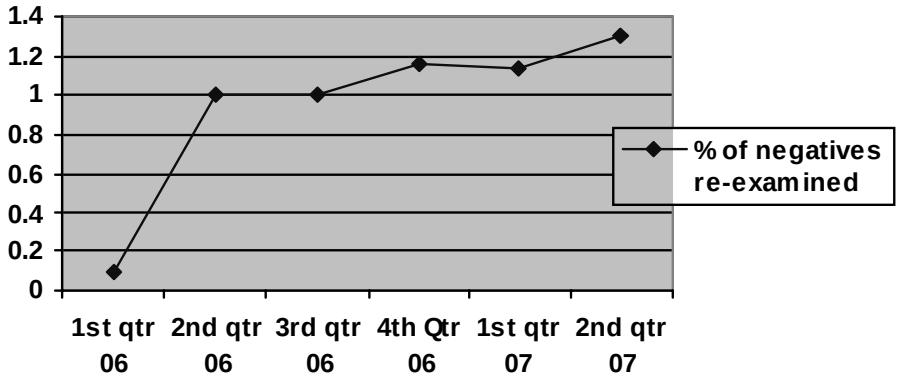


Figure 1: Percentage of smear negative patients in whom repeat sputum examination was done during the study

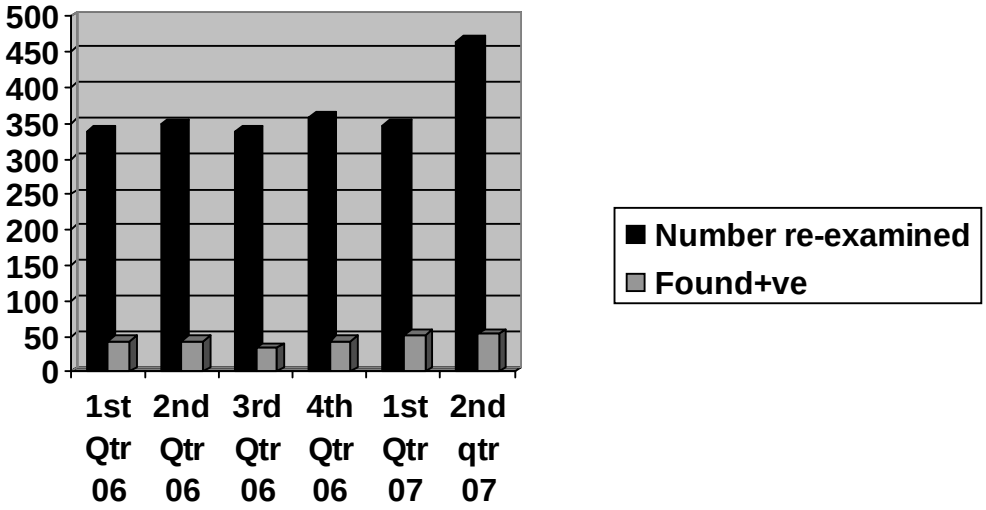


Figure 2: Patients re-examined and found sputum positive during six quarters

Random Blinded Rechecking of slides(RBRC) and On site evaluation (OSE) of DMCs has been going on in all the districts of the State since December 2005.

STUDY DESIGN

This was a retrospective study. The laboratory reports of chest clinics sent to the State TB Demonstration Centre as well as the quarterly RNTCP performance reports for all the 24 districts of Delhi were retrospectively analysed w.e.f. first January of 2006 to 30th June 2007 i.e. for six quarters after the application of the repeat sputum examination policy came into being.

During this period, three sputum samples of patients presenting with cough of more than three weeks duration were examined for acid fast bacilli (AFB) microscopy according to RNTCP guidelines using Ziehl-Neelsen staining[6] . Patients with at least two smears positive for AFB were registered for smear positive tuberculosis and depending on whether patient was a new or re-treatment case, started on CAT I or CAT II regimen containing four and five primary drugs respectively during the intensive phase. Patients in whom all three samples were smear negative, were prescribed symptomatic treatment with broad spectrum antibiotics for 10-14 days. Patients, who remained symptomatic after antibiotic course, were advised a repeat sputum smear examination (3 samples) as per the revised algorithm[6]. If two or more smears out of three were positive, the patient was diagnosed as having smear positive tuberculosis and prescribed CAT I or CAT II regimen as described above. X-ray was advised if the result of repeat three sputum samples' examination revealed either one sample positive or all the three negative and the patient was symptomatic. In case of all three sputum samples negative and suggestive X-ray consistent with pulmonary tuberculosis, patients were diagnosed as cases of sputum smear negative tuberculosis and administered CAT III regimen containing three drugs in intensive phase of treatment as per RNTCP guidelines[6].

RESULTS

A total of 2,43,244 of TB suspects were examined for diagnosis during the six quarters w.e.f. january 2006. Out of these, 37,666 were found positive on sputum smear microscopy giving a positivity rate of 15.4% (range: 14.16-16.67%).

During the same period, of the 2,05,578 initial smear negatives (ISN) patients, a total of 2,195 (1%) TB suspects underwent repeat sputum examination after antibiotic course, of which 272 were found positive giving a mean positivity rate of 12.3% (range: 10-15%). A steady increase in the number of repeat examinations performed was observed over the six quarters (Table and Figures 1 and 2)

DISCUSSION

Smear negative tuberculosis presents a diagnostic challenge to the treating physicians around the world. The exact proportion of smear positive TB cases among suspected TB cases depends on the quality of the microscopy, local TB and HIV prevalence and the index of suspicion[7]. In the vast majority of cases however, diagnosis is made on clinical and radiological grounds[4].

It is interesting to note that many of these smear negative TB patients can be easily diagnosed by a simple repeat examination of their sputum. In a study from Malawi, of 352 patients registered for treatment of smear negative tuberculosis, 22% were confirmed as sputum positive TB by mere repeat sputum smear microscopy[8]. In the present study, 1% of 2,05,455 initial smear negatives underwent repeat sputum examination with average positivity rate of 12.3% (range: 10-15%), which is as good as the expected range of 10-15% smear positivity of the new cases in RNTCP[6]. The initial smear negativity in all these patients cannot be explained on the basis of laboratory error only since External Quality assurance programme is being meticulously implemented in the entire state of Delhi. Poor quality of the sputum sample is one of the most common causes of a false negative result [9]. Besides, since mycobacteria are excreted intermittently into sputum from cavities[1], some of these cases could have been genuinely sputum negative on initial examination. In some initial sputum negative patients, disease progression may have also led to sputum positivity during the repeat examination[8]. In the absence of repeat sputum examination, the above 272 patients diagnosed as sputum positive after repeat sputum examination would have undergone X-ray examination as per the previous RNTCP algorithm and could have been labelled as having smear negative Pulmonary tuberculosis in case of suggestive CXR and given a less potent three drug CAT III regimen if earlier untreated . This would have probably increased their chances of sub-optimal response or treatment failure. TB suspects with normal /minimal abnormal changes on CXR may not be diagnosed as pulmonary tuberculosis and continue to transmit the infection to the healthy population. In early stages of TB, especially the HIV positive patients may have normal or minimal changes on X-ray. In a study carried out by Harries et al in a high HIV prevalent area , 16(21%) of Pulmonary TB suspects with negative sputum smears and a normal /minimally abnormal CXR were culture positive for *M. tuberculosis*. Seven of these 16 patients developed CXR suggestive of TB by three months [4]. Normal X-ray appearance was seen in a significant number of culture positive patients in a low HIV setting in neighbouring Pakistan [10]. Such cases may stand more chances of diagnosis on repeat sputum examination.

Reliance on X-rays may also increase chances of over-diagnosis. In a study in Kenya, the number of patients labelled as having TB using CXR with a negative culture that were placed on treatment was rather high; 22% among all suspects and 45% among smear negative suspects[4].

It is interesting to note that during the study period, 14,316 (apprx. 7% of 2,05,578 ISN) patients were registered in the State as sputum smear negative tuberculosis cases and administered Cat III treatment. A majority of these patients (12,393; barring those, who were smear negative after repeat sputum examination) were diagnosed without undergoing a repeat examination[11-16].

To conclude, a significant number of apparently smear negative TB cases may in fact be smear positive due to various reasons. A simple repeat sputum examination gives a chance of detection of these sputum positive cases among the symptomatic initial sputum negatives. Yield of sputum positive cases in sputum re-examination is almost the same as in initial sputum examination i.e. 10-15%.

Therefore, the policy of repeat sputum examination in symptomatic initial sputum negative cases should be meticulously followed as advocated in the RNTCP diagnostic algorithm. Though, this policy would lead to an increase in laboratory burden, but would also spare the possibility of diagnostic errors due to over/ under-diagnosis and the increased cost with radiology.

Besides, a sound repeat sputum examination policy would prove useful in the long run in case there is a switch to two sputum sample guidelines instead of the present three for detection of new sputum positive cases. The small loss in the detection of smear positive cases due to deletion of the third sample during initial examination would be compensated later on during repeat examination besides giving immense gains due to increase in efficacy of microscopic examination.

REFERENCES

1. World Health Organisation. Toman's Tuberculosis: case detection, treatment and monitoring (second edition). Geneva: *World Health Organisation*,2004:1-332.
2. Global Tuberculosis Programme. Treatment of Tuberculosis: Guidelines for national programmes. 2nd ed. WHO/TB/97.220. Geneva, Switzerland: World Health Organisation,1997.
3. Aber VR, Allen BW, Mitchison DA, Ayuma P, Edwards EA , Keyes AB. Quality Control in Tuberculosis Bacteriology.1. Laboratory studies on isolated positive cultures and the efficiency of direct smear examination. *Tubercle* 1980; **61**:123-33.
4. Harries AD, Banda HT, Boeree MJ, Wirima JJ, Subramanyam VR, Maher D, Nunn P. Management of Pulmonary Tuberculosis suspects with negative sputum smears and normal or minimally abnormal chest radiographs in resource poor settings. *Int J Tuberc Lung Dis* 1998; **2**:999-1004.
5. World Health Organisation. Treatment of Tuberculosis. Guidelines for National Programmes Geneva: *World Health Organisation*,2003.
6. Central TB Division. Directorate General of Health Services, Ministry of Health and Family Welfare. Nirman Bhawan, New Delhi; A training course on Managing the Revised National Tuberculosis Programme in your area, New Delhi April 2005.
7. Hawken MP, Muhindi DW, Chakaya JM, Bhatt SM, Ng'ang'aLW and Porter JD. Under diagnosis of smear positive pulmonary tuberculosis in Nairobi , Kenya. *Int J Tuberc Lung Dis* 2001; **5**:360-63.
8. Hargreaves NJ, Kadzakumanja O, Phiri S, Nyangulu DS, Salaniponi FML, Harries AD, Squire SB. What causes smear negative pulmonary tuberculosis in Malawi, an area of high HIV seroprevalence? *Int J Tuberc Lung Dis* 2001; **5**:113-22.
9. 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.
10. Siddiqi K, Walley J, Khan MA, Safdar N. Clinical guidelines to diagnose smear negative tuberculosis in Pakistan, a country with low HIV prevalence. *Trop Med Int Health* 2006; **11**:323-31.
11. Central TB Division. Directorate General of Health Services, Ministry of Health and Family Welfare. Nirman Bhawan, New Delhi; *RNTCP Performance Report,India* First quarter,2006.
12. Central TB Division. Directorate General of Health Services, Ministry of Health and Family Welfare. Nirman Bhawan, New Delhi; *RNTCP Performance Report,India* Second quarter,2006.
13. Central TB Division. Directorate General of Health Services, Ministry of Health and Family Welfare. Nirman Bhawan, New Delhi; *RNTCP Performance Report,India* Third quarter,2006.
14. Central TB Division. Directorate General of Health Services, Ministry of Health and Family Welfare. Nirman Bhawan, New Delhi; *RNTCP Performance Report,India* Fourth quarter,2007.
15. Central TB Division. Directorate General of Health Services, Ministry of Health and Family Welfare. Nirman Bhawan, New Delhi; *RNTCP Performance Report,India* First quarter,2007.
16. Central TB Division. Directorate General of Health Services, Ministry of Health and Family Welfare. Nirman Bhawan, New Delhi; *RNTCP Performance Report,India* Second quarter,2007.

Original Article

ISOLATION, CHARACTERISATION AND KINETIC STUDIES ON SEVA TB ES-31 ANTIGEN, A METALLO-SERINE PROTEASE OF INTEREST IN SERODIAGNOSIS

Vijay J Upadhye[1], Ashok V. Gomashe[2], Satish Kumar[3] and Bhaskar C. Harinath[4]

(Received on 29.4.2008. Accepted after revision on 20.11.2008)

Summary

***Background*:** SEVA TB Excretory secretory -31 (ES-31) antigen, a glycoprotein isolated from *M. tb* H_{37}Ra culture filtrate, was found to be useful in the serodiagnosis of pulmonary tuberculosis (TB), extrapulmonary TB and in HIV-TB coinfection. Further, it has been shown to be a zinc containing serine protease.

***Aim*:** To isolate and purify SEVA TB ES-31 antigen from *M. tb* H_{37}Ra culture filtrate and study of its enzyme properties and peptide sequence.

***Methods*:** ES-31 antigen was purified from culture filtrate of *M. tuberculosis* H_{37}Ra strain by ammonium sulphate precipitation, SDS-PAGE fractionation and FPLC. Protease activity of ES-31 antigen was studied using azocasein as substrate. ES-31 antigen was further fractionated by two dimensional polyacrylamide gel electrophoresis (2D PAGE) followed by LCMS-T analysis.

***Results*:** Mycobacterial metallo-serine protease was purified 3096 fold from *M. tb* H37Ra culture filtrate protein. Purified enzyme showed optimum activity at pH 7.0 at 37 ^{0}C. Of the four substrates explored, the enzyme has shown maximum activity with azocasein and had a Km value of 0.01 mM with specific activity of 6250 x 10^{-6} U/mg protein. Further, analysis of ES-31 antigen by 2D PAGE showed two protein spots (A and B).

***Conclusion*:** Kinetic studies on SEVA TB ES-31 protein, an immunogen with metallo serine protease activity are reported for the first time. Purified enzyme had a Km value of 0.01 mM with azocasein as substrate. Further, study on structure and biological role of serine protease will be of interest. **[*Indian J Tuberc 2009; 56: 22-29*]**

***Key words*:** Mycobacterium tuberculosis, SEVA TB ES- 31 Ag, Metallo-Serine Protease.

INTRODUCTION

Tuberculosis continues to be a major scourge of mankind. Each year, approximately million people develop active tuberculosis and two million people die of this disease[1]. Microbial pathogens frequently utilize extra-cellular proteases as virulence factors, which often contribute significantly to pathology. These proteases participate in tissue destruction, inactivation of host defense molecules, activation of key regulatory proteins or peptides, nutrient acquisition and the processing of secreted signaling molecules which regulate gene expression. The genome of *M. tuberculosis* H_{37}Rv encodes over 30 proteases[2] but little is known about the biology and role of these enzymes in this organism. Earlier studies from our laboratory showed that ES-31, a glycoprotein antigen with metallo-serine protease activity was found to be promising in the diagnosis of pulmonary tuberculosis (TB), some forms of extra-pulmonary TB like TB Lymphadenopathy, TB meningitis and in HIV-TB co-infection.[3,4] Recently Brown et al identified five *M. tuberculosis* genes (myc P 1-5) that encode a family of serine proteases (mycosins-1 to 5), ranging from 36 to 47% identity present in *M. tuberculosis*, *M. bovis* BCG and other virulent mycobacteria.[5] Amongst them, Mycosin-1, a membrane and cell wall associated serine protease was identified in M tuberculosis culture filtrates[6]. Very few studies have been reported on mycobacterial serine proteases[5-8]. Hence studies related to characterization and kinetic

1. Senior Research Fellow* 2. Head, Dept. of Microbiology** 3. Professor, Dept. of Biochemistry*** 4. Director*
* Jamnalal Bajaj Tropical Disease Research Centre, Mahatma Gandhi Institute of Medical Sciences, Sevagram, Wardha
** Shivaji College of Science, Nagpur (Maharashtra)
*** Mahatma Gandhi Institute of Medical Sciences, Sevagram, Wardha
Correspondence: Dr. B. C. Harinath, Director, JB Tropical Disease Research Centre (JBTDRC), & Department of Biochemistry, Mahatma Gandhi Institute of Medical Sciences, Sevagram-442 102 (Wardha) (Maharashtra); Tele Fax: (07152) 284038; E-mail: bch@jbtdrc.org, bc_harinath@yahoo.com

properties of mycobacterial serine proteases may be helpful in understanding the biology and function of these enzymes in the mycobacterial cell and host-pathogen interaction. In this study, we report the isolation, characterization and kinetic studies on SEVA TB ES-31 antigen, a metallo-serine protease of interest in sero-diagnosis of TB.

MATERIAL AND METHODS

M. tuberculosis H_{37}Ra Excretory secretory (ES) protein

Excretory secretory protein was isolated from culture filtrate of *M. tuberculosis* H_{37}Ra strain grown in thyroxine-supplemented Sauton liquid medium at 37 ^{0}C on an orbital shaker (150 rpm) for 10 days, as described earlier. [9]

Purification of SEVA TB ES-31 antigen:

SEVA TB ES-31 antigen was purified from culture filtrate of *M. tuberculosis* H_{37}Ra strain by ammonium sulphate precipitation, SDS-PAGE fractionation followed by separation using resource 'S' (1 ml) cation exchange column by fast protein liquid chromatography (Pharmacia Biotech, Sweden) as reported earlier.[10]

Enzyme assay

Protease activity of SEVA TB ES-31 antigen was studied at pH 7.5 using Azocasein (Fluka Biochemicals, USA), azocoll, bovine serum albumin and human serum albumin (Sigma chemicals Co. USA) as substrates and absorbance was measured at 440 nm, 520 nm,680 nm and 680 nm respectively. After finding that azocasein is a better substrate, specific activity of ES-31 antigen enzyme was assayed at optimum pH 7.5 and temperature 37^{0}C using azocasein assay.[11] Briefly, 5 ml of azocasein assay incubation mixture consists of the mycobacterial serine protease (ES-31 Ag) antigen (100 µg) with 25 mg azocasein in 0.5 M Sodium Bicarbonate buffer (pH 8.3). Azocasein assay mixture without ES-31 antigen served as control. The azocasein assay mixture was incubated at 37 ^{0} C for 6 hrs. Further, 1 ml aliquot from both test and control solution was removed and 4 ml of trichloroacetic acid (5 %) was added to the solution. After mixing and filteration using 0.45 µm syringe filters, again 1 ml aliquot from both test and control solution was removed and 3 ml of 500 mM NaOH solution was added to the solution. Absorption of the liberated dye at 440 nm was measured using a spectrophotometer (Ultra-spec, Elico Ltd, India). Km value for azocasein and Vmax of serine protease enzyme were determined by a Lineweaver-Burk plot by using Graph Pad Prism Software.

Determination of optimum pH and temperature for SEVA TB ES-31 antigen:

The optimum pH for SEVA TB ES-31 antigen (serine protease) was determined by incubating the enzyme in different pH buffers (citrate phosphate, pH 3, 4 & 5, sodium phosphate, pH 6 & 7, Tris-HCl, pH 8.0 and glycine NaOH, pH 9,10,11 & 12) followed by assay of enzyme activity. The effect of temperature on the enzyme was similarly determined by incubating the enzyme at different temperatures viz. 0^{0}C, 4^{0}C, 20^{0}C, 30^{0}C, 37^{0}C, 50^{0}C and 60^{0}C and assay of enzyme activity.

Two dimensional polyacrylamide gel electrophoresis (2D PAGE):

SEVA TB ES-31 antigen protein was further resolved by 2D PAGE consisting of isoelectric focusing (IEF)/SDS-PAGE as described by O'Farrell PH [12] with certain modifications on the 2D GEL System – MINIGEL (Biotech, Salem India). ES-31 antigen (100 µg) was run from cathodic reservoir (100 mM NaOH) to anodic reservoir (10 mM H_3PO_4). IEF tube gel was composed of 10 M urea, 4 % acrylamide, 2 % Triton X-100 and 0.5 % ampholyte pH 3.0 to 10.0. SEVA ES-31 antigen protein was dissolved in the sample buffer (IEF buffer) containing 10 M urea, 2 % Triton X-100, 3 % Dithiothreitol, 0.5 % ampholyte pH 3.0 to 10.0 and 0.001 % bromophenol blue, and then the sample was loaded into the IEF tube gel. The IEF was conducted at 200 V for 2 hours, 500 V for two hours and at 800 V for 16 hours. After the electrophoresis, the tube gel was equilibrated with SDS-sample buffer consisting of 62.5 mM Tris-HCl

Table 1: Purification of serine protease (SEVA TB ES-31 antigen) from *M. tuberculosis* H_{37}Ra culture fluid.

Table 2: Activity of mycobacterial serine protease on different protein substrates

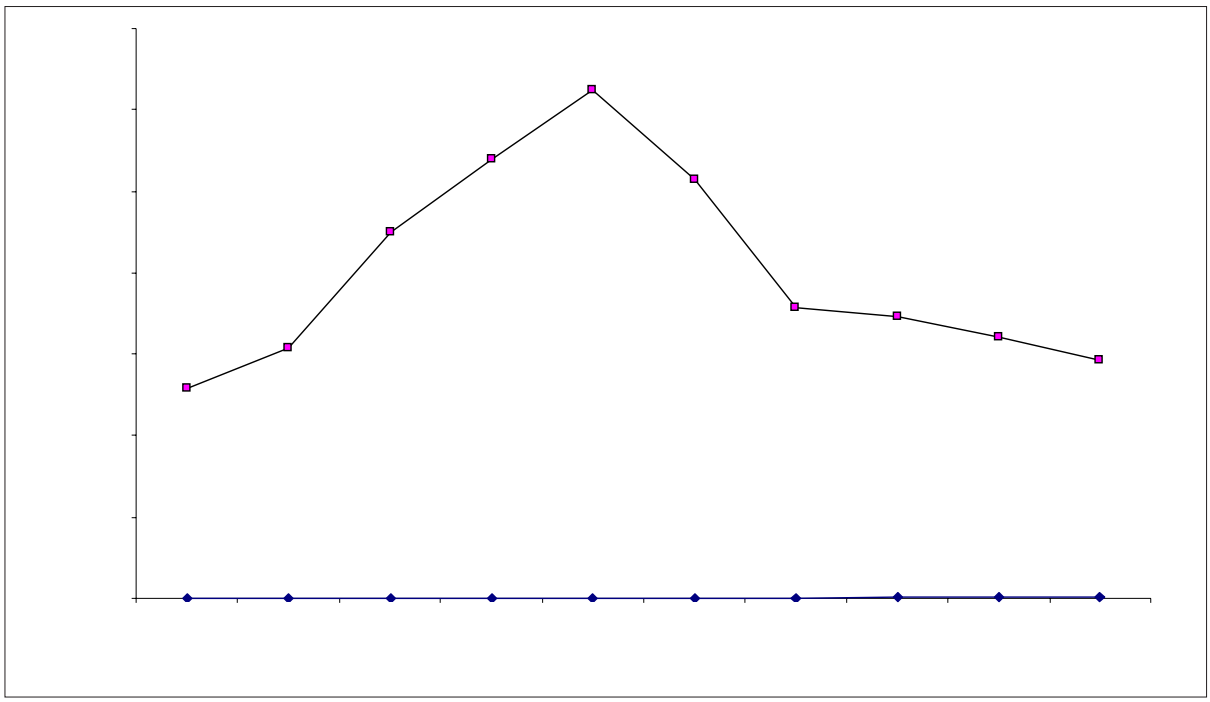


Figure 1: Effect of pH on SEVA TB ES-31 antigen

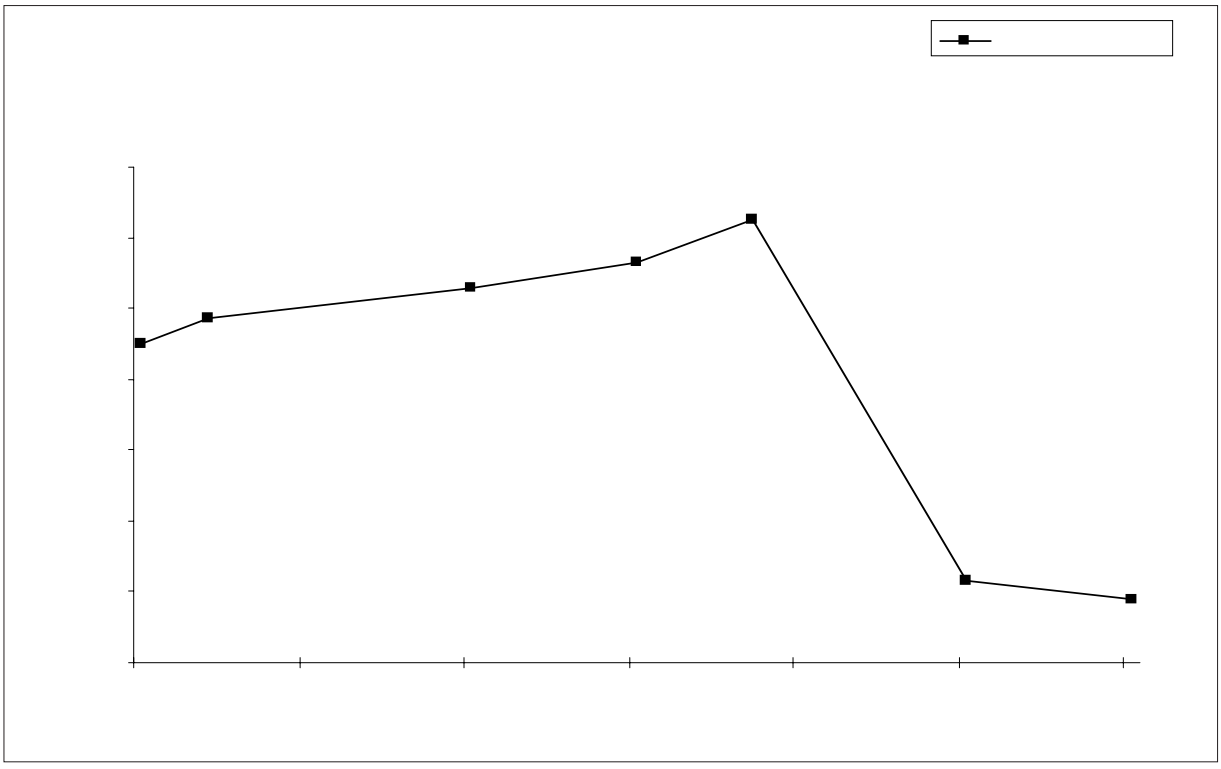


Figure 2: Effect of Temperature on SEVA TB ES-31 antigen

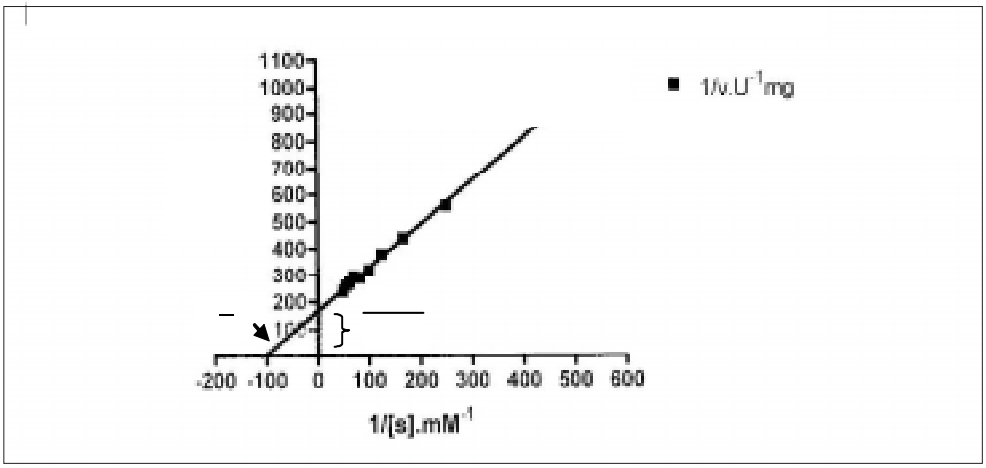
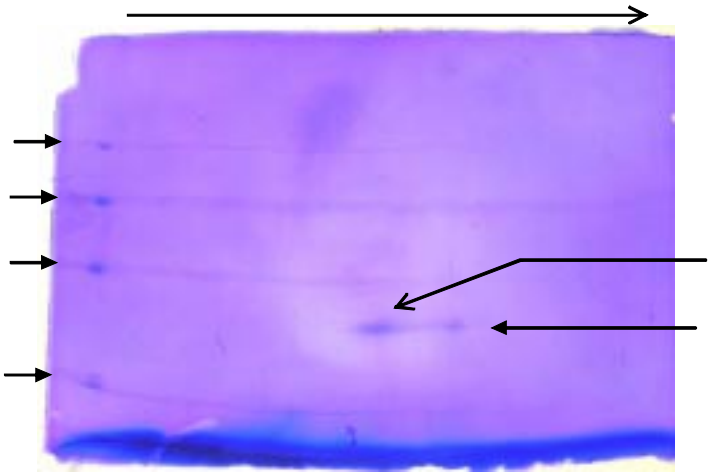


Figure 3: Lineweaver Burk Plot for Serine protease using azocasein substrate

Note: Lineweaver Burk plot was plotted using Graph Pad Prism Softwre



ES-31 antigen applied	:	100 µg
Molecular weight marker range	:	14-100 kDa
Phosphorylase b	:	97.4 kDa
Bovine serum albumin	:	66 kDa
Ovalbumin	:	43 kDa
Soyabean trypsin inhibitor	:	20.1 kDa

Figure 4: Two dimensional Poly-acrylamide Gel Electrophoresis (2D PAGE) of SEVA TB ES-31 antigen

(pH 6.8), 10% SDS, 5.0% β-mercaptoethanol, and 10% glycerol and then applied to 10 % SDS-PAGE for the electrophoresis of second dimension. The gel was stained with Coomassie stain.
Molecular weight marker (Banglore Genei): 14 – 100 kDa
Phosphorylase b (97.4 kDa), Bovine serum albumin (66 kDa), Ovalbumin (43 kDa) and Soyabean trypsin inhibitor (20.1 kDa) were used.

LCMS-T analysis of SEVA ES-31A antigen

The predominant 2D-PAGE gel spot (ES-31A) from ES-31 antigen was analyzed by Liquid Chromatography- Mass-spectometry (LCMS-T) at The Centre for Genomic Application (TCGA), New Delhi for peptide sequencing.

RESULTS

SEVA TB ES-31 antigen with protease activity was purified from *M tb* H_{37}Ra culture filtrate antigen by different steps namely ammonium sulphate precipitation, SDS-PAGE fractionation and FPLC as reported earlier[10]. In this study, ideal optimum substrate concentration, purification fold and yield of enzyme were studied in detail.

Different purification steps gave 3096 fold purification of mycobacterial serine protease with 26% yield and specific activity of 6250 x 10^{-6} U/mg protein (Table 1). Amongst protease substrates tested, maximum catalytic activity was observed with azocasein. The substrate preference was in the order azocasein > azocoll> BSA> HSA (Table 2). The enzyme showed optimum pH of 7.0 and incubation temperature at 37 ^{0} C (Figures 1 and 2). The activity of serine protease at optimum pH and temperature was assayed using different concentrations of azocasein (5 mg/ml - 50 mg/ml) and the reciprocal of the enzyme activity was plotted versus reciprocal of substrate concentration. A Km value for azocasein was found to be 0.01mM (Figure 3).

Further, 2D PAGE analysis of ES-31 antigen showed two protein spots (A and B) (Figure 4). LCMS-T analysis of predominant gel spot (ES-31A) has shown two conserved amino acid sequences K.AEIEGEIGDSHMGLAAR.M and K.TCAFIDAEHALDPIYAK.K (Mascot database search). On blasting the two conserved amino acid sequences of SEVA TB ES-31 antigen, the sequence showed match with *Rec A* (recombinase) protein of *M. tuberculosis* H_{37}*Ra , M. tb* H_{37}*Rv, M. tb bovis BCG strain, M. tb CDC1551, M. tb strain C, M. tb Harleem, and M. tb F11 strain.* However, sequences didn't show homology with known mycobacterial serine proteases.

DISCUSSION

Mycobacterium tuberculosis, the primary etiologic agent of tuberculosis, is the world's leading cause of death and hence, new vaccines and drugs to combat the disease are urgently needed. Extracellular proteins of *M. tuberculosis* figure prominently in new vaccine and drug development[13]. Extra cellular proteins of *M. tuberculosis* are proteins that are secreted or otherwise released by the bacterium into its extra cellular milieu. In comparison to the proteins of other cellular fraction of *M. tuberculosis* (cell wall, cell membrane and cytoplasm), the culture filtrate proteins are being studied in terms of function, immunogenecity and composition. Many of these proteins have been found to have enzyme activity, including the 58-kDa protein, glutamine synthetase [14] the 23-kDa protein, superoxide dismutase [15] and the 30- and 32-kDa complex of proteins, mycolyl transferase[16]. Markaryan et al[17] reported secretion of zinc metalloprotease in Aspergillosis infection which possibly aids the pathogen to invade the host. Most of the bacterial enzymes and proteases are likely to contribute in host pathogenecity and may be potential drug targets. However, biology and function of most of the mycobacterial proteases is still not clear. Several studies have demonstrated that glycosylated proteins are key aspects of the immunogenecity and pathogenecity of the host organism.[18-19] Earlier studies from our laboratory showed that ES-31, a glycoprotein antigen showing metallo-serine protease activity was found to be promising in the diagnosis of pulmonary tuberculosis (TB), some forms of extrapulmonary TB like TB Lymphadenopathy, TB meningitis and in HIV-TB co-infection.[2,3] The

presence of 31 kDa antigen has also been shown *in vivo*, with identical epitope of *in vitro* released ES-31 antigen and was found to be useful as an immunological marker for confirming active TB infection.[20] Based on the inhibitory studies on secretory glutamine synthetase by pathogenic mycobacteria, *Harth et al* proposed that extracellular enzymes released by a bacterium may be an additional site for drug targeting.[21] In our laboratory, the specific serine and metallo protease inhibitors inhibited growth of mycobacteria that secretes a serine protease in culture showing importance of serine protease for mycobacterial growth (unpublished observations). There is a need to focus on the identification and characterization of specific mycobacterial proteases to understand their biological role and importance in mycobacterial cell growth if any. In the present study, an effort has been made to purify SEVA TB ES-31 antigen, a metallo-serine protease of interest in serodiagnosis of TB and study its kinetics.SEVA ES-31 antigen was purified 3096 fold from *M tb* H_{37}Ra culture filtrate antigen by ammonium sulphate precipitation, SDS-PAGE fractionation and FPLC (Table 1). Amongst protease substrates tested, maximum catalytic activity was observed with azocasein and the Km value was 0.01mM (Fig 3). The enzyme showed optimum pH of 7.0 and incubation temperature of 37 ^{0} C (Fig 1 and 2). ES-31 antigen was further resolved by 2D-PAGE into two protein spots, *M tb* 31 A (predominant band) and M tb 31 B of molecular weight 31 kDa (fig 4). LCMS-T analysis of predominant ES-31 A protease showed two conserved amino acid sequences AEIEGEIGDSHMGLAAR.M and K. TCAFIDAEHALDPIYAK.K. On blasting these sequences with genomic database of H_{37}Ra and H_{37}Rv strains of mycobacteria, it doesn't show homology with known serine proteases. This needs further study as no serine protease is reported in the range of 31 kDa in TB genome. As far as we are aware, this is the first kinetic study of 31 kDa mycobacterial metallo-serine protease with strong immunogenic activity. Further, study on structure and biological role of this excretory secretory immunogenic serine protease will be of interest.

CONCLUSION

SEVA TB ES-31 protein, an immunogen with metallo serine protease activity from *Mycobacterium* tuberculosis bacilli has been purified about 3000 fold for the first time. The enzyme showed maximum activity with azocasein with a Km value of 0.01 mM. The enzyme showed optimum pH of 7.0 and incubation temperature at 37 ^{0} C. Further, study on structure and biological role of this excretory secretory immunogenic serine protease will be of interest.

ACKNOWLEDGEMENTS

This study was supported by a Tropical Disease Research grant from the Kasturba Health Society, Sevagram. We thank Shri. Dhiru S. Mehta, President, KHS, Dr. (Ms.) S. Chhabra, Dean, MGIMS for their keen interest and encouragement for this study. The technical assistance of Mrs. S. Ingole is appreciated.

REFERENCES

1. Goldman RC and Laughon BE. Tuberculosis drugs and drug targets. *Infectious disorders –drug targets*. 2007; 7 **(2)** :71-72.
2. Cole ST et al.Deciphering the biology of *Mycobacterium tuberculosis* from the complete genome sequence. *Nature* 1998; **393**:537-544.
3. Nair ER, Banerjee S, Gupta S, Kumar S, Reddy MVR and Harinath BC. Seroreactivity and characterization of 31 kDa *M.tb*. secretory protease of diagnostic interest in tuberculosis. *Trends Clin Biochem Lab Med* 2003; 330-338.
4. Shende N, Gupta S, Bhatia AS, Kumar S and Harinath BC. Detection of free and immune-complexed serine protease and its antibody in TB patients with and without HIV co-infection. *Int J Tuberc Lung Dis* 2005; **9(8):** 915-919.
5. Brown GD, Dave JA, Gey van Pittius NC, Stevens L, Ehlers MRW and Beyers AD. The mycosins of *Mycobacterium tuberculosis* H_{37}Rv: a family of subtilicin-like serine proteases. *Gene* 2000; **254**: 147-155.
6. Dave JA, Pittius NCG, Beyers AD, Ehlers MRW and Brown GD. Mycosin-1, a subtilisin-like serine protease of *Mycobacterium tuberculosis*, is cell wall-associated and expressed during infection of macrophages. *BMC Microbiol* 2002; **2**: 1-8.
7. Skeiky YA, Lodes MJ, Guderian JA, Mohamath R, Bement T, Alderson MR and Reed SG. Cloning, expression, and immunological evaluation of two putative secreted serine

protease antigens of *Mycobacterium tuberculosis. Infect Immun* 1999; **67(8):** 3998-4007.

8. Cameron RM, Stevenson K, Inglis NF, Klausen J and Sharp JM. Identification and characterization of a putative serine protease expressed *in vivo* by Mycobacterium avium subsp. paratuberculosis. *Microbiology* 1994;**140** (Pt 8):1977-82.
9. Pramanik J, Lodam AN, Reddy MVR, Narang P and Harinath BC. Increased yield of excretory secretory antigen with thyroxine supplement in *in vitro* culture of tubercle bacilli. *Indian J Tuberc* 1997; **44**:185– 190.
10. Nair ER, Banerjee S, Kumar S, Reddy MVR. and Harinath BC. Purification and characterization of a 31kDa mycobacterial excretory – secretory antigenic protein with a diagnostic potential in pulmonary tuberculosis. *Ind J Chest Dis Allied Sci* 2001; **43**: 81– 90.
11. SSAZOC03 - revised 10/14/98, Suitability Assay for Azocasein as a substrate for Trypsin http://www.sigmaaldrich.com/img/assets/18160/Azocasein_substrate_trypsin.pdf. The date last assessed 16-01-2007
12. O'Farrell PH. High resolution two-dimensional electrophoresis of proteins. *J Biol Chem* 1975; **250**: 4007-4021.
13. Harth G, Lee BY, Horwitz MA. High-level heterologous expression and secretion in rapidly growing nonpathogenic mycobacteria of four major *Mycobacterium tuberculosis* extracellular proteins considered to be leading vaccine candidates and drug targets. *Infect Immun* 1997; **65(6):** 2321– 2328.
14. Harth G, Clemens DL and Horwitz MA. Glutamine synthetase of *Mycobacterium tuberculosis*: extracellular release and characterization of its enzymatic activity. *Proc Natl Acad Sci USA* 1994; **91**:9342–9346.
15. Zhang Y, Lathigra R, Garbe T, Catty D, and Young D. Genetic analysis of superoxide dismutase, the 23 kilodalton antigen of *Mycobacterium tuberculosis*. *Mol Microbiol* 1991; **5**: 381–391.
16. Belisle JT, Sievert T, Takayama K and Besra GS. Identification of a mycolyltransferase from *Mycobacterium tuberculosis*: extracellular release and characterization of its enzymatic activity. 1995 p. 212–216. *In:* Programme of the 30th Joint Conference on Tuberculosis and Leprosy 1995. U.S.-Japan Cooperative Medical Science Program, National Institute of Allergy and Infectious Diseases, National Institute of Health, Ft. Collins, Colo.
17. Markaryan A, Morozova I, Yu H. and Kolattukudy P. Purification and characterization of an elastinolytic metalloprotease of *Aspergillus fumigatus* and immunoelectron microscopic evidence of this enzyme by the fungus invading the murine lung. *Infect Immun* 1994; **62(6):** 2149-2157.
18. Ishioka GY, Lamont AG, Thomson D, Bulow A, Sette FCA, Grey HM. MHC interaction and T-cell recognition of carbohydrate and glycoproteins. *J Immunol* 1992; **148**:2446-51.
19. Virji M, Saunders Jr, Sims G, Makepeace K, Maskell D, Ferguson DJP. Pilus- facilitated adherence of Neisseria meningitis to human epithelial and endothelial cells: Modulation of adherence phenotype occurs concurrently with changes in primary amino acid sequence and the glycosylation status of pilin. *Mol Microbiol* 1993; **10**: 1013-28.
20. Nair ER, Banerjee S, Kumar S and Harinath BC. Isolation and characterization of a 31 kDa mycobacterial antigen from tuberculous sera and its identification with *in vitro* released culture filtrate antigen of *M.tb*. H_{37}Ra bacilli. *Scand J Infect Dis* 2000; **32**: 551-556.
21. Harth G, Lee BY, Horwitz MA. An inhibitor of exported *Mycobacterium tuberculosis* Glutamine synthetase selectively blocks the growth of pathogenic mycobacteria in axenic culture and in human monocytes:extracellular proteins as potential novel drug targets. *J Exp Med* 1999; **189(9)**: 1425-1435.

The Editor-in-Chief and the members of the Editorial Board of the *Indian Journal of Tuberculosis* wish you all a very Happy and Prosperous New Year 2009

M.M. SINGH
EDITOR

PROGRESS TOWARDS MILLENNIUM DEVELOPMENT GOALS FOR TB CONTROL IN SEVEN ASIAN COUNTRIES*

V. K. Chadha**

Tuberculosis (TB) is known to afflict mankind since ancient times. With the advent of chemotherapy during 1940s and establishment of standardized short course treatment regimen in 1970s and 1980s, it was believed that TB would surely be conquered soon. Steady declines in case notifications were observed in most of the developed countries from 1950s to 1980s[1]. While no such declines were seen in most developing countries, a reversal of the declining trend occurred in the developed world during late 1980s[1]. New challenges emerged in the form of HIV epidemic and evolution of multi-drug resistant (MDR) TB. Consequently, TB was declared a global emergency by the World Health Assembly (WHA) in 1991 and a frame work for TB control was developed in the form of DOTS (the internationally recommended strategy for TB control)[2]. Within the framework of DOTS, annual targets of 70% case detection of new smear positive Pulmonary TB (PTB) cases and 85% treatment success were set to be achieved by the year 2000, which were later revised to 2005. The principle target of United Nations' ***Millennium Development Goals (MDGs)*** for TB control adopted in the year 2000 is to ensure that the incidence rate of TB is declining by 2015[3]. The supplementary targets are to halve the prevalence of TB and TB mortality rates by 2015 as compared to 1990[4]. The ultimate goal is to eliminate TB by 2050, when the annual incidence should be less than one case per million population[4].

To control TB, the DOT strategy was introduced in late 1990s in all the seven countries of the region - India, Bangladesh, Pakistan, Myanmar, Nepal, Sri Lanka and Bhutan. Population coverage with DOTS was expanded in a phased manner to 100% in all countries of the region by the year 2006[5]. There have been steady improvements in case notification and case detection rates with cure rates consistently in excess of 80%[5]. Efficient logistics and surveillance systems have been put in place and large strides made in creating a large pool of manpower trained in TB control. Efforts are being made to implement all other components of the Stop TB Strategy. In some countries like India and Myanmar, good progress has been made in collaborative TB/HIV activities and there are ambitious plans to scale-up the availability of culture and drug sensitivity testing and provision of second line drugs to multi-drug resistant (MDR) TB cases. Preparations are under way for introduction of these activities in other countries.

It is time now to assess whether the immense efforts of about 15 years are making impact on TB situation in the community and whether we are making enough progress towards the mid-term goals of TB control set to be achieved by the year 2015.

Measuring progress towards MDGs

There epidemiological indicators for measuring the progress towards MDGs are:-

i. Prevalence of TB disease

It is a product of incidence and duration of illness. A good chemotherapy programme reduces the average duration of illness. Therefore, in the presence of an efficient TB control programme, prevalence of disease declines more rapidly than incidence.

---

*TAI Oration delivered at the First International Conference of South East Asia Region of the Union and 63rd National Conference of Tuberculosis and Chest Diseases (SEAR-NATCON 2008) – New Delhi – September, 8-10, 2008

**Sr. Epidemiologist, National Tuberculosis Institute, No. 8, Bellary Road, Bangalore-560003; email: vin_chadha@yahoo.com; vin_chadha@rediffmail.com; Phones: 080-23441192 (office); 080-23620942 (Residence) 09916493109 (Mobile)

ii. Incidence of TB disease

It is the most important indicator of progress towards MDGs since it reflects the number of people who become victims to the disease. However, it changes slowly even in presence of efficient TB control activities

iii. TB specific Mortality rate

Mortality rates also change quickly in response to efficient TB control activities. Direct estimation of TB mortality rates in the community is only practicable through an efficient system of vital registration or through verbal autopsy studies.

Annual Risk of TB Infection (ARTI) which is another epidemiological indicators of TB has been extensively used for the study of epidemiological trends of TB, due to its practicability. Parallel trends in prevalence of TB disease and ARTI have also been observed in many countries. With all its limitations, ARTI remains an important tool to monitor epidemiological trends of TB.

Review of epidemiological data

All available epidemiological data is reviewed here under to derive possible inferences on the epidemiological trends of TB in the seven countries of our region.

I. India

Prevalence of TB

First reliable information on the magnitude of TB in India came forth in 1955-58 when a nation-wide survey was carried out by Indian Council of Medical Research to estimate the prevalence of Pulmonary TB (PTB). About 3,00,000 individuals $\geq$ 5 years of age residing in a sample of 150 villages, six cities and 30 towns in and around Calcutta, Delhi, Hyderabad, Madanapalle, Patna and Trivandrum were screened using mobile Mass Miniature Radiography (MMR) of the chest and one sputum specimen was collected from eligible individuals, for examination by smear and culture for Acid Fast Bacilli (AFB). The survey findings revealed the average prevalence of ***bacillary PTB*** (sputum positive for AFB, either on microscopy or culture, irrespective of MMR report) in the country at 400 per 1,00,000 populations[6]. It was found to be similar in rural and urban areas. Considering the country's population at that time, about 80% of the cases lived in villages. These findings were of great value in formulating National TB Program (NTP), which was launched all over the country in the year 1962.

Subsequently, several surveys to estimate the prevalence of PTB were carried out in defined geographical areas of the country, selected by the respective investigators on the basis of operational feasibility (Table 1). The age group of the study population varied from >4 years to >14 years. Most of the investigators used either MMR or symptom elicitation for screening while others used both. Two sputum specimens were collected from each eligible individual, during all the surveys. While some investigators examined sputum specimens either by culture or smear, others performed both. Similarly, there were variations in the methods used for smear microscopy (Ziehl-Neelsen technique / flourescent microscopy). With all these variations, the estimated prevalence of bacillary PTB varied between 180-1270 per 100,000 populations in different areas and at different points of time[7-24].

Nevertheless, serial surveys in seven different areas of the country over different time periods provided opportunities to evaluate the impact of TB control programs on prevalence of PTB in the community (Table 2). For the study of trends in these areas, prevalence estimates at subsequent surveys were standardized by age and sex to the study population at the baseline surveys.

Three surveys in Madnappalle during 1960-68 and two in Tumkur district during 1960-1973 revealed increases in prevalence of bacillary PTB, which were not statistically significant[14,15]. However, the increase in prevalence observed in Car Nicobar Islands between two surveys carried out 15 years apart in 1986 and 2001, was statistically significant[23]. Eight serial surveys in an urban area in Delhi during 1962-1991 and seven

Table 1: Prevalence of PTB per 1,00,000 populations

(a) Single survey in selected areas

State & District	Study period	Age (yrs)	Sample Size	Screening Method	Prevalence of PTB cases/ 100,000 population		
					Culture + ve	Smear + ve	culture and/or smear + ve
National Sample Survey[5]	1955–1958	>4	290 758	MMR	-	-	400
Srinagar, Baramulla & Anantnag (J & K)[7]	1978	>4	14226	MMR	-	-	280
North Arcot - KV Kuppam Block (T.N)[8]	1981	>9	18 688	Symptoms	-	241	-
North Arcot—plains (T.N)[9]	1989	>14	a) 64 077 b) 25 485	a) Symptoms b) MMR +symptoms	-	-	a) 430 b) 790
North Arcot—tribal (T.N)[10]	1889	>14	16 017	MMR +symptoms	520	470	840
Wardha (Mah.)[11]	1982–1988	>4	687 401	Symptoms	71	80	182
Raichur (Kar.)[12]	1988–1989	>14	40 496	Symptoms	-	760	1090
Morena (M. P)[13]	1991–1992	>14	11 097	Symptoms	-	-	1270

J& K: Jammu & Kashmir, T.N: Tamil Nadu, Mah.: Maharashtra, Kar.: Karnataka, M.P: Madhya Pradesh
-: Not available

Table 1: Prevalence of PTB per 1,00,000 populations

(b) Serial surveys in selected areas

State & District	Study period	Age (yrs)	Sample Size	Screening Method	Prevalence of PTB cases/ 100 000 population		
					Culture + ve	Smear + ve	culture and/or smear + ve
Tumkur (Kar.)[14]	1960-1961	>9	21 021 24785	MMR	314 399	281 262	410 444
Madanappalle (A.P)[15]	1960–1961 1962–1964 1965–1968	>14	46 888 46 296 84 486	MMR	-	-	681 501 483
Delhi[16]	1962 1964 1967 1969 1972 1976 1982 1991	>4	21 344 22 621 24 803 24 808 26 132 26 686 25 583 27 838	MMR	-	-	400 700 700 210 280 320 360 330
Bangalore rural[17-20] (Kar.)	1960–1961 1962–1963 1964–1965 1966–1968 1974–1975 1984–1986	>4	43 889 40 633 40 405 41 213 15 243 21 924	MMR	406 372 337 393 320 438	-	-
Chingleput (T. N)[21-22]	1968–1970 1971–1973 1973–1975 1976–1978 1979–1981 1981–1983 1984–1986	>9	206 609 101 331 104 789 112 142 115 164 120 118 121 877	MMR	800 819 868 815 759 767 689	570 476 496 406 436 471 423	1070 -
Car Nicobar (A & N)[23]	1986 2000–2001	>14	9514 10 570	Symptoms	-	410 729	-
Thiruvallur (T. N)[24]	1999–2001 2001-2003 2004-2006	>14	83 425 85474 89413	MMR + symptoms	609 451 311	326 257 169	

Kar.: Karnataka, A.P: Andhra Pradesh, T.N: Tamil Nadu, A &N: Andaman & Nicobar; -: Not available

surveys each in selected areas of Rural Bangalore and Chingleput districts during 1960-1989 and 1968-1986 respectively revealed minimal declines in prevalence of bacillary PTB, which were not statistically significant[16,22].

The above mentioned serial surveys were carried out before the implementation of Revised National TB Control Programme (RNTCP) adopting DOTS. After the introduction of RNTCP, three surveys carried out in Thiruvallur between 1999 and 2005 revealed statistically significant decline in prevalence of bacillary PTB - 12.6% per year in prevalence of culture positive PTB and 12.3% per year in prevalence of smear positive PTB[24]. During this period, the estimated average case detection rates of new sputum smear positive cases of PTB in this area was 86% and their treatment success was 77%. On the other hand, statistically non-significant declines of about 2.5% per year had been observed during 1968-1986 in the same areas.

Diagnosis of bacteriologically negative PTB (sputum negative for AFB on microscopy and culture but X-ray suggestive of PTB) requires follow-up of X-ray suspects with a course of antibiotics which was not followed in these surveys. Therefore, the estimated prevalence of bacteriologically negative PTB at 1.5-4 times the prevalence of bacillary PTB during different surveys should be viewed with caution.

Incidence of TB

Direct estimates of incidence of PTB have been made during a limited number of community based studies (table 2).

In rural Bangalore, during the 1960-1968 four serial surveys, new cases of culture positive PTB were identified at the subsequent surveys[17]. Uneven trends in disease incidence did not permit any interpretation regarding its trends (Table 3). Moreover, many new cases occurring in the

Table 2: Annual incidence rate of culture /smear positive PTB per 1,00,000 population in selected areas

Study Area	Study Period	Age group (in Years)	Sample Size	Incidence rate /100,000 population	
				Culture +ve PTB	Smear +ve PTB
Bangalore Rural[17]	1961-62	>4	34 476	132	-
	1962-64		32 194	79	
	1964-68		31 381	99	
Chingleput[21,22]	1971-73	>9	213 307	352	157
	1973-75		38 461	250	142
	1976-78		44 091	251	106
	1979-81		50 794	207	104
	1981-83		56 067	209	127
	1984-86		65 826	189	113
North Arcot[8]	1981-82	>9	19 129	-	110
	1982-83		19 570		
Thiruvallur[25]	2001-03	>14	60009	-	126

Years indicate period of survey -: Not available

intervening period were missed as a result of cure or death.

In Chingleput, in addition to serial surveys every 2.5 years during 1968-86, selective case finding and passive case detection were also undertaken[21,22]. Selective case finding was undertaken every 10 months among individuals with any abnormal pulmonary shadow on X-ray or pulmonary symptoms at the previous survey and also among those absent at the previous survey. Passive case detection was undertaken among persons reporting with pulmonary symptoms at the health centers in the area during the period intervening between two surveys. During different rounds of these surveys, incidence of culture positive PTB varied between 189-352 per 100 000 populations[21,22]. The incidence of smear positive PTB varied from 113-157 per 100 000 populations[21,22]. The observed declines of 4.3% and 2.3% per year respectively in the incidence of Culture positive PTB and smear positive PTB during this period, were statistically significant.

Table 3: TB disease prevalence surveys in other countries

	Area	Survey period	Age[?]	Sample Size	Screening Method	Prevalence of PTB cases/ 1,00,000 population	
						Smear +ve	Culture &/or smear + ve
Bangladesh	National level	1964-66[45]	>9	24590	Symptoms[$]	180	
	National level	1987-88[46]	>14	25102	Symptoms*	870	
	74 Upzillas[48]	2001	>11	266189	Symptoms**	24	
	Matlab[49] (a rural area)	2004-05	>14	59395	Symptoms**	95	
Pakistan	Karachi, Rawalpindi & Lahore divisions[50]	1961-62	>9	6456	No screening[$]	186	
	National level[51]	1987	>14	22457	Symptoms*	174	
Myanmar	National level[52]	1972	>14	35206	Symptoms*		183
	National level	2006[#]					
Sri Lanka	National level[53]	1970-71	>14	27500	Symptoms+ MMR		234

[?] Age in years, [$]1 sputum specimen collected from all eligible, *2 sputum specimen; ** 3 sputum specimen;
[#]Report not yet available

In North Arcot, incidence of smear positive TB was estimated at 110 per 100 000 population during 1981-83[8].

In Thiruvallur, incidence of smear positive cases among adults, between two surveys carried out between 1999 and 2003 was 126 per 100,000 populations[25]. Passive case finding at the health centers was also included in this estimation.

Ratio of prevalence to incidence of PTB can also provide important clues about the efficiency of TB control programs. A poorly functioning program increases this ratio by increasing the duration of illness due to diagnostic delay and failure of treatment in a significant proportion of cases. A well functioning programme, on the other hand, reduces this ratio by decreasing duration of the disease. During the pre-chemotherapy era, the average duration of infectiousness of a smear positive case was found to be about two years and therefore the ratio of prevalence to incidence was observed as 2:1. In Chingleput, this ratio was observed to increase from 2.5 to 3.9 for culture positive cases and from 2.5 to 4.2 for smear positive cases during 1968-86[22]. This increase signified further accumulation of old cases due to poor treatment completion rates besides low case finding efficiency under the NTP. However, this ratio was observed as 2:1 in Thiruvallur during 2004 in the period of RNTCP implementation[24,25].

Annual Risk of TB Infection

A number of tuberculin surveys were carried out in different parts of the country during the later half of last century, during which period ARTI rates were found to vary between 1-2%[26-33]. Serial surveys were carried out in rural Bangalore, Dodaballapur and Chingleput. While an increase in ARTI was observed in Dodaballapur during 1974-79 and in Chingleput during 1969-84, an average annual decline of 3% per year was observed in rural Bangalore during 1961-84.

Table 4: Tuberculin surveys in other countries

	Area	Year	Sample size	Age group (Years)	Estimated ARTI
Bangladesh	National Level[45]	1964-66	4971	5-9	0.6%(5-9 years) 1.2% (10-14 years)
Pakistan	Selected urban and rural areas	1949-54		7-14	5.2%
	Karachi, Rawalpindi, Lahore divisions[50]	1961-62	622	0-9	2.8%
	National Level[51]	1974-78	4425	0-9	1.1%
Myanmar	National Level[52]	1972			1.2%
Nepal	sub-national surveys in 17 selected areas[54]	1985-95		6-10 years	2.1%*
	National Level[54]	2006-07		5-7	0.9%
Sri Lanka	National Level[53]	1970-71	1160	5-9	1.8%
Bhutan[55]	Thimphu, Mongar and Bumthang dsitricts	1991	1736	6-14	1.9%

*National level estimate for 1994 on compiling data from all areas.

Since nearly all the earlier surveys were carried out in limited geographical areas in South India, a nation–wide tuberculin survey was carried out during 2000-2003. For this purpose, the country was divided into four zones, each comprising 25% of the country's population. The objective was to estimate the average ARTI in each of the zones. The estimated ARTI rates were: South zone-1%, East zone-1.3%, West zone-1.6%, North zone-1.9% [34-37]. In all the zones, higher levels of transmission of infection were observed in urban areas compared to the rural.

Recently, state level surveys have been carried out in Orissa, Andhra Pradesh and Kerala. ARTI in Orissa and Andhra Pradesh were estimated at 1.7% and 1.5% respectively[38,39]. In Kerala, data suggested a low rate of transmission of infection, even though ARTI could not be estimated due to the absence of identifiable cut-off points (anti-mode, mode) on the frequency distribution of reaction sizes[40].

During the period of RNTCP implementation, estimated ARTI rates during three rounds of surveys in Thiruvallur during 1999-2005, varied between 1.2 -1.6%. There was an average decline of 6% per year during this period[41].

Trends are also available from Bangalore city and peri-urban villages of Rural Bangalore from the period overlapping the pre- RNTCP and RNTCP period. RNTCP was introduced in Bangalore districts during 1997 and full population coverage was achieved in 2001. Average yearly decline of 4% was observed in Bangalore city between two surveys carried out in 1998 and 2006[42]. In peri-urban villages of Bangalore district, an annual decline of 3% was observed between two surveys carried out in 1992 and 2006[43].

TB-specific mortality rates

Annual TB-specific mortality rate in rural Bangalore was estimated at 80 per 100 000, when study population was followed-up during the serial surveys from 1960 to 1968[44].

Verbal autopsy based studies to estimate TB-mortality rate have been undertaken in two states namely Andhra Pradesh and Orissa under the leadership of Tuberculin Research Centre, Chennai. The results are in the process of finalisation.

II. Other countries

Prevalence of TB disease

Four other countries in the region have carried out surveys to estimate prevalence of PTB (Table 3) [45-53].

In Bangladesh, two national level surveys have been carried out in 1964-66 and 1987-88[45-47]. During the first survey, one sputum specimens were collected from persons with pulmonary symptoms while only two specimens were collected during the second survey. The age-groups of the study population also differed. Consequently, the results of the two surveys were vastly different and not comparable.

A national level survey was also carried out in Pakistan during 1987 and the prevalence of smear positive PTB was estimated at 174 per 100 000 populations[51].

In Myanmar, 2 national surveys have been carried out in 1972 and 2006 respectively[52]. The results of the second survey are awaited and shall throw light on the epidemiological trend of TB in Myanmar.

A classical survey employing all available tools was carried out in Sri Lanka during 1970-71 when the prevalence of culture positive PTB was estimated at 234 per 100 000 population[53].

Annual risk of TB infection

One national level survey has been carried out in most countries and the latest available estimates in these countries varied between 1-1.8%[45,50-54] (Table 4).

In Nepal, a number of sub-national surveys were also carried out during 1985-95, from which the national level estimate of ARTI was derived for 1994[54]. However, this was not comparable to the ARTI estimates from the national survey in 2006, since most of the earlier sub-national surveys were carried out in areas with high expected rates of transmission of infection. Therefore, trends in ARTI are not available from any of these countries.

In Bhutan, a tuberculin survey was carried out during the year 1991 in a sample of easily accessible schools of 3 districts. The ARTI was estimated at 1.9%.

No studies have been carried out on incidence of TB or TB-mortality in any of these countries.

Summary of epidemiological data

The above review suggests that the data on epidemiological trends of TB has been available from a limited number of areas in India. In the pre-DOTS period, either there were no declines in the estimates of various epidemiological parameters or the declines were minimal. The significant decline in prevalence of PTB in Thiruvallur during the DOTS-period is on expected lines since prevalence should change quickly in response to efficient TB control activities. However, the direction and scale of change in other areas of the country would depend on the initial magnitude of disease burden and efficiency of control activities. Significant changes in risks of infection have been observed from two areas in south India during the period of RNTCP implementation. However, no comment whatsoever can be made about the epidemiological trends in other countries.

Trends of TB based on epidemiological models

In the absence of sufficient data from epidemiological surveys, mathematical models have been developed by Corbett et, al[56]. The World Health Organization, (WHO) uses these models to estimate prevalence of TB, incidence of TB and TB specific mortality rates for each country[5,57]. The estimates are revised every year, using new information generated through epidemiological studies in the community and routine surveillance data under NTPs. The estimates of incidence thus obtained also provide the basis for estimating case detection rates under NTPs of individual countries.

The estimates of incidence are generally derived as under :-

(i) Numbers of new cases reported under NTP are divided by the estimated fraction of all new cases occurring in the community that have been reported.

(ii) Application of Styblo's equation: According to this equation, every one per cent of ARTI estimated in children corresponds to an annual incidence of 50 sputum smear-positive cases of PTB per 100 000 population[58]. Incidence of smear negative PTB and extra-pulmonary TB is estimated based on the published data on proportion of cases that are smear positive in each age group.

The estimate of prevalence of TB is derived on multiplying the estimated incidence of TB cases by their average duration. Average duration is collated after estimating the durations of all types of TB cases by HIV status treated under DOTS and outside DOTS programs and those untreated. All available information on delay in diagnosis, case detection and outcome of treatment among cases treated is used for this purpose.

The estimate of TB-specific annual mortality rate is derived on multiplying the estimated incidence of TB cases by their average case fatality rate. Average case fatality rate is collated after estimating the case fatality rates among all types of TB cases by HIV status treated under DOTS and outside DOTS programs and those untreated.

These modeling exercises have revealed the following trends :-

i ***Prevalence of TB***: Compared to 1990 levels, prevalence of TB (all cases) declined in all the seven countries of the region[5]. The decline by the year 2006 varied from a minimum of 27% in Sri Lanka to 74% in Bhutan. In India, this decline was 48%[5].

ii ***TB-specific mortality rate***: TB-mortality rates also declined in all countries ranging from 20% in Sri Lanka to 70% in Myanmar[5]. In India, this decline was 31%.

iii ***Incidence of PTB***: Except Nepal and Bhutan, no reduction in incidence of smear positive PTB cases is estimated to have occurred in other countries[5]. Perhaps, it is too early to expect the incidence to fall, since most of the TB incidence today is a consequence of infections acquired long ago when the rates of transmission of infection and life time risk of developing disease would have been higher than at present.

The trends as above may be considered reliable since the estimates in different years have been derived using a uniform approach.

Present TB situation

About 24% of the world's population living in our region bears about 30% of the disease burden in terms of incidence of TB cases[5]. The absolute incidence of all TB cases for the year 2006 was estimated at 2.7 million (174 per 100 000), of which 1.2 million (78 per 100 000) were smear positive cases[5]. In India alone, about 1.8 million new episodes of disease accounted for one in every five cases in the world[5]. India is thus ranked first in the list of 22 High Burden Countries (HBCs)[5]. Bangladesh, Pakistan and Myanmar are other HBCs in the region ranked 6th, 8th and 19th respectively[5]. Sri Lanka and Bhutan were estimated to have the lowest rates of incidence and TB-mortality among the regional countries[5].

About 0.4 million (25 per 100 000 population) people died of TB in the region during 2006[5]. India alone contributed to about 20% of TB deaths globally[5]. Unfortunately, these rates translate into case fatality rates of about 15% suggesting introspection into implementation of program activities.

There are about one million people co-infected with HIV and tubercle bacilli in the region. India has the highest number of PLWHIVs after South Africa while HIV prevalence per capita is highest in Myanmar among the countries of the region. Higher incidence rates of TB cases among dually infected individuals also threaten the health of HIV negative individuals.

MDR-TB cases have been reported by all countries in the region. In six countries (except Sri Lanka), about 3% of all new TB cases are MDR. One case out of every five MDR-TB cases in the world occurs in India[5]. There is thus a considerable burden of MDR cases in the region.

Case detection and treatment success

All the countries either achieved or were close to achieving the target of 85% treatment success in the target year of 2005[57]. Overall, about 1.2 million estimated new smear positive cases emerged in 2005, of which about 0.7 million were detected[57]. Therefore, the overall case detection rate in the region was 60%. While Myanmar and Sri Lanka achieved the CDR targets of 70% in 2005, only Sri Lanka achieved both the targets in 2005[57]. Myanmar and Bhutan have achieved both the targets in 2006 and India in 2007[5,59]. Other three countries are close to achieving these targets. However, three countries of Indian sub-continent also account for 30% of the undetected cases in the world.

Prospects of achieving MDG targets

It is estimated that the impact targets of changes in prevalence and TB-mortality rates may be met in the region as a whole by 2015 provided the estimated rate of change between 2001- 2006 continue in future[5]. However, the expected decline in mortality may not be achieved in high HIV prevalence States of India according to another modeling exercise[60]. This underlines the role of anti-retroviral therapy (ART) to HIV-positive TB cases.

With roughly 30-40% of the population presently harboring tubercle bacilli, new TB cases shall continue to emerge in large numbers for a number of years to come. However, the rates of transmission of infection seem to be declining as a result of DOTS programs as also observed in many other countries. Therefore, once the cohorts of older people with higher prevalence of infection are replaced by cohorts of younger people with lower prevalence of infection, the incidence rates of TB disease are expected to start falling. Also, with the change in age at first infection, the life time risk of developing TB disease should change. Reduction in incidence is also crucial for further reductions in prevalence and mortality.

On the other hand, implications of HIV epidemic will depend on the extent to which it increases transmission of tuberculous infection. Any slackening in control efforts, rapid urbanization and MDR -TB shall also have opposing influences. These factors may reverse the biological balance in favour of the tubercle bacillus.

TB could eventually be eliminated by reducing the infection rates to zero. But since the incubation period of TB can be a life time, the zero infection rates will have to be maintained for at least three generations to eliminate all infected populations.

Monitoring epidemiological trends of TB in future

Control of TB would ultimately, depend upon multiple factors which interact with one another. It is, therefore, too complex to make predictions. Therein lies the importance of continued monitoring of the epidemiological trends of TB in the years to come.

Mathematical models are based on various assumptions like proportions of incidence TB cases that are reported by NTPs, trueness of Styblo's equation, error free reporting of treatment outcomes, proportion of cases treated outside DOTS programs or untreated, etc. Consequently, it is important to monitor the disease trends through community based epidemiological studies. WHO task force on TB impact measurement has recommended all HBCs to carry out two disease prevalence surveys between now and 2015. In case a survey has been carried out between 1990 and 2007, like in Myanmar, then one survey may be carried out in future[5].

Consequently, as stated above, India has planned serial surveys to estimate prevalence of TB in six more districts besides Thiruvellur- Rural Bangalore, Wardha (Maharashtra), Jabalpur (Madhya Pradesh), Banda (Uttar Pradesh), Mohali (Punjab) and Balabhgarh (Haryana). Presently, surveys to obtain baseline estimates of prevalence of bacillary PTB are in progress in these areas. It has also been planned to repeat the zonal tuberculin surveys. National level surveys to estimate prevalence of PTB have also been planned in Bangladesh and Myanmar and sub-national surveys have been planned in Pakistan. National level tuberculin surveys have been planned in Bangladesh, Pakistan, Bhutan and Sri Lanka.

While the community based epidemiological studies play an important role to monitor the epidemiological trends of TB in future, these are operationally cumbersome and cost prohibitive. Such studies may also become impracticable once disease burden declines to sufficiently low levels. Therefore, all efforts must be undertaken to strengthen the routine surveillance under NTPs as the ultimate tools to reliably reflect on disease trends. When most of the incidence cases get reported under NTPs, the trends in annual case reporting rates would reflect the trends in incidence provided the case finding efficiency remains stable over the years. The mean age of reported new cases

can also provide clues to the epidemiological trends of TB, provided the accessibility of services to different age groups is uniform. In presence of a well-functioning TB control program, the mean age of notified cases should increase over the years and vice versa. Strengthening of death certification and vital registration systems are also needed to monitor trends in TB-mortality rates on a long term and continual basis.

CONCLUSION

TB remains a major cause of ill health and death in the region accounting for 30% of the global burden of disease. Significant advances have been made in TB control and we are in a position to achieve the mid-term goals of halving prevalence of TB and TB-mortality by 2015. Whether we shall achieve the goal of reversing the trends in incidence of TB would depend upon how well we perform and face various challenges in the coming years.

REFERENCES

1. Rieder HL: Epidemiological basis of tuberculosis control. International Union against Tuberculosis & Lung Diseases 1999. Paris.
2. World Health Organization. Forty-fourth World Health Assembly, Resolutions and Decisions. Resolution WHA 44.8. Geneva: World Health Organization; 1991. Report No.: WHA44/1991/REC/1.
3. United Nations Statistics Division. Millennium Indicators Database. 2007 [cited 2007; Available from: mdgs.un.org/unsd/mdg/Default.asp
4. The Global Plan to Stop TB, 2006-2015. Stop TB Partnership and World Health Organization, 2006, WHO/ HTM/STB/2006.35.
5. World Health Organization, Geneva. WHO report 2008: Global Tuberculosis control – surveillance, planning, financing. WHO/HTM/TB/2008.393.
6. Indian Council of Medical Research: Tuberculosis in India: A National Sample Survey; ICMR special report series No.34, 1955-58. ICMR, New Delhi.
7. Mayurnath S, Anantharaman DS, Baily GVJ, Radhamani MP, Vallishayee RS, Venkataraman P, Tripathy SP. TB prevalence survey in Kashmir valley. *Indian J Med Res* 1984; **80**: 129-40.
8. Ray D, Abel R. Incidence of smear positive pulmonary TB from 1981-83 in a rural area under an active health care programme in South India. *Tubercle & Lung Dis* 1995; **76**: 190-5.
9. Dutta M, Gopi PG, Appegowda BN, Bhima Rao KR, Gopalan BN. Tuberculosis in North Arcot district of Tamil Nadu – A sample survey. *Indian J Tuberc* 2000; **47**: 147-154.
10. Datta M, Radhamani P, Sadacharam K, Selvaraj R, Satyanaryana R, Nagabushanrao RS. Survey for tuberculosis in a tribal population in North Arcot district. *Int J Tuberc Lung Dis* 2001; **5**:240-249.
11. Narang P, Nayar S, Mendiratta DK, Tyagi NK, Jajoo U. Smear and culture positive cases of pulmonary TB found among symptomatics surveyed in Wardha district. *Indian J Tuberc* 1992; **39**: 159-64.
12. Gopi PG, Vallishayee RS, Appegowda BN, Paramasivan CN, Ranganatha S, Venkataramu KV, et al. A Tuberculosis prevalence survey based on symptoms questioning and sputum examination. *Indian J Tuberc* 1997; **44**: 171-80.
13. Chakma T, Vinay Rao P, Pall S, Kaushal LS, Datta M, Tiwary PS. Survey of pulmonary TB in a primitive tribe of Madhya Pradesh. *Indian J Tuberc* 1996; **43**: 85-89.
14. Gothi GD, Chakraborty AK, Nair SS. Ganapathy KT, Banerjee GC: Prevalence of tuberculosis in a south Indian district twelve years after initial survey. *Indian J Tuberc* 1979; **26**: 121-135.
15. Moller FJ, Acharyulu GS, Kesava Pillai K. A controlled study of the effect of a domiciliary TB chemotherapy programme in a rural community in South India. *Indian J Med Res* 1981 (suppl); **73**: 1-80
16. New Delhi TB Centre. Study of epidemiology of Tuberculosis in an urban population of Delhi-report on 30 year follow up. *Indian J Tuberc* 1999; **46**: 133.
17. National Tuberculosis Institute, Bangalore. TB in a rural population of south India – A five year epidemiological study. *Bull World Health Organ* 1974; **51**: 473-488.
18. Gothi GD, Radha Narayan, Nair SS, Chakraborti AK, Srikantaramu N. Estimation of prevalence of bacillary TB on the basis of chest X-ray and symptomatic screening. *Indian J Med Res* 1976; **64**: 1150-59.
19. Chakraborty AK, Singh H, Srikanatan K, Rangaswamy KR, Krishna Murthy VV, Stephen JA. TB in a rural population of south India:report on five surveys. *Indian J Tuberc* 1982; **29**: 153-167.
20. Chakraborty AK, Suryanarayana HV, Krishna Murthy VV and Shashidhara AN: Prevalence of TB in a rural area by an alternative survey method without prior radiographic screening of the population; *Tubercle and Lung Dis* 1995; **76**: 20.
21. Tuberculosis Prevention Trial, Madras. Trial of BCG vaccines in south India for tuberculosis prevention. *Indian J Med Res* 1980 (suppl); **72**: 1-74.
22. Tuberculosis Research Centre, Chennai. Trends in the prevalence and incidence of tuberculosis in south India. India. *Int J Tuberc Lung Dis* 2001; **5**:142-157.
23. Muthrekar MV, Kolappan C, Gopi PG, Chakraborty AK, Sehgal SC. Tuberculosis situation among tribal population of Car Nicobar, India, 15 years after intensive tuberculosis control project and implementation of a national tuberculosis programme. *Bull World Health Organ* 2004; **82**:836-843.
24. Subramani R, Radhakrishna S, Frieden TR, Kolappan C, Gopi PG, Shanta T, et al. Rapid decline in prevalence of pulmonary tuberculosis after DOTS implementation in a rural area of South India. *Int J Tuberc Lung Dis* 2008; **12**:916-920.

25. Chakraborty AK, Chaudhuri K, Sreenivas TR, Krishnamurthy MS, Shashidhara AN, Channabasavaiah R. Tuberculous infection in a rural population of south India : 23-year trend. *Tubercle and Lung Dis* 1992; **73**: 213-218.
26. Gopi PG, Subramani R, Santha T, Kumaran PP, Kumaraswami V, Narayanan PR. Relationship of ARTI to incidence and prevalence of tuberculosis in a district of south India. *Int J Tuberc Lung Dis* 2006; **10**:115-117.
27. Chadha VK, Suryanarayana HV, Krishnamurthy MS, Jagannatha PS, Sashidhara AN. Prevalence of under-nutrition among peri-urban children and its influence on the estimation of annual risk of tuberculosis infection. *Indian J Tuberc* 1997; **44**: 67-71.
28. Chadha VK, Jagannatha PS, Shashidhar Savanur J. Annual risk of tuberculosis infection in Bangalore City. *Indian J Tuberc* 2001; **48**:63-71.
29. Kurthkoti AG, Hardan Singh. Changes in the prevalence rates of infection in younger age groups in a rural population of Bangalore district over a period of 5 years. *NTI Newsletter* 1985; **21**: 28.
30. Mayurnath S, Vallishayee RS, Radhamani MP, Prabhakar R. Prevalence study of tuberculosis infection over 15 years in a rural population in Chingleput district (South India). *Indian J Med Res* 1991; **93**:74-80.
31. Directorate of Health Services A and N Islands: Intensified tuberculosis control programme in the isolated tribal population of Car-Nicobar island, WHO - Government of India HSR project No. IND/HSR 001/D., A and N Administration Port Blair, 1989.
32. Kumari Indira KS, Sivaraman S, Joshi M, Sivanandan Pillai N. Annual risk of Tuberculosis infection: an estimate from ten year old children in Trivandrum district. *Indian J Tuberc* 2000; **47**:211-218.
33. Siddiqi D, Ghose S, Krishnamurthy MS, Shashidhara AN. Tuberculosis infection rate in a rural population of Bikaner district. *Indian J Tuberc* 1996; **43**:91-97.
34. Kolappan C, Gopi PG, Subramani R, Chadha V K, Kumar P, Prasad V et.al. Estimation of annual risk of tuberculous infection among children aged 1-9 years in the south zone of India. *Int J Tuberc Lung Dis* 2004. **8**:418-423.
35. Chadha VK, Vaidyanathan PS, Jagannatha PS, Unnikrishnan KP, Savanur SJ, Mini PA. Annual risk of tuberculous infection in the western zone of India. India. *Int J Tuberc Lung Dis* 2003; **7**:536-542.
36. Chadha VK, Vaidyanathan PS, Jagannatha PS, Unnikrishnan KP, Mini PA. Annual risk of tuberculous infection in the northern zone of India. *Bull World Health Organ* 2002; **81**:573-81.
37. Chadha VK, Kumar P, Gupta J, Jagannatha PS, Lakshminarayana, Magesh V, et al. Annual risk of tuberculous infection in the eastern zone of India. *Int J Tuberc Lung Dis* 2004; **8**:537-544.
38. Shashidhara AN, Chadha VK, Jagannatha PS, Ray TK, Mania RN. The annual risk of tuberculous infection in Orissa State, India. *Int J Tuberc Lung Dis.* 2004; **8** :545-51.
39. Chadha VK, Kumar P, Satyanaryana AVV, Gupta J. Annual Risk of Tuberculous infection in the State of Andhra Pradesh. *Indian J Tuberc* 2007; **54**:177-183.
40. Kumar S, Radhakrishna, Chadha VK, Jeetendra R, Kumar P, Chauhan LS, et al. Prevalence of tuberculus infection among school children in kerala. Under Publication.
41. Gopi PG, Subramani R, Narayanan PR. Tuberculosis Research Centre, Chennai, Trend in the prevalence of TB infection and ARTI after implementation of a DOTS programme in South India. *Int J Tuberc Lung Dis* 2006; **10 (3):** 346-348.
42. Chadha VK, Jitendra R, Kumar P, Shashidhara AN, Kirankumar R, Suganthi P, et.al. Change in the risk of tuberculous infection over a 8-year period among school children in Bangalore city. *Int J Tuberc Lung Dis*. Under Publication.
43. Singh S, Chadha VK, Gupta J, Magesh V, Lakshminaryana, Ahmed J, et al. Prevalence and risk of tuberculous infection among school children in Bangalore rural district. *NTI Bulletin* 2006, **42**:68-73.
44. Chakraborty AK, Gothi GD, Dwarkanath S, Singh H. Tuberculosis mortality rate in a south Indian rural population. *Indian J Tuberc* 1978; **25**:181-86
45. The tuberculosis survey in Bangladesh, 1964-66.National Tuberculosis control and research project, Government of the people's Republic of Bangladesh,Dacca.
46. Report of the National Prevalence survey on Tuberculosis in Bangladesh, 1987-88. Directorate General of Health Services, Office of the Director, Tuberculosis and Leprosy Control sections, Dhaka, Bangladesh,1989.
47. Begum V, Van der Werf MJ, Becx-Bleumink M, Borgdorff MW. Do we have enough data to estimate the current burden of tuberculosis? The example of Bangladesh. *Trop Med Int Health* 2007;**12(3):**317-22.
48. Hamid Salim MA, Declercq E, Van Deun A, Saki KAR. Gender differences in tuberculosis: a prevalence survey done in Bangladesh. *Int J Tuberc Lung Dis* 2004;**8(8):**952-957.
49. Zaman K, Yunus M, Arifeen SE, Baqui AH, Sack D.A, Hossain S, Rahim Z. et.al. Prevalence of sputum smear-positive tuberculosis in a rural area in Bangladesh. *Epidemiol Infec* 2006;**134(5):**1052-9.
50. Report of the Tuberculosis survey in Karachi, Rawalpindi and Lahore division of West Pakistan. Directorate of tuberculosis control (health revision, Govt. of Pakistan, 1962)
51. Report on National Tuberculosis prevalence survey 1987-88, the Directorate of Tuberculosis control, Govt. of Pakistan, Islamabad, 1989.
52. Tuberculosis Baseline survey, Burma 1972; Final Report.
53. Report on the National TB Survey of Ceylon, 2nd May -5th October 1956 by Dr. James Deeney. SEA/TB/8, Part I , 1957.
54. Shrestha KB, Malla P, Jha KK, Shakya T M, Akhtar M, Gunneberg C, et.al. First national tuberculin survey in Nepal. *Int J Tuberc Lung Dis* 2008; **12(8):**909–915
55. International Tuberculosis Surveillance Center. Skin sensitivity of human PPD, school children in the kingdom of Bhutan 1991. ITSC/20002/OM/MJH/23-01-1992.
56. Corbett EL, Watt CJ, Walker N, Maher D, Williams BG, Raviglione MC, et.al. The Growing Burden of

Tuberculosis –Global Trends and Interactions With the HIV Epidemic. *Arch Intern Med* 2003;**163**:1009-1021.

57. World Health Organisation, Geneva: WHO report 2007. Global Tuberculosis control – surveillance, planning, financing. WHO/HTM/TB/2007.376.
58. Styblo K: The relationship between the risk of tuberculous infection and the risk of developing infectious tuberculosis. *Bull Int Union Tuber Lung Dis* 1985; **60**: 117-119.
59. Central TB Division, Directorate General of Health Services, Ministry of Health and Family Welfare, New Delhi. TBC India 2007, Performance Reports. URL: http://www.tbcindia.org. Accessed on 31st May 2008.
60. Williams BG, Granich R, Chauhan LS, Dharmshaktu NS, Dye C. The impact of HIV/AIDS on the control of tuberculosis in India. PNAS 2005; 102:9619-9624. URL: www.pnas.org/cgi/doi/10.1073/pnas.0501615102.

Indian J Tuberc 2009; 56:44-47

STATUS REPORT ON RNTCP*

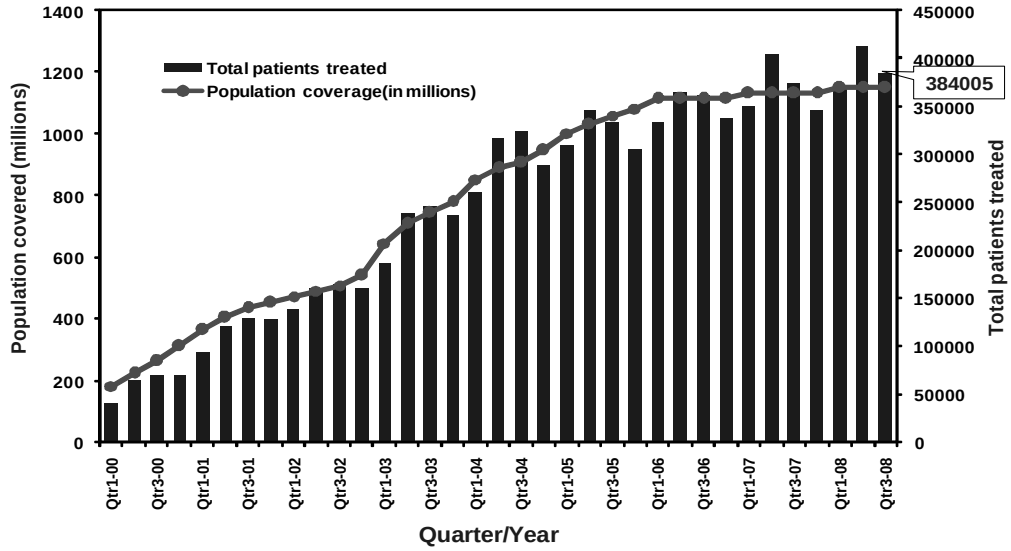
RNTCP has nearly achieved its twin objectives of NSP case detection and NSP success rate at the national level again during the 3rd quarter, 2008. With this, the annualized New Smear Positive Case Detection Rate (NSP CDR) for the year 2008 from the first three quarters is 73%.

RNTCP performance in third quarter 2008

During this quarter, over 1.7 million suspects were examined, 228,198 sputum positive cases were diagnosed, and 384,005 TB cases were registered for treatment. The annualized total case detection rate is 134 cases per 100,000 population. The new smear positive TB case detection rate (annualized) for the third quarter 2008 was 72% of the estimated cases, with a total of 154,598 new smear positive cases being registered for treatment. In addition, 98,334 new smear negative cases, 56,084 new extra pulmonary cases, 51,909 smear positive re-treatment cases and 22,605 'Others' were also initiated on treatment in this quarter. The treatment success rate amongst the new smear positive PTB cases registered in the third quarter 2007 is 87%. The sputum conversion rate and cure rate among the new sputum positive cases was 90% and 84% respectively. The default rate among NSP (5.9%), NSN (7.4%) and re-treatment cases (14.4%) is showing a declining trend but the current rates are still a cause of concern.

During the third quarter, the internal evaluations undertaken at the national and state level have once again highlighted the need that in few districts there is a need for improvement in supervision and monitoring, training/update training of the field level staff and validation of data prior to submission of quarterly reports. It is, therefore, important that we focus on patient centered supervision and monitoring at all levels, training both -initial and update- be undertaken periodically and continue to observe 'zero tolerance' to falsification of data under the programme from all levels.

Population in India covered under DOTS and Total Tuberculosis Patients put on treatment each quarter



* 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 (2008 third quarter), Smear Conversion (2008,second quarter), and Treatment Outcome (2007,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]	Annualised total case detection rate	New smear positive patients registered for treatment	Annualized new smear positive case detection rate (%)		No of new smear negative cases registered for treatment	No of new EP cases registered for treament	No. of smear positive re-treatment cases registered for treatment	Three month conversion rate of new smear positive patients	Cure rate of new smear positive patients	Success rate of new smear positive patients
Andaman & Nicobar	4	239	114	197	192	79	77	103%	49	43	19	97%	81%	83%
Andhra Pradesh	822	159	18520	28609	139	12358	60	80%	7798	3054	4128	92%	87%	89%
Arunachal Pradesh	12	246	295	633	211	208	69	92%	164	107	75	90%	85%	87%
Assam	299	123	5747	10142	136	4397	59	78%	2802	1222	974	90%	86%	88%
Bihar	938	91	11366	21544	92	8428	36	48%	7348	1549	2061	88%	78%	86%
Chandigarh	11	337	460	619	233	212	80	84%	116	180	64	92%	89%	93%
Chhatisgarh	236	113	3019	6723	114	2476	42	52%	2694	873	353	88%	83%	87%
D & N Haveli	3	224	79	109	166	43	66	82%	21	20	16	91%	91%	91%
Daman & Diu	2	342	34	62	132	12	26	32%	25	7	5	92%	83%	93%
Delhi	171	231	6372	12816	300	3604	84	89%	2074	4029	1887	89%	87%	87%
Goa	16	192	272	466	114	144	35	44%	105	117	66	90%	80%	80%
Gujarat	564	176	15001	19961	142	8805	62	78%	2676	2467	4496	91%	87%	87%
Haryana	238	156	5724	9109	153	3304	56	59%	1781	1519	1931	89%	85%	86%
Himachal Pradesh	66	233	2038	3343	204	1237	76	80%	649	691	532	91%	87%	90%
Jammu & Kashmir	124	123	1653	2677	87	1254	41	43%	417	592	336	91%	87%	88%
Jharkhand	300	126	5450	10165	135	4338	58	77%	3489	788	701	90%	83%	90%
Karnataka	574	192	10240	16568	115	6378	44	59%	3679	3145	2282	86%	77%	79%
Kerala	342	186	3406	6122	72	2636	31	62%	1167	1470	634	83%	82%	84%
Lakshadweep	1	58	0	0	0	0	0	0%	0	0	0	100%	100%	100%
Madhya Pradesh	693	115	11960	20839	120	7479	43	54%	6575	2387	2951	88%	83%	86%
Maharashtra	1069	150	19161	34395	129	13113	49	61%	8372	5883	4075	90%	84%	86%
Manipur	26	139	363	1071	163	256	39	52%	362	222	74	85%	81%	83%
Meghalaya	25	159	584	1247	197	396	62	83%	262	335	133	84%	82%	83%
Mizoram	10	247	273	698	285	212	87	115%	191	182	36	95%	95%	95%
Nagaland	22	148	376	773	141	305	56	74%	188	120	110	92%	88%	88%
Orissa	399	136	7357	12665	127	5658	57	67%	3146	2180	1012	86%	82%	86%
Puducherry	11	293	388	322	120	147	55	73%	47	82	44	89%	84%	85%
Punjab	266	157	5948	9853	148	3833	58	61%	1875	2096	1622	87%	84%	87%
Rajasthan	646	139	18182	29085	180	10720	66	83%	8235	3524	5530	92%	88%	90%
Sikkim	6	345	203	412	277	125	84	112%	79	118	56	90%	87%	87%
Tamil Nadu	664	206	11195	21239	128	8366	50	67%	5492	4463	2226	90%	85%	86%
Tripura	35	145	437	685	78	379	43	58%	107	115	59	96%	87%	90%
Uttar Pradesh	1909	155	43206	70200	147	29786	62	66%	20137	7500	9768	91%	85%	88%
Uttarakhand	95	178	2347	3432	145	1265	53	56%	830	543	626	88%	83%	87%
West Bengal	879	156	16428	27224	124	12645	58	77%	5382	4461	3027	88%	84%	86%
Grand Total	**11477**	**152**	**228198**	**384005**	**134**	**154598**	**54**	**72%**	**98334**	**56084**	**51909**	**90%**	**84%**	**87%**

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, include new sputum smear positive cases, new smear negative cases, new extra-pulmonary cases, new others ,relapse,failure,TAD and retreatment others

Major activities during the quarter

Involvement of Medical Colleges

The Zonal Task Force (ZTF) workshops for the involvement of medical colleges were held during the months of August and Septmeber'2008 at Patiala (North Zone), Trivandrum (South Zone), Raipur (East Zone) and Goa (West Zone). The workshops highlighted the highly impressive progress that has been made in all the zones and by the end of 3Q08, 263 medical colleges have been involved for providing RNTCP diagnostic and treatment services for TB patients seeking health care from them. This has been possible largely because of the leadership and commitment shown by the programme managers and the faculties of the medical colleges in addressing the problem of tuberculosis in the country. The implementation of the recommendations made during the workshop will further strengthen the involvement of medical colleges.

Accreditation of Intermediate Reference Laboratories

Two intermediate reference laboratories of Andhra Pradesh and Delhi were accredited for *M TB* culture and DST during this quarter. Another nine IRLs (Kerala, Haryana, Rajasthan, West Bengal, Tamil Nadu, Uttarakhand, Jharkhand, Uttar Pradesh, and Orissa) and four medical college labs are presently under the accreditation process and are expected to be accredited for *M TB* culture and DST in 2008-09. The state level drug resistance surveillance has started in the State of Andhra Pradesh from September 2008.

DOTS-Plus Services

DOTS Plus services for the management of MDR TB have been expanded to another four states (AP, Delhi, Haryana and Kerala) bringing the total number of states which have initiated DOTS Plus services to six. These four states have started enrolling MDR suspects for culture and DST and are expected to commence the Cat-IV treatment services shortly. Gujarat and Maharashtra which began the Cat IV services in August 2007 are making encouraging progress and at the end of this quarter 182 MDR-TB cases were on treatment in these two states. Both these states are now in the process of expanding the DOTS-Plus services to a few more districts each. The national level DOTS-plus training was undertaken for the States of Tamil Nadu, West Bengal and the phase two districts of Gujarat and Maharashtra.

TB-HIV collaboration

Under the revised framework an 'intensified TB/HIV package of services' has been introduced for states with higher burden of HIV (Andhra Pradesh, Goa, Karnataka, Maharashtra, Manipur, Mizoram, Nagaland, Pondicherry and Tamil Nadu). During the quarter, training material for Intensified Package has been prepared in July 2008 and put on the website of tbcindia.org for wide dissemination in nine high HIV prevalence states. Training of master trainer on intensified package of services has already completed in all the 9 states. With the development of the district level action plan for the implementation of the intensified package during the training, it is expected that all the 9 states will implement the intensified package of services including the recording of HIV status, CPT and ART on the treatment card and TB registers during the 4th Q08.

Involvement of NGOs and PPs

The revised RNTCP NGO-PP schemes were approved by the Government of India during the quarter from 1st October 2008.It is expected that these revised schemes will further encourage participation and contribution of the NGOs and PPs for the tuberculosis control activities in the country.

The Catholic Bishops' Conference of India (CBCI) is facilitating implementing RNTCP services in all Catholic health facilities in 11 states of India. During the quarter, state level personnel were recruited and six state level workshops were conducted. The training and sensitization at

institutional level has started. Assessments of Catholic health facilities prior to signing of MoUs for the new NGO-PP schemes are in progress. It is expected that all dispensaries, major hospitals, Community Care Centres and Medical Colleges under the aegis of the CBCI will participate in RNTCP as per national policy.

The RNTCP PPM IMA Project supported by round-6 of the GFATM (April 2007 to March 2012) has successfully completed the first year of the project. A national level review workshop of the IMA was held at Kovalam on 9th -10th August 2008 which was attended by President and Secretaries of all State branches.

Advocacy, Communication and Social Mobilization (ACSM)

During the quarter, the preparation for ACSM capacity building training workshops was initiated with the discussion with National Institute of Health and Family Welfare (NIHFW). TORS were discussed and field visits by the NIHFW were facilitated by CTD and detailed budget and plan have been finalized. The proposed workshops are planned to be held for State TB officers, IEC Officers and Communication Facilitators in four batches of 30 each in the months of November and December 2008.

TUBERCULOSIS HEALTH VISITORS' COURSE

The 2009-2010 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, 2009.

Case Report

EXTENSIVELY DRUG RESISTANCE (XDR) TB IS NOT ALWAYS FATAL

N.K. Jain[1] S.P. Agnihotri[2], Krishan K. Sharma[3], Shikha Gupta[3] and Pawan Gupta[3]

(Received on 11.6.2008; Accepted after revision on 4.12.2008)

Summary: The chance of incidence of XDR TB is on the rise due to improper use of second line anti-tubercular drugs. XDR-TB is very difficult to treat successfully and is often referred to as "virtually untreatable form of TB". We herein report a case of XDR TB confirmed by bacteriological examination in a WHO recognised laboratory who after 12 months of regular treatment improved both clinically and radiologically with sputum smear conversion. To the best of our knowledge, there has been no previous report of any similar case in literature. ***[Indian J Tuberc 2009; 56:48-50]***

Key Words:XDR-TB, Case report, Improved

INTRODUCTION

XDR-TB definition was revised in October 2006 to describe cases that involve resistance to isoniazid, rifampicin, any flouroquinolone, and any of three injectable drugs; capreomycin, kanamycin and amikacin[1]. XDR-TB reflects a failure to implement the measures recommended in the WHO's Stop TB Strategy[2]. XDR-TB is very difficult to treat successfully, as there are very few drugs left to which the organism is still sensitive. Tuberculosis Research Centre (TRC) in Chennai, India is a part of the WHO Supra-National Reference Laboratory Network, and is recognized to perform drug sensitivity testing for second-line drugs. We herein report a patient with XDR-TB who responded to revised anti-tuberculosis therapy.

CASE REPORT

A 30-year old male, known case of drug resistant pulmonary tuberculosis, presented to outpatient department in February 2007 with complaints of cough with expectoration, low grade fever, weakness and malaise. Patient was on second line anti-tuberculous treatment since last seven years from multiple physicians. Drugs given in various combinations were: Kanamycin(K), PAS(P), Ofloxacin/ Ciprofloxacin/Levofloxacin(Q), Cycloserine(Cy), Ethionamide(Et), Roxithromycin(Ro), Isoniazid(H), Etambutol(E), Pyrazinamide (Z). In year 2000-01, patient took nine month KEtPQHEZ, followed by 16 month EtPQHEZ irregularly. In 2003, mycobacterial culture and sensitivity report (Myco C/S) showed resistance to HERK and patient was put on EtPQZE ,which he defaulted after seven months. Myco C/S report in 2005 showed resistance to HSRQ. During 2005-06, patient took CyQRoHEZ irregularly for 15 months.

Examination was normal except for evidence of grade II clubbing and bilateral crepitations with occasional rhonchi on auscultation. Investigations showed normal haemogram, urine analysis and biochemical investigations. ECG was normal. ELISA for HIV was negative. Three separate samples of sputum examined for Acid Fast Bacilli(AFB) were positive. Chest roentgenogram showed left upper and mid zone(MZ) fibrocavitary disease with right MZ thick walled cavity and old healed bilateral diffuse calcified lesions (Figure 1). Due to persistently positive sputum for AFB with Myco C/S reports of MDR-TB

1. Professor 2. Associate Professor 2. Resident Doctor
Department of Chest Diseases and Tuberculosis, SMS Medical College, Jaipur.
Correspondence: Dr. N.K. Jain, A-123, Shopping Complex, Subhash Nagar, Jaipur-302 016.
E-mail: jainnkdr@yahoo.co.in, dr_kksharma@yahoo.co.in; Contact No. +91(141)2290348, 2281000, 2281001; Mobile: +919314504774

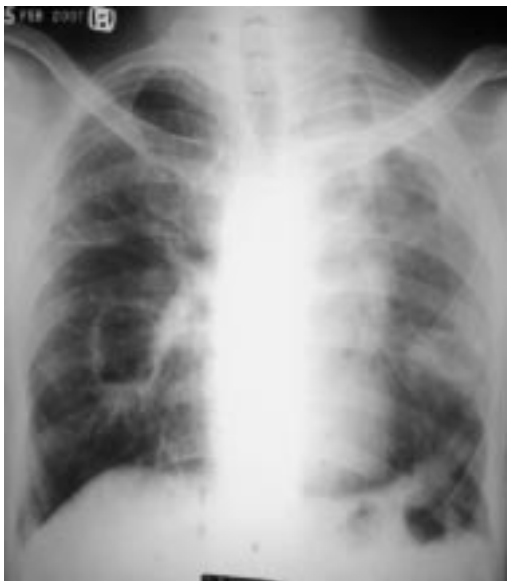


Fig.1: Chest roentgenogram showing left upper and mid zone (MZ) fibrocavitary disease with right MZ thick walled cavity and old healed bilateral diffuse calcified lesions.

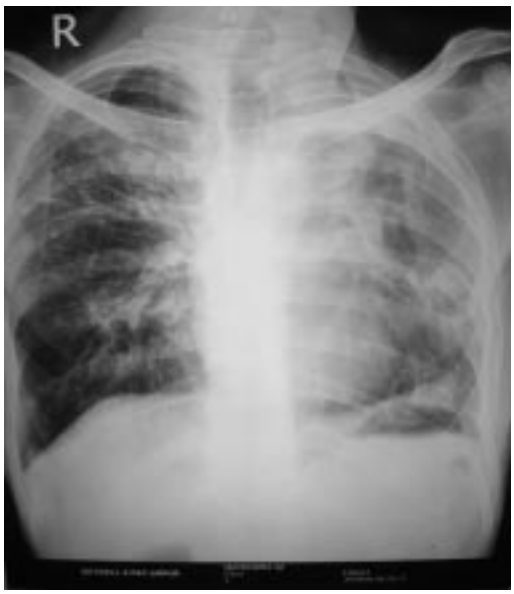


Fig. 2: Chest roentgenogram showing marked reduction in left upper MZ fibrocavitary disease with almost complete resolution of right MZ cavity.

and history of multiple spells of ineffective chemotherapy, his sputum was sent to TRC, Chennai on 27 December 2006 for Myco C/S to second line drugs. Report received on 28 March 2007 was suggestive of resistance to Kanamycin, Ethionamide, Ofloxacin, Streptomycin, Isoniazid and Rifampicin, putting the patient under definition of XDR-TB. Now, the treatment was revised and the patient was put on injection Capreomcin with oral Moxifloxacin, PAS, Clarithromycin, Clofazimine, Ethambutol. Capreomycin was stopped after six months with continuation of rest of the medicines.

Patient is on regular therapy since April 2007. On Follow-up, his smear for AFB and mycobacterial culture done in January 2008 and April 2008 were negative. As on 30-08-2008 patient's sputum smear was negative, culture report was awaited. Chest roentgenogram showed marked reduction in left upper MZ fibrocavitary disease with almost complete resolution of right MZ cavity (Figure 2). Now the patient is afebrile with improvement in weight and appetite. In between, patient's only respiratory complaint was mild occasional cough, others being reddish discolouration of skin and tingling and numbness in hand and feet. With further symptomatic treatment, these complaints have decreased.

DISCUSSION

As XDR-TB is resistant to first and second line drugs, treatment options are seriously limited. It is, therefore, vital that TB control is managed properly. WHO estimates that there were almost half a million cases of Multi-Drug Resistant TB (MDR-TB) worldwide in 2004, and MDR-TB usually has to occur before XDR-TB arises. Findings from the only global study carried out so far showed that in some places as many as 19% of MDR-TB cases were in fact XDR-TB[3].

The first outbreak of XDR-TB was seen in the KwaZulu-Natal province in South Africa in early 2005. Of the 53 who tested positive

for XDR-TB, 52 died within 25 days of infection, showing the severity of fatality of XDR TB. Many of those who died were HIV positive. Genotyping analysis revealed that 85% of the 46 isolates tested had similar strains[4].

CDC and the WHO report during 2000-2004 summarizes that, of 17,690 TB isolates, 20% were MDR and 2% were XDR and population based data on drug susceptibility of TB isolates from United States, Latvia, and South Korea, showed that 4%, 19%, and 15% of MDR TB cases, respectively, were XDR[5].

The incidence and prevalence XDR-TB in India is currently not available. In India at Mumbai, a study from Hinduja Hospital revealed that of 1,324 samples received, 724 were culture positive and 45% of these were MDR, of which 11% were XDR-TB cases[6]. This laboratory is also not accredited for second line drug testing. So, the results should be interpreted in that context.

As indicated in previous reports, the high mortality of XDR-TB is most probably due to deadlier combination with other comorbid factors like HIV in KwaZulu-Natal. These reports address XDR-TB as virtually untreatable, and there is a lot of debate about its fatality. **But it is not always true as proper treatment of XDR-TB is possible keeping in mind the golden principle of chemotherapy i.e. at least 3-4 unused drugs should be given in revision of therapy.** In our case we used capreomycin, Clarithromycin, clofazimine, moxifloxacin as new addition. Patient improved with treatment, showing that adherence to therapy can improve the outcome but at the cost of some unwanted side effects. **So, there is still a ray of hope with currently available drugs and patient is always encouraged for adherence to the treatment, which may improve the outcome and survival of the patient with XDR-TB.**

WHO also stated that several countries with good TB control programmes have shown that cure in XDR-TB is possible for up to 50–60% of affected people. But successful outcomes also depend greatly on the extent of the drug resistance, the severity of the disease and whether the patient's immune system is compromised[3].

REFERENCES

1. CDC. Revised definition of extensively drug-resistant tuberculosis. *MMWR* 2006;**55**:1176
2. Raviglione MC, Uplekar MW. WHO's new Stop TB Strategy. *Lancet* 2006;**367**:952-955
3. World TB day 2007 , WHO, XDR-TB factsheet; page 2
4. Gandhi NR, Moll A, Sturm AW, et al. Extensively drug-resistant tuberculosis as a cause of death in patients co-infected with tuberculosis and HIV in a rural area of South Africa. *Lancet* 2006;**368**:1575-1580
5. Emergence of Mycobacterium tuberculosis with extensive resistance to second-line drugs — worldwide, 2000-2004. *MMWR Morb Mortal Wkly Rep* 2006;**55**:301-305
6. Jain S, Rodrigues C, Mehta A, Udwadia ZF. High prevalence of XDR-TB from a tertiary care hospital in India. American Thoracic Society 2007 International Conference, San Francisco, USA;May 2007.Abstract A510.

Editor's note

Some of the authors believe that with the proper treatment with the available drug combinations, XDR cases are not always fatal.

Case Report

ISONIAZID INDUCED GYNAECOMASTIA: A CASE REPORT

R. Garg[1], Vaibhav[2], Sumit Mehra[2] and R. Prasad [3]

(Received on 27.11.2007; Accepted after revision on 10.12.2008)

Summary*:** Gynaecomastia due to anti-tubercular chemotherapy is a rare side effect. Isoniazid causing breast tissue enlargement has been very rarely reported. We report a 60-year old, male patient of Pulmonary Tuberculosis who was started on anti-tuberculous treatment(ATT) with rifampicin (R), isoniazid (H), ethambutol (E) and pyrazinamide (Z) together for initial two months and R, H & E thereon. After five months of initiation of treatment, while receiving RHE, he developed painful bilateral gynaecomastia. Isoniazid was stopped and patient was continued on R & E till completion of the treatment up to nine months. After stopping isoniazid, his breast swelling subsided to some extent and became non-tender. Follow up, at six months, after stopping the course of treatment, patient was asymptomatic except for slight bilateral non-tender breast enlargement. ***[Indian J Tuberc 2009; 56:51-54]

***Key Words*:** Isoniazid, Gynaecomastia

INTRODUCTION

Side-effects to anti-tubercular drugs are fairly common but there are a few side effects that belong to the rare category. Isoniazid is one of the most effective and cheapest among anti-tuberculous drugs. It is bactericidal against metabolically active bacilli and bacteriostatic against resting bacilli. Isoniazid is well tolerated at recommended dose. It is rarely associated with serious adverse effects that include hepatitis, peripheral neuropathy, cutaneous reactions and mental changes[1]. Gynaecomastia i.e. enlargement of breast tissue as opposed to adipose tissue due to isoniazid[2] is one of the rare but non-serious side-effects of it. Though this has also been implicated as a cause of gynaecomastia[3] but the description in literature is very sparse. Online search on Pub Med revealed only one case report from India[4], two reports from France[5,6] and one from Italy[7]. The first report from France was published in 1953 and the second report on it was from Italy in 1957. In the literature, only one case report from India is available in English and elsewhere this finds mention in the long list of drugs causing gynecomastia. The rarity of such an extent prompted us to report this case.

CASE REPORT

SN, a 60-year-old male smoker, non alcoholic patient presented to Department of Pulmonary Medicine, King George's Medical University, Lucknow with complaints of cough, expectoration, low grade fever, decreased appetite and streaking. On further evaluation, he was diagnosed as a case of sputum positive pulmonary tuberculosis. Patient was put on isoniazid 300 mg, rifampicin 450 mg, ethambutol 800 mg and pyrazinamide 1000mg once daily. Pyrazinamide was stopped after two months. Patient responded well to the treatment and gained 5-kilogram weight during the initial intensive phase of treatment. Under treatment follow up at 5th month while continuing with rifampicin, isoniazid and ethambutol patient complained of pain and swelling in both breasts. On examination, patient had bilateral tender mobile breast lump (Figs. 1,2), about 5x6 cm in diameter. Suspecting it to be drug induced gynaecomastia, isoniazid which appeared to be most obvious cause of it was withdrawn immediately from the treatment and rest were continued with further investigation of the patient to know the cause of gynaecomastia. On examination, secondary sexual characters and

1. Associate Professor 2. Junior Resident 3. Professor and Head
Department of Pulmonary Medicine, Chhatrapati Sahuji Maharaj Medical University, Lucknow (Uttar Pradesh)
Correspondence: Dr. Rajiv Garg, Associate Professor, Department of Pulmonary Medicine, Chhatrapati Sahuji Maharaj Medical University, Lucknow-226 003; Phone No. 9415002386; e-mail: rgarg70@rediffmail.com

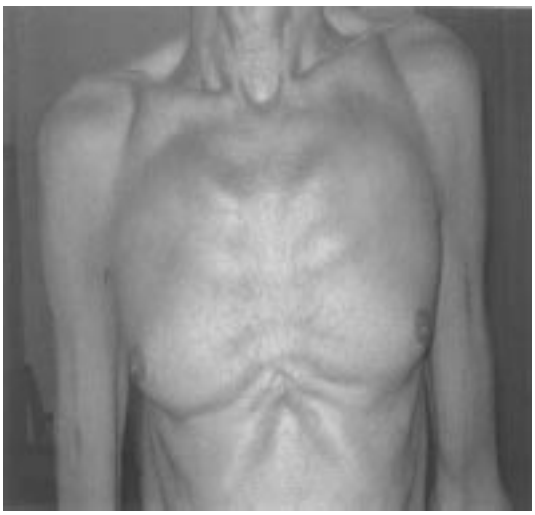


Fig. 1: Frontal view of the patient showing Gynaecomastia developed during isoniazid based anti-tuberculosis treatment.

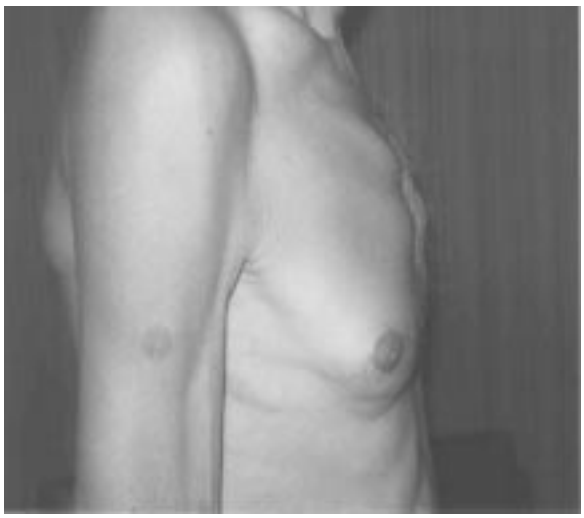


Fig. 2: Lateral view of the patient showing Gynaecomastia

external genitalia were found normal, his mammogram showed features suggestive of, bilateral benign mammary tissue hyperplasia (Fig. 3). His ultrasonogram showed it to be glandular tissue hyperplasia. Patient's thyroid stimulating hormone level was 0.77uIU/mL (0.3-6.0), Leutinizing hormone was 6m IU/ml [0.7-7.4], follicle stimulating hormone 6.8 mIU/ml [1.0-14.0], prolactin 11.0 ng/mL [1.8-17 ng/mL], testosterone 4.0 ng/mL [3-20 ng/mL] and estradiol was 54.9 pg/mL [21-79 pg/mL]. His hepatic and renal functions were within normal limits. Ultrasonogram of external

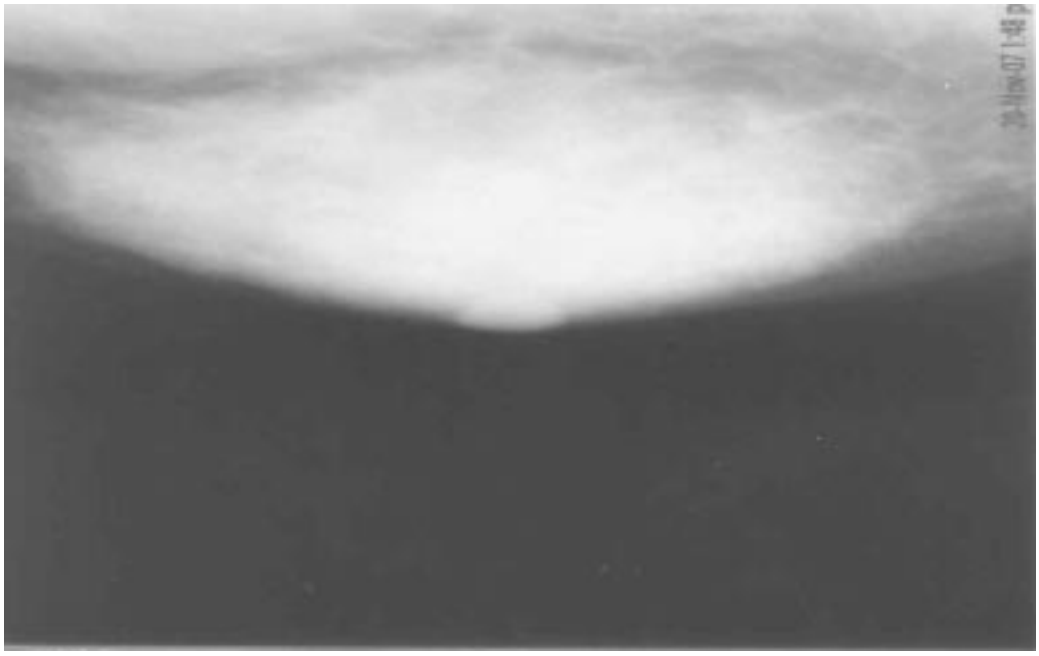


Fig. 3: Mammogram of the patient showing glandular hypertrophy indicating true gynaecomastia.

genitalia revealed no abnormality. He recuperated well after stopping isoniazid, pain from the breast subsided within a month. Follow-up at six months after the prescribed nine months of treatment revealed an asymptomatic patient with non-tender slightly enlarged breast.

DISCUSSION

Gynaecomastia is one of the most common breast problems in men and was first described by Paulus Aegineta (AD 625-690), who thought it was due to formation of fat[2]. It can occur due to numerous causes which include developmental gynaecomastia, congenital causes like Klinefelter syndrome, hermaphroditism, enzyme defects of testosterone production, acquired causes like trauma, infection, torsion (twisted testicles), radiation, mumps, chemotherapy, malignancies like bronchogenic carcinoma, alcoholism, systemic causes like congenital Adrenal hyperplasia, cirrhosis, renal failure, thyrotoxicosis and drugs.

Drugs are a very common cause of gynecomastia[8,9] and should always be entertained as the possible causal agent of such a condition. Most of the drugs causing gynaecomastia have been reported by means of case reports, which document temporal association of the offending drug[10]. Clinically significant gynecomastia caused by drugs may be due to an impaired balance in the serum estrogen to androgen ratio or a rise in prolactin level[11]. In case of isoniazid induced gynaecomastia it has been hypothesized that disturbance in vitamin B_6 complex activation in liver leads to altered oestrogen-androgen metabolism. It has also been postulated that isoniazid probably acts by phenomenon called 'Refeeding gynaecomastia', which is supposed to be caused by restoration of weight, gonadotrophin secretion and gonadal functions[12].

Among anti-tubercular drugs, Isoniazid, Thioacetazone and Ethionamide[11,13] have been implicated as causes of gynaecomastia. First report of isoniazid induced gynaecomastia came in 1953 from France, a year after its introduction in 1952, then from Italy in 1957 and another French report came in 1976. The only Indian report was published in 2003. French report described painless, bilateral gynaecomastia in 52-year old man who was receiving 600 mg of isoniazid daily for four months along with rifampicin and ethambutol[6]. On investigation, he was slow acetylator. Strikingly similar to the only other Indian case report, ours was also receiving isoniazid 300 mg daily and also had bilateral painful enlargement of breast in contrast to other foreign reports which had reported it with higher dose of isoniazid and painless enlargement of the breast. The acetylator status was not carried out in this case and the same is also not available in other Indian report. In contrast to other Indian report we stopped isoniazid only, rather than all the ATT after initiation of symptoms and were able to achieve good symptomatic relief. There appears to be temporal association with this offending drug as in all the reports it has occurred in later part (continuation phase) of the therapy.

It is difficult to distinguish true breast enlargement from increased adipose tissue (lipomastia). True glandular tissue is often palpable, especially around the areola, as it is firmer and contains cord like features distinct from the texture of adipose tissue. In difficult cases, true gynaecomastia can be identified by ultrasound, which is recommended as the first-line imaging investigation although mammography may be added to confirm the diagnosis. In this case both were done to confirm the true gynaecomastia which was not done in any of the previous reported cases.

Most patients with gynaecomastia require no treatment other than the removal of any inciting cause. Specific treatment of enlarged breast tissue is indicated if it is causing sufficient pain, embarrassment, or emotional discomfort to interfere with patient's daily life.

The other anti-tuberculosis drugs which are implicated in the list of drugs causing gynaecomastia are thioacetazone and ethionamide only. As of now, there are no reports implicating

rifampicin and ethambutol causing gynaecomastia which prompted us to attribute isoniazid as the inciting etiology.

Our case appears to be the first well worked up case after the similar case report in English literature and highlights the fact that isoniazid therapy can lead to development of painful gynecomastia which may be very embarrassing to the patient, especially if the patient is elderly.

REFERENCES

1. Girling DJ. Adverse effects of anti-tuberculousis drugs. *Drugs* 1982; **23** : 56-74.
2. Park AJ and Lamberty BGH Gynaecomastia :Have Webster's Lesson Been Ignored? J.R.Coll.Surg.Edinb, 43, April1998, 89-92.
3. Braunstein GD. Gynaecomastia. *N Engl Med* 1993; **328** : 490-95.
4. Khanna P, Panjabi C, Maurya V, Shah A. Isoniazid associated, painful, bilateral gynaecomastia.*Indian J Chest Dis Allied Sci.* 2003; **45:** 277-279.
5. Guinet P, Garin JP, Morpex A. *Un cas de gynecomastie chez un tuberculeux pulmonaire grave en cours de traitment par I'hydrazide de I'acide isonicotinique*. (A case of gynecomastia in pulmonary tuberculosis during the course treatment with isonicotinic acid hydrazide) *Lyon Med* 1953; **85:** 281-284.
6. Bergogne-Berezin E. Nouhouayi A. Letonturier P, Tourneur R. Gynaecomastia caused by isoniazid: Value of determination of inactivation of phenotype. *Nouv Presse Med* 1976; **5**: 213-214.
7. Borsella C, Merelli B. Appearance of gynaecomastia in pulmonary tuberculosis patients during isoniazid therapy. *G Clin Med*. 1957; **38**: 1744-58.
8. Rodriguez Garcia LA, Jick H. Risk of gynaecomastia associated with cimetidine,omeprazole and anti -ulcer drugs. *BMJ* 1994; **308**:503-506
9. Hugues FC, Gourlot C, Le Jeunne C: Drug induced gynecomastia .*Ann Med Interne* [Paris] 2000; **151**: 10-17.
10. Thompson DF, Carter JR. Drug induced gynecomastia. *Phrmacotherapy* 1993; **13**: 37-45.
11. Jean D Wilson. Endocrine disorders of the breast. In : Braunwald E etal. Harrison's principles of internal medicine. XVth ed; Vol. II, New York; Mc Graw-Hill Co.; 2001: 2170-2171.
12. Shirley A Bembo and Harold E Carlson: Gynaecomastia:Its Feature, and when and how to treat it. *Cleveland Clinic Journal of Medicine* 2004; **7**: 6.
13. Chunhaswasdikul B. Gynaecomastia in association with administration of thiacetazone in the treatment of tuberculosis. *J Med Assoc Thai.* 1974; **57**: 323-7.

ABSTRACTS

Factors associated with a clinician's decision to stop anti-tuberculosis treatment before completion

Chiang, C-Y. Enarson, D.A. Bai, K-J. Suo, J. Wu, Y-C. Lin, T-P. Luh, K-T. *Int J Tuber Lung Dis* 2008; **12(4)**: 441-446

The objective was to investigate the diagnosis of pulmonary tuberculosis (PTB) and factors associated with a clinician's decision to stop anti-tuberculosis treatment before completion. The medical charts of all citizens of Taipei City, Taiwan, reported to have received treatment for PTB in 2003 were investigated. Of 1126 PTB patients, 512 (45.5%) started treatment immediately based solely on chest X-ray (CXR) findings; treatment for 214 (19.0%) was based on a positive sputum smear for acid-fast bacilli, for 261 (23.2%) it was based on other findings and for 139 (12.3%) it was based on a positive mycobacterial culture. Of the 1126 PTB patients, 156 (13.9%) had their diagnosis of TB changed by a clinician. Multivariate analysis shows that patients whose diagnosis was based on CXR or other findings, female patients, patients who interrupted treatment for two months, patients who continued care at other health facilities (transfer) and patients with lung cancer were significantly more likely to have their diagnosis changed than other groups. A substantial proportion of patients were prescribed anti-tuberculosis treatment based on CXR findings alone, and a considerable proportion were advised to stop treatment before completing a full course, findings that require the immediate attention of Taiwan's National Tuberculosis Programme.

Physician-initiated courtesy MODS testing for TB and MDR-TB diagnosis and patient management

Nic Fhogartaigh, C.J. Vargas-Prada, S. Huancaré, V. Lopez, S. Rodríguez, J. Moore, D.A.J. *Int J Tuber Lung Dis* 2008; **12(5)**: 520-526.

The objective was to evaluate the contribution of unselected (courtesy) Microscopic Observation Drug Susceptibility (MODS) testing to the diagnosis and/or Drug Susceptibility Testing (DST) of tuberculosis and their subsequent impact upon patient management. It is a retrospective data base analysis and case note review of MODS culture-positive cases. *Mycobacterium tuberculosis* was isolated in 28.9% of 225 samples (209 patients); 22.2% of 63 positive cases were multi-drug-resistant. In 58 MODS culture-positive cases with follow-up data available, MODS provided culture confirmation of diagnosis, DST or both in 82.8%, before any standard method. In 41.4%, this result should have prompted a modification in patient management. Delays between laboratory result and initiation or change of treatment, where applicable, took on average 42 and 64 days, respectively, of which a delay of respectively 17 and 48 days occurred after the receipt of results by the health facility. MODS provides important data for clinical management within a meaningful time-frame and should contribute positively to patient outcome due to earlier initiation of appropriate therapy. Although clinicians may successfully select patients likely to benefit from MODS, ongoing work is required to identify optimal implementation of the assay and to reduce logistical and health system derived delays.

High levels of resistance to second-line anti-tuberculosis drugs among prisoners with pulmonary tuberculosis in Georgia

Jugheli, L. Rigouts, L. Shamputa, I.C. Bram de Rijk, W. Portaels, F. *Int J Tub Lung Dis* 2008; **12(5)**: 561-566.

The objective was to determine the prevalence of resistance to second-line drugs among Prisoners with Pulmonary Tuberculosis (PTB). It was a retrospective evaluation of resistance to second-line drugs in tuberculosis (TB) patients treated from 2001 to 2003. The overall observed prevalence of multi-drug-resistant TB (MDR-TB) was 14.4% (39/270). The lowest resistance was found for ofloxacin (OFX), which was 2.2% (6/

270) overall and 5.1% (2/39) among MDR patients. Isolates from four non-MDR patients who had never received anti-tuberculosis treatment were found to be resistant to OFX. Resistance to kanamycin and capreomycin occurred simultaneously only among MDR patients and was observed in 17/39 cases (43.6%). High rates of resistance to e"2 second-line drugs (18/39, 46.2%) and e"3 second-line drugs (10/39, 25.6%) were observed among all MDR-TB patients, reaching respectively 59.3% and 29.6% among previously treated MDR-TB cases. Only one patient was found to be resistant to four second-line drugs. No extensively drug-resistant TB (XDR-TB) according to the latest definition was detected.

Risk factors for delay in the diagnosis and treatment of tuberculosis at a referral hospital

Lorent, N. Mugwaneza, P. Mugabekazi, J. Gasana, M. Van Bastelaere, S. Clerinx, J. Van den Ende, J. *Int J Tuber Lung Dis* 2008; **12(4)**: 392-396.

The objective was to evaluate delays in the diagnosis and treatment of tuberculosis (TB) and associated risk factors. It was a prospective data collection of patients treated for pulmonary TB (PTB) or extra-pulmonary TB (EPTB) between June and September 2006. Of 104 patients with a mean age of 35 years (range 17-84) recruited into the study, 62% were HIV-positive. EPTB was diagnosed in 60 cases. The median total, health care and patient delays were respectively 57, 28 and 25 days. The health system delay before referral was significantly longer than the delay at our institution (18 vs. 6 days, $P < 0.0001$). Risk factors for a longer health system delay at our institution were smear-negative PTB or EPTB (OR 5.12) and a trial of antibiotics (OR 2.96). The latter was also found to significantly prolong total delay (OR 2.85), as did rural residence (OR 4.86). No significant association was found between patient delay and age, sex, profession or health insurance status. Smear-negative PTB and EPTB were associated with longer health system delays. A trial of antibiotics significantly increased the health system delay. Its use, recommended by the World Health Organization in case of smear-negative TB and EPTB in developing countries, needs validation at the tertiary health care level.

Poor outcome is associated with delayed tuberculosis diagnosis in HIV-infected children

Viani, R.M. Lopez, G. Chacón-Cruz, E. Hubbard, P. Spector, S.A. *Int J Tuber Lung Dis* 2008; **12(4):** 411-416

The objective was to describe the morbidity and mortality associated with tuberculosis (TB) in human immuno-deficiency virus (HIV) infected children in Baja California, Mexico. It was a retrospective review of the medical records of all children with perinatally acquired HIV infection evaluated at Tijuana General Hospital with a diagnosis of TB between 1998 and 2007. The clinical criteria for the diagnosis of TB were used. A total of 73 HIV-infected children were followed during the study period. Thirteen (18%) children were diagnosed with TB; one was confirmed by culture to be positive. Among these children, the mean ages at HIV and TB diagnosis were respectively 3.6 and 5.3 years. There were a cumulative 29 hospital admissions prior to TB diagnosis; 24 of these were due to pneumonia. The mean duration of symptoms at TB diagnosis was 73 days. The most common symptoms were cough (92%) and anorexia (85%). Seven patients (54%) had disseminated TB and five (39%) died as a consequence of TB. We observed high morbidity, hospital utilization and high mortality associated with TB among HIV-infected children in Baja California.

Evaluating the effects of providing financial incentives to tuberculosis patients and health providers in China

H. Yao, X.Wei, J. Liu, J. Zhao, D. Hu and J.D. Walley. *Int J Tuber Lung Dis* 2008; **12(10)**: 1166-1177

A project was implemented in 50 low-income counties of Shanxi, where transport incentives were provided to poor patients for their first visit for tuberculosis (TB) diagnosis as well as for referral and supervision incentives for doctors. Objective was to evaluate the effects of providing incentives on TB case detection and treatment. A group of 51 control counties in Shanxi comparable to the intervention counties was selected. Routine TB reporting was reviewed at baseline (January-September 2004) and during the project period

(January- September 2005) in both groups. A patient survey was conducted in two counties in each group, with interviews of 119 new smear-positive patients treated during the intervention. Patients who received travel incentives had an annual individual income similar to those who did not. The notification rates of new smear-positive cases improved in both groups; however, improvement was less marked in the intervention group (70%) than in the control group (99%). Travel incentives did not reduce patient and doctor delays in the intervention group compared with the control group ($P > 0.05$). Providing incentives was not effective in improving TB control. There are two possible reasons for this: the poor were not well-targeted due to a lack of operational tools, and more influential health systems issues were not addressed.

Costs and cost-effectiveness of tuberculosis cultures using solid and liquid media in a developing country

D.H. Mueller, L. Mwenge, M. Muyoyeta, M.W. Muvwimi, R. Tembwe, R. McNerney, P. Godfrey-Faussett amd H.M. Ayles. *Int J Tuber Lung Dis* 2008; **12(10)**: 1196-1202

The expansion of culture has been proposed to aid tuberculosis (TB) control in developing countries. The objective was to examine the cost and cost-effectiveness at the.Zambian National TB Reference Laboratory of homemade and commercially produced Lowenstein-Jensen culture (HLJ and CLJ) as well as automated and manually read liquid culture (AMGIT and MMGIT). Costs were estimated from the provider's perspective and based on the average monthly throughput. Cost-effectiveness estimates were based on yield during the study period. All techniques showed comparable costs per culture (between US$28 and $32). Costs per *Mycobacterium tuberculosis* specimen detected were respectively US$197, $202, $312 and $340 for MMGIT, AMGIT, CLJ and HLJ. When modelled for the maximum through-put, costs were above US$95 per *M. tuberculosis* specimen detected for all techniques. When only performed among smear-negative specimens, costs per additionally identified *M. tuberculosis* would be US$487 for MMGIT and higher for other methods. Based on cost-effectiveness grounds, liquid media compared well with conventional solid media, especially where yield of MGIT was substantially higher than that of LJ media. The results indicated high overall costs per culture; the expansion of culture to decentralize levels with lower throughputs may result in even higher costs.

Isolation of *Mycobacterium bovis* and *M. tuberculosis* from cattle of some farms in north India - Possible relevance in human health

K. Srivastava, D.S. Chauhan, P. Gupta, H.B. Singh, V.D. Sharma, V.S. Yadav, Sreekumaran, S.S. Thakral, J.S. Dharamdheeran, P. Nigam, H.K. Prasad and V.M. Katoch. *Indian J Med Res* 2008; **128**: 26-31

Infection due to *Mycobacterium bovis* typically occurs in cattle and animals transmit infection to each other. The choice of appropriate clinical specimen is very important for isolation of *M. bovis* and *M. tuberculosis* from cattle. The present study reports the isolation of *M. tuberculosis* and *M. bovis* from different types of specimens from cattle suspected to be suffering from tuberculosis in certain organized cattle farms in north India. A total of 768 specimens (heparinized or EDTA containing blood (162), fine needle aspirates from prescapular lymph gland (PSLG,160), milk (154), pharyngeal swab (PhS, 98), rectal pinch (RP, 97) and faecal sample (97) from 161 cattle of organized cattle farms in North India suspected to be suffering from tuberculosis were analyzed. After decontamination by modified Petroff's method isolation of *M. tuberculosis* complex was done on Lowenstein-Jensen medium (with and without pyruvate). The culture isolates were identified as *M. tuberculosis* and *M. bovis* on the basis of biochemical tests. A total of 54 *M. tuberculosis* complex isolates were obtained, of them 40 were identified as *M. bovis* and 14 as *M. tuberculosis. M. bovis* were isolated from 12 of 38 animals in group A (Tuberculin +ve with signs of tuberculosis), 7 of 37 animals in group B (Tuberculin +ve and apparently healthy), 9 of 21 group C animals in (Tuberculin -ve with clinical signs of tuberculosis), 4 of 26 animals in group D (Tuberculin -ve and apparently healthy), 4 of 27 group E animals (having non-mycobacterial infection) and 4 of 12 animals in group F (having clinical signs such as debilitated condition, cough, decreasing milk production, *etc).*

Maximum number of *M. bovis* (19/40,47.5%) and *M. tuberculosis* (5/14,35.7%) isolates were grown from prescapular lymph gland biopsy (PSLG) followed by blood from which 9/40 (22.5%) *M. bovis* and 4/14 (28.5%) *M. tuberculosis* were isolated. *M. bovis* [6/40(15%)] and *M. tuberculosis* [4/14(28.5%)] were also isolated from milk. Only 3/40 (7.5 %) isolates of *M .bovis* could be isolated from 97 rectal pinch followed by 98 pharyngeal swab 2/40 (5%) and 97 fecal samples 1/40 (2.5%) while 1/14 (7.1%) *M .tuberculosis* isolates were obtained from pharyngeal swab. Among the samples analyzed, PSLG was found to be most suitable specimen for isolation of *M. tuberculosis* complex from cattle and is thus of diagnostic importance. *M. bovis* in milk indicates the need to investigate the transmission to human in such settings. Isolation of *M. bovis* and/or *M. tuberculosis* from apparently healthy cattle indicates sub-clinical infection in the herd. Further, isolation of a significant number of *M. tuberculosis* from cattle suggests possible human-to-cattle transmission which need to be confirmed by prospective studies including tools like DNA fingerprinting.

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. 56 : No. 1 **January 2009**

Subscription

Inland	
Annual	Rs.800
Single Copy	Rs.200
Foreign	
For SAARC countries	US $ 30
For South East Asian and Eastern countries	US $ 35
For other countries	US $ 40

Cheques/D.Ds. should be drawn in favour of "Tuberculosis Association of India, New Delhi"

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). ***IJT*** **is indexed in MEDLINE of National Library of Medicine, USA.**

Published and printed by S.C. Goyal, on behalf of the Tuberculosis Association of India, 3, Red Cross Road, New Delhi-110001 Phone: 011-23711303; 23715217 and printed at Cambridge Printing Works, B-85, Naraina Industrial Area-II, New Delhi-110 028 Phone : 25893439.