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Editorial

NOSOCOMIAL TUBERCULOSIS IN THE ERA OF DRUG RESISTANT TUBERCULOSIS

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Occupational exposure to *Mycobacterium tuberculosis* constitutes a potential health hazard for health care workers (HCWs) worldwide. Recent reports from developing countries have shown that HCWs caring for patients with infectious TB are at high risk of acquiring *M. tuberculosis* infection and disease. With the emergence of XDR strains of TB, there is growing concern about nosocomial transmission and there is a need to protect HCWs from TB. The risk of transmission of *Mycobacterium tuberculosis* from patients with TB to other patients and HCWs has been recognized for many years[1]. The level of risk varies by patient population and effectiveness of TB infection control measures. The risk is higher in places where large numbers of infectious TB patients are being treated, who are not rapidly diagnosed, isolated and treated, particularly in the absence of other infection control measures such as respiratory protection[2]. The World Health Organization (WHO) has proposed practical and low-cost interventions to reduce nosocomial transmission in settings where resources are limited[3,4]. These recommendations emphasize prompt diagnosis and rapid treatment of TB rather than expensive technologies, such as isolation rooms and respirators.

Most developed countries implement TB infection control programmes to reduce the risk of nosocomial transmission[5]. However; such control programmes are not routinely implemented in underdeveloped countries. Most healthcare facilities in these countries lack the resources to prevent nosocomial transmission of TB[6]. The primary focus of national TB programmes in high-prevalence, low-income countries is to expand basic DOTS services and the nosocomial transmission is ignored, but several factors illustrate that nosocomial TB must be addressed, even in such areas. First, nosocomial transmission is of concern because it affects not only patients who are exposed but also the healthcare workforce, which could adversely affect healthcare services over time. Secondly, transmission of TB can have serious consequences, particularly with drug-resistant TB. Several outbreaks in the developed countries demonstrated the role that hospitals can play as focal points of MDR-TB transmission. Therefore, interventions to reduce nosocomial transmission of TB are useful and cost-effective preventive measures to control TB, including XDR and MDR-TB, particularly in tertiary care settings.

Nosocomial TB in India

A recent review of several Indian studies showed that nosocomial transmission of TB is an important but poorly documented problem in India[1]. The prevalence of LTBI and annual risk of TB infection appears to be high (about 5% per year, much higher than the national average of about 1.5%) among young HCWs and medical and nursing trainees suggesting an increased risk for acquiring TB in the hospital setting. The rate of LTBI is 50% and active disease appears to be exceedingly high in sub-groups such as interns, residents and nurses[7]. The predominance of extra-pulmonary (mostly pleural) disease

among healthcare workers[8] may indicate progression to disease from newly acquired primary infection rather than reactivation of latent TB.

Factors facilitating nosocomial transmission

Several factors may facilitate nosocomial transmission in Indian hospitals. The overwhelming number of TB patients and repeated exposures to smear-positive TB patients are main factors. Delays in diagnosis and initiation of treatment and failure to separate or isolate patients with smear-positive TB from other patients also contribute to transmission risk[9]. Poor adherence to treatment, lack of continuous drug supply, use of sub-optimal treatment regimens, and insufficient treatment duration have been known to prolong infectiousness of TB patients and thereby facilitate nosocomial transmission. Outbreaks of drug resistant tuberculosis have been reported in areas with high prevalence of HIV infection[10].

Few hospitals in India have established infection control procedures. Hospitals, especially publicly owned facilities, tend to be crowded, poorly ventilated, and have limited or no facilities for respiratory isolation. Most respiratory care procedures (including sputum collection) are routinely carried out in a general ward setting, rather than in respiratory isolation rooms. Further, few of these hospitals offer routine screening programmes to detect and treat TB among healthcare workers. Since nearly half the Indian population is infected, healthcare workers do not view latent TB infection as a problem. Hence, latent infection is rarely treated.

Implementing TB infection control in India

Effective TB infection control in healthcare settings depends on early identification, isolating infected persons, and rapidly and effectively treating persons with TB[5]. After assessing the disease prevalence, risk factors, and resources, India must implement effective strategies to reduce nosocomial transmission. In all healthcare settings, a basic TB infection control programme should be implemented, as recommended by WHO and other agencies[2,4]. WHO also recommends developing an infection control plan, educating healthcare workers and patients, improving sputum collection practices, performing triage and evaluation of suspected TB patients in outpatient settings, and reducing exposure in the laboratory[4].

Implementing many of the recommended engineering controls is not feasible in most healthcare facilities because of the high costs of such measures (e.g., negative-pressure isolation rooms). However, separation or segregation of smear-positive TB patients in rooms with simple mechanical exhaust ventilation (e.g., window fans) could be feasible in some settings. At such centres, patients with infectious TB, especially XDR and MDR-TB, must not be admitted to the same wards as patients with HIV infection[11]. Personal respiratory protection measures (e.g., N95 respirators) are probably not feasible because of the high cost[1]. Respirators may be relatively costly to implement and of limited effectiveness in high-incidence, resource-limited settings. But the N 95 respirators should be used while managing XDR and MDR-TB patients.

Efforts should be made to improve the quality of TB care in the private sector through better coordination between the RNTCP and the private sector. By improving TB diagnosis and treatment practices, smear-positive TB patients are more likely to receive rapid diagnosis and treatment, thereby directly and indirectly reducing the overall transmission in the community and in the nosocomial setting. DOTS PLUS strategy needs to be expanded and all patients with XDR-TB and MDR-TB should be identified and treated in hospital in isolation to prevent infected individuals from possibly spreading infection to others.

In summary, there is growing evidence that TB is an important occupational problem among HCWs in developing countries like India. The available evidence clearly underscores the need to design and implement simple, effective and affordable TB infection control programmes in healthcare facilities in these countries. The need for implementing interventions is made more urgent because of a new threat identified recently—extensively drug-resistant tuberculosis[12]. Because of the XDR-TB threat, the WHO and the Stop TB Partnership are beginning to highlight the need to implement TB infection control measures in hospitals in poor countries[13]. In fact, the Global Task Force on XDR-TB has outlined a series of measures that countries must put in place to effectively combat the emergence of XDR-TB. Infection control is one of the recommended interventions. Thus, efforts are ongoing to update existing infection control guidelines in the wake of XDR-TB, and to develop programmes that are suitable for resource-limited countries[14]. In the era of XDR-TB which is practically incurable, it is critical that we protect the health of our HCWs because they are the main weapons in the battle against TB, and their health needs to be protected as well.

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Original Article

UTILIZATION OF RNTCP SERVICES IN RURAL AREAS OF BELLARY DISTRICT, KARNATAKA, BY GENDER, AGE AND DISTANCE FROM HEALTH CENTRE

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Summary

***Background*:** Knowledge on utilization pattern of RNTCP shall provide important inputs towards its strengthening in rural areas.

***Aims*:** To find out the utilization of RNTCP services by age, sex and distance from residence to designated microscopy centres and treating health centres.

***Methods*:** The study was carried out in Sandur TU of Bellary District, Karnataka. Information on age, sex and residence of persons with pulmonary symptoms and detected new sputum smear positive cases during third quarter 2003 to second quarter 2004; and their treatment outcome was obtained from the respective RNTCP records. Age and sex distribution of out-patients was collected from OPD registers of one randomly selected DMC and its PHCs.

***Results*:** A lesser number of males accessed the health care services. However, larger number of males with pulmonary symptoms and new sputum smear positive cases utilized RNTCP services than females in the ratio of 1.6:1 and 2.5:1 respectively. This was due to higher prevalence of persons with pulmonary symptoms and sputum positivity rate among males. Sputum positivity rates were also lower among the elderly. Male symptomatics and cases were on an average older than females. About 70% symptomatics and 53% cases resided at more than four kilometers from the respective DMCs and treating health centres. Treatment outcome was poorer among males with higher proportion of initial defaulters and among those residing at more than 20 kms.

***Conclusion*:** There is need to make health services available to the male working population at convenient hours and to be more vigilant to screen persons with pulmonary symptoms among the elderly. Collection of sputum specimen from eligible persons may be undertaken at PHCs which may later be transported to DMC. Supervision and motivation of treatment for male TB cases and those residing more than 20 kms from the treating health centres requires to be strengthened.

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Key words: RNTCP, Utilization, Chest symptomatic, New sputum smear positive TB, age, sex, distance.

INTRODUCTION

Tuberculosis (TB) still exists in India as a significant public health problem. About 1.8 million new cases of tuberculosis occur every year, with about half of them being infectious cases of sputum smear positive pulmonary TB (PTB)[1]. TB kills more adults in the productive age group than any other infectious disease thus leading to socio-economic problems in the community. To overcome this enormous burden of TB, the DOTS strategy was introduced in the country in 1997 in the form of Revised National Tuberculosis Control Programme (RNTCP) and has been expanded in a phased manner to cover the entire population by 2006[1]. Under RNTCP, priority is given to detection and treatment of sputum smear positive cases, which are responsible for majority of transmission of infection in the community. Sputum microscopy services are thus provided at the designated Microscopy Centres (DMCs) usually set up in the PHCs having adequate out patient attendance, trained microscopist and facilities for sputum microscopy. Each DMC caters to a population of approximately one lakh residing in about 50-100 villages[2]. The other health centres present in its jurisdiction function as referral centres. Free treatment is given to the detected TB cases, under direct observation using

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standardized regimen through a network of health centers and other designated DOT (directly observed treatment) centres.

The primary objectives of RNTCP are to detect at least 70% of the estimated new sputum smear positive cases and to cure 85% of them[2]. While the cure rates in excess of 80% have been consistently achieved at the national level as well as in Karnataka State, the case detection rates of new sputum smear positive cases have shown considerable improvement in the recent years to meet the 70% target[1]. Nevertheless, there is scope for further improvement in case detection and treatment as we march towards effective TB control in the community. Utilization of services by the community is one of the key factors in effective implementation of RNTCP and may be related to age, gender and distance from residence to DMC and treating health centre, especially in rural areas. Knowledge on these aspects shall provide important inputs towards further strengthening the delivery of RNTCP services. Therefore, a study was undertaken in rural areas of Bellary District in Karnataka State with the following objectives:-

1. To find out the distribution of persons with pulmonary symptoms, new sputum smear positive cases of pulmonary TB and initial defaulters by age, gender and distance from residence to DMC.

2. To find out the sputum smear positivity rate by age and sex.

3. To find out treatment outcome among new sputum smear positive cases of pulmonary TB by age, gender and distance from residence to the treating health centre.

METHODOLOGY

The study was carried out in Sandur TU (Tuberculosis Unit) of Bellary District. Bellary was the first rural district in the State to be implemented with RNTCP in the year 2000 and therefore the utilization patterns of RNTCP were perceived to have stabilized in the district. Sandur TU was selected by simple random sampling out of four (TUs) in the district.

The data collection team consisting of three officials of National Tuberculosis Institute, Bangalore (NTI)-two field investigators and one statistical assistant visited Sandur TU and all of five DMCs and 19 PHCs under its jurisdiction. Information on persons with pulmonary symptoms (PPS) subjected to sputum smear examination and detected new sputum smear positive cases of PTB with respect to their age, gender and village of residence was obtained from the respective laboratory registers. Information regarding the distance from village of residence to DMCs and treating health centres was obtained from the respective health staff. Information on initial defaulters was collected by comparing the laboratory register with TB register and information on treatment outcomes was obtained from TB register and treatment cards. For the purpose of the study, initial defaulter was defined as the one found to be sputum smear positive but not initiated on treatment within one month of diagnosis.

Age and sex distribution of out-patients was collected from OPD registers of one randomly selected DMC (Sidiginamola) and all PHCs under its jurisdiction to find out the proportion of persons with pulmonary symptoms among new out-patients by age and sex.

Data collection as above for persons with pulmonary symptoms, new sputum smear positive pulmonary TB cases, initial defaulters, treatment outcome and out-patients was undertaken for the patients registered in the laboratory register, TB register and OPD register respectively during third and fourth quarters of 2003 and first and second quarters of 2004.

Definitions

For the purpose of the study, initial defaulter is defined as a sputum smear positive case detected under RNTCP but had not been initiated on treatment in the same TU.

DOT centre is defined as the place of consumption of anti-TB drugs under supervision by a designated provider.

Treating health centre is defined as the health centre where cases are registered for treatment.

Definitions of chest symptomatic, new sputum smear positive case, Tuberculosis unit and various treatment outcomes are as per RNTCP criteria[2].

RESULTS

Persons with pulmonary symptoms

A total of 1983 adult persons with pulmonary symptoms: males-1229 (62%), females-754 (38%) were subjected to sputum smear microscopy under Sandur TU during the four quarters, with male to female ratio as 1.6:1. Age and sex distribution of PPS is given at Table 1. About half of the PPS among males were in the age groups of 45 years and above while half of the female PPS were in 25-44 year age group.

About 70% of the PPS resided at more than four kilometers from the respective DMCs. Their distribution by distance from village of residence to DMC is given at Table 2.

New Sputum Smears (NSS) positive cases

A total of 232 NSS cases were diagnosed in Sandur TU during the four quarters, with male to female ratio as 2.5:1. About two-thirds of the cases were in the peak productive age group of 25-54

Table 1: Distribution of persons with pulmonary symptoms, new sputum smear positive by age and sex.

Age-group	Male		Female		Total	
	No. PPS	No. NSS	No. PPS	No. NSS	No. PPS	No. NSS
15-24	153 (12.4)	26 (15.8)	120 (15.9)	18 (26.9)	273 (13.7)	44 (19.0)
25-34	233 (18.9)	32 (19.4)	221 (29.3)	23 (34.3)	454 (22.8)	55 (23.7)
35-44	257 (20.9)	46 (27.9)	175 (23.2)	12 (17.9)	432 (21.7)	58 (25)
45-54	259 (21.0)	31 (18.8)	124 (16.4)	8 (11.9)	383 (19.3)	39 (16.8)
55+	327 (26.6)	30 (18.2)	114 (15.1)	6 (9)	441 (22.2)	36 (15.5)
Totals	**1229**	**165**	**754**	**67**	**1983**	**232**

PPS: Persons with Pulmonary Symptoms, NSS: New Sputum Smear Positive cases detected
No. : Numbers, ( ): vertical percentages

Table 2: Distribution of Persons with Pulmonary Symptoms (PPS) and NSS by distance from village of residence to DMC

Distance	Total (PPS)	Total (NSS)
<4 kms	622 (30.4)	65 (28.0)
5-9	138 (6.7)	31 (13.3)
10-14	254 (12.4)	43 (18.5)
15-19	202 (9.9)	17 (7.3)
≥ 20	830 (40.5)	76 (32.7)
Total	2046*	232

* Including 0-14 yrs

Table 3: Treatment outcome of NSS cases by sex

Treatment outcome	Male	Female	Total
No. evaluated	**126**	**60**	**186**
Cured	89 (70.6)	51 (85.0)	140 (75.3)
TC	9 (7.1)	2 (3.3)	11 (5.9)
Failure	3 (2.4)	1 (1.7)	4 (2.2)
Dead	16 (12.7)	4 (6.7)	20 (10.8)
Default	6 (4.8)	2 (3.3)	8 (4.3)
Transfer-out	3 (2.4)	0	3 (1.6)

No.: Number, (): vertical percentages.

years (Table 1). About two-thirds of the cases among males were aged 35 years and above while 60% the female cases were below 35 years. The peak age group was 35-44 years in males and 25-34 years in females with mean age being 40 years and 34 years respectively.

About 70% of the detected cases belonged to villages located at more than four kilometers from the respective DMCs (Table 2). About 53% of the cases resided at more than four kilometers from the respective treating health centres (Table 4).

Sputum positivity

About 12% of the persons with pulmonary symptoms were found to be positive for AFB (Acid fast bacilli) on sputum smear microscopy. The sputum positivity rate was higher among males at about 13% compared to females at about 9% (P=0.003). Differences in sputum positivity rate between males and females were also seen in individual age groups (tables not given). Sputum positivity rate was lower in 45+ age group in both sexes compared to the younger age groups. This trend was statistically significant among males (P=0.008) as well as females (P=0.004)

Initial defaulters

Of 232 detected NSS cases, 43 (18.5%) were initial defaulters. This was higher among males at about 23% compared to females at 9% (P=0.004). Proportion of initial defaulters was observed to increase with age in males while such trend was not clearly seen among females owing to small numbers. There was no relationship between the proportion of initial defaulters among NSS cases by distance from the village of residence either to DMC or PHC (Table not given).

Treatment outcome

Treatment outcome was available for 186 (80%) cases (Table 3). The overall treatment success was about 81% (Cured -75%, treatment completed-6%). It was significantly higher among females at about 88% compared to males at about 78%. Failures, defaults, transfer-outs and case fatality rates were significantly higher among males compared to females. There was no difference in treatment outcomes by age group (Table not given).

Table 4: Treatment outcome of NSS cases, by distance from village of residence to treating health centre

Distance in kms	No. of cases	No. Cured	No. Treatment completed	No. Dead	No. Defaulted	No. Failure on treatment	No. Transferred - Out
<4	86	64 (74.4)	8 (9.3)	7 (8.1)	2 (2.3)	2 (2.3)	3 (3.5)
5-9	50	39 (78.0)	0	6 (12.0)	3 (6.0)	2 (4.0)	0
10-14	19	15 (78.9)	2 (10.5)	1 (5.3)	1 (5.3)	0	0
15-19	12	11 (91.7)	0	1 (8.3)	0	0	0
>20	15	7 (46.7)	1 (6.7)	5 (33.3)	2 (13.3)	0	0
Totals	**182**	**136 (75.3)**	**11 (5.9)**	**20 (10.8)**	**8 (4.3)**	**4 (2.2)**	**3 (1.6)**

No. : Numbers, ( ): percentages out of number of cases

The treatment outcome by distance from the village of residence to the treating health centre is given at Table 4. It reveals that the outcome for cases residing at more than 20 kms was significantly poorer with lower cure rate and higher case fatality rate than those residing nearer.

Out-patients

A total of 31,200 new adult out-patients were registered in five health centres under Sidiginamola DMC (four PHC and one DMC). Considering that the total population covered by five health centres was 100327, the adult out-patient attendance was 31 / 100,000 population / year. The ratio of male to female out-patients was 0.9:1. About 1.1% of the total adult out-patients under Sidiginamola DMC were found to be PPS. Proportion of PPS was higher among males at 1.4% compared to females at 0.9% (P=.0003). Though this proportion was lowest in the 65+ age group, there was no significant trend by age group.

DISCUSSION

The analysis of data on out-patients under Sidiginamola DMC revealed that the number of males accessing health care services was lower than females. The out-patient male attendance was particularly lower in the peak productive age group of 15-54 years which accounts for majority of the TB cases occurring in the community thus raising concerns about accessibility of health services to male TB cases. The lower proportion of males seeking health care was consistent with findings of other studies and may be related to their primary role of wage earners and the consequent time constraints to access primary health care services during working hours [3-5].

However, larger number of males with pulmonary symptoms utilized RNTCP services than females in the ratio of 1.6:1 due to a higher prevalence of persons with pulmonary symptoms among male out-patients. The mean age of PPS in the present study was lower among females considering that the number of PPS increased with age among males while the maximum numbers among females were in 25-34 year age-group. The prevalence of pulmonary symptoms was also lower in elderly age group of 65+ years in both the genders when compared to younger age-groups. This was contrary to the findings observed in some of the community based studies thus underlining the need to be more

vigilant to screen persons with pulmonary symptoms among the elderly[6,7]

Among persons with pulmonary symptoms examined for sputum smear microscopy, the positivity rate for AFB was higher in males ultimately resulting in the male to female ratio of New Sputum Smear Positive Cases detected at 2.5:1. The difference in sputum positivity rate may be related to differences in the incidence / prevalence of other disorders of the respiratory system and needs to be investigated further. Even though, the sputum positivity rate decreased with age in both the sexes, a majority of the cases among males were in the age group of 35 years and above while majority of cases among females were in the younger age groups. Similar patterns in sex and age distribution of persons with pulmonary symptoms and TB cases have been observed during the various community based studies[6-13].

The proportion of initial defaulters was found to be significantly higher than expected, perhaps due to absence of a well-established mechanism of referral and recording of the same at the point of conducting this study.

Poorer treatment outcome in terms of cure, failure and deaths among male TB cases coupled with higher proportion of initial defaulters among them underline the need for greater efforts on the part of health workers to motivate male patients for initiating treatment and also to ensure treatment regularity as advised under the programme.

Long distance travelled by majority of persons with pulmonary symptoms and new sputum smear positive cases for accessing RNTCP services as observed in this rural area are attributable to one DMC for every 100,000 population- the norm having been fixed for maintaining quality of sputum smear microscopy. The poorer outcome among those residing at more than 20 kms may be attributable to lack of adequate supervision of DOT worker by the health centre staff from the respective treating health centre since direct observation of treatment (DOT) was being undertaken by Auxiliary Nurse Midwives (ANM) within the respective villages.

To summarize, study results indicate the need to make health services available to the male working population at convenient hours and also to be more vigilant to screen persons with pulmonary symptoms among the elderly. To overcome the problem of long distances travelled by majority of persons with pulmonary symptoms and new sputum smear positive, collection of sputum specimen may be undertaken at PHCs which may later be transported to DMC. Supervision and motivation of treatment for male TB cases and those residing more than 20 kms from the treating health centres requires to be strengthened.

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Essay Competition For Medical Students-2009

The Tuberculosis Association of India awards every year a cash prize of Rs. 500/- to a final year medical student in India for an original essay on tuberculosis. The subject selected for the year 2009 competition is 'Drug Resistant Tuberculosis'. The essay should be written in English, typed double spaced, on foolscap size paper and should not exceed 15 pages (approximately 3,000 words, including tables, diagrams, etc.). Four copies of the typescript should be forwarded through the Dean or Principal of a College/University to reach the Secretary-General, Tuberculosis Association of India, 3 Red Cross Road, New Delhi-110 001, before 30th June, 2009 along with a certificate that the author is a final year medical student.

Original Article

POST-HAART TUBERCULOSIS IN ADULTS AND ADOLESCENTS WITH HIV IN INDIA: INCIDENCE, CLINICAL AND IMMUNOLOGICAL PROFILE

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Summary

***Bacground*:** Highly Active Antiretroviral Therapy (HAART) was introduced in National AIDS Control Programme in 2004 to reduce the morbidity and mortality among those affected with HIV/AIDS. Tuberculosis, being an important co-infection, its emergence / occurrence in post-HAART period has potential implications.

***Objective:*:** Primary objectives were to study the incidence of post- HAART tuberculosis in HIV patients and to identify the possible risk factors. It was also intended to understand the clinical and immunological profile of this important condition.

***Methodology*:** Eligible adults and adolescents with HIV disease enrolled on HAART at Government Hospital of Thoracic Medicine, Tambaram Sanatorium, Chennai, from April, 2004 to March, 2007, formed the study population. They were monitored and screened for the occurrence of tuberculosis after commencing HAART. Clinical details and immunological profile of these patients were analysed.

***Results*:** Two hundred and sixty-two patients (5.1%) of 5099 patients followed-up for one to four years were found to have Post HAART TB with 100-person year risk of 2.83. Post HAART TB occurred predominantly in men (67.6%) and in 31-44 years age group (69.8%) with 100-person year risk being 3.26 and 2.83 respectively. Pulmonary, Extra-pulmonary and disseminated tuberculosis were found to occur in the frequencies of 78%, 16% and 6% respectively. A total of 144 patients (54.9%) developed tuberculosis within six months and this number increased to 202 (77%) by 12 months. 230 patients (87.7%) had base level CD4 cell count < 200 / mm^3.

***Conclusion*:** Tuberculosis was found to occur pre-dominantly in adult male patients with HIV during the first year after the initiation of HAART. Significantly, occurrence of Post HAART TB remained almost the same (5%) among patients treated for TB prior to the initiation of HAART. **[*Indian J Tuberc 2009; 56:69-76*]**

***Key Words*:** HIV, Post-HAART TB, Incidence, Risk factors.

INTRODUCTION

Morbidity and mortality resulting from Human Immunodeficiency Virus (HIV)-Tuberculosis co-infection continue to be the major challenge to both the National AIDS Control Programme and the National TB Control Programme in India, like in many other resource-limited settings. HIV infection is the greatest known risk factor for progression of TB disease from latent TB infection and Highly Active Antiretroviral Therapy (HAART) can reduce the progression to TB disease, TB relapse, and death[1]. While Tuberculosis remains a major cause of mortality among patients with HIV, even after successful initiation of HAART[2], age and sex-standardized mortality rates among HIV-infected TB patients decreased significantly over time[3]. HAART has an important role to play in controlling TB, as it reduces the incidence of TB in treated cohorts by approximately 80%[4]. In a population-based study conducted in Brazil, an 80% reduction in TB incidence was demonstrated in HAART-treated HIV-infected patients compared to ART-naïve patients under programmatic conditions[5]. It is also equally true that active TB may develop or sub-clinical TB may become apparent, after initiation of HAART, as a component of Immune Reconstitution[6], Though one of the recent Indian studies showed

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that the IRIS-TB could be to the extent of 3.5% of patients initiated on HAART[7], the incidence of Post-HAART TB is not clearly known. This study aims to identify the incidence, possible risk factors, clinical and immunological profile of POST-HAART TB in Indian Public Health setting.

MATERIAL & METHODS

The Government Hospital of Thoracic Medicine, Tambaram Sanatorium, Chennai (GHTM, Tambaram) is the largest care and support centre in the country, in identifying HIV-TB co-infection and in providing treatment for them. As the patients with HIV are being screened for eligibility for Anti-Retroviral Therapy, they are also evaluated for WHO clinical staging by identifying the possible presence of HIV related co-morbid conditions and tuberculosis is one of them. As part of routine screening exercise, all the patients are subjected to routine questions on respiratory symptoms, details of previous and current treatment history for tuberculosis, if any, and contact and family history of tuberculosis. All the responses are entered in the standardised pre-printed clinical assessment forms, which are subsequently electronically entered and stored in the data base of specially developed TB/HIV information system of GHTM, Tambaram. Those patients, who were on anti-TB treatment at the time of initiating HAART, were not included in this study.

All adults and adolescents with the HIV disease were screened for the possible co-existence of tuberculosis by performing sputum smear microscopy for AFB and radiological investigations including chest radiography. Among patients with abnormal chest x-ray lesions, in the absence of sputum smear positivity for AFB, differential diagnosis for other possible opportunistic infections were considered, investigated and ruled out. Those patients, who had persistent respiratory symptoms of cough and sputum even after getting treated with a course of antibiotics and cotrimoxozole for two weeks had repeat sputum smear examination and chest skiagram. Diagnosis of sputum smear negative pulmonary tuberculosis was made by a senior TB and Chest specialist in those patients who had persistent clinical symptoms and radiological pulmonary lesions with negative sputum smear microscopy on repeat examination, taking into the clinical course, radiological and other investigation results into consideration. Other specimens given by patients from extra-pulmonary sites were also subjected to smear microscopy for AFB before initiating HAART.

HAART Protocol

Persons living with HIV, found to be eligible by National AIDS Control Organization (NACO) Guidelines (2004), were enrolled in ART Programme

Table 1: Age and sex distribution of post-HAART TB

Age Group (in years)	Study Population	Post-HAART TB					
		Male		Female		Total	
		patients	%	patients	%	patients	%
15-29	981	20	11.3	29	34.2	49	18.7
30-44	3511	131	74.0	52	61.2	183	69.8
45 & Above	607	26	14.7	4	4.7	30	11.5
Total	5099	177	100	85	100	262	100

and provided with HAART. For the purpose of this study adults and adolescents, who were enrolled on HAART from April 2004 to March 2007, were selected for the analysis. They received their free treatment and clinical check-up during their monthly visits to GHTM. HAART regimens consisted of Zidovudine or Stavudine and Lamivudine and Nevirepine or Efavirenz. Zidovudine was preferred drug except in patients with anaemia. Efavirenz was preferred in the place of Nevirapine, when the latter drug was found to be intolerant. Patients identified a treatment guardian, such as a family member, friend, or drawn from NGO organization, who would be assisting them during treatment. In addition to a monthly clinical assessment, patients on HAART are provided with multiple counselling sessions, which addressed social, psychological issues, drug adherence and follow-up support. Body weight and haemoglobin level were measured every month. Laboratory monitoring, including the CD4 cell count by Flow cytometry, was done every six months.

Screening for Tuberculosis

During every visit of the patient at the Anti-Retroviral Therapy Centre (ART Centre), he or she was subjected to a detailed clinical history and clinical examination. Patients with respiratory symptoms were investigated for the possible development of pulmonary tuberculosis by resorting to sputum smear microscopy for AFB and chest radiography. Investigations including ultrasonography, CT/MRI scan and Fine Needle Aspiration Cytology and/or Tissue Biopsies were performed, as and when necessary, to confirm or exclude possible development of extra-pulmonary tuberculosis. All the patients under analysis had at least one year period of follow-up after getting initiated on HAART.

Statistical Methods

Incidence of POST-HAART TB in patients who had follow-up period varied from one to four years, 100-person year risk was calculated. Person-year risk was compared between groups using log-rank test. Chi-square was done to compare between groups percentages. Data was analyzed using SPSS ver 13.0.

RESULTS

Study Population

In all, 5099 adults and adolescents with HIV, attending GHTM Tambaram enrolled on HAART in National ART Programme from April 2004 to March 2008 formed the study population. There were 3036 males (59.5%) and 2063 females (40.55). While 30-44 years age group had the largest cohort of 3511 patients (68.9%), 15-29 years and >44 years group had 981 (19.2%) and 607 (11.9%) patients respectively, reflecting the attendance pattern of symptomatic HIV patients at an ART center under programme conditions. Significantly, 674 of female patients (32.7%) belonged to the 15-29 years age group.

Post-HAART Tuberculosis

Two hundred and sixty-two patients (5.1%) developed post-HAART TB of the 5099 patients initiated on HAART during the follow-up period,

Table 2: Post-HAART TB: Time of occurrence

Occurrence of Post HAART TB (Months)	Patients	%
Up to 6	144	54.9
7 - 12	58	22.1
13 - 18	30	11.5
18 -24	12	4.6
> 24	18	6.9
TOTAL	262	100

extending from one to four years. Table 1 provides the details of age and sex distribution of patients developing post-HAART TB. 177(67.6%) were males and 85(32.4%) females (p<.001). Predominantly, 183 patients were aged between 30 and 44 years. Increasing incidence of post-HAART TB was witnessed in both male and female groups from age 15 up to 44 years and reduced with age increasing to and beyond. Apparently, more female patients, 29 of 85(34.1%), in 15 – 29 age group were found to develop TB as against their male counterparts. However, when considered the study population 29 of 674 females studied (4.3%) and 20 of 307 males assessed (6.5%) developed post-HAART TB. However, the difference was not statistically significant (p=.93). Most (59.1%) had CD4 cell count <100.

Clinical Manifestations of Post-HAART Tuberculosis

Pulmonary tuberculosis was found to develop in 204 (77.9%) patients. In another 16 (6.1%) patients disseminated tuberculosis was

Table 3: Development of Post-HAART TB in relation to prior Anti-TB treatment

Prior Anti TB Treatment	Total Number of ART Patients Assessed	Post-ART TB			
		No		Yes	
		Patients	%	Patients	%
No	2615	2478	94.8	137	5.2
Yes	2484	2359	95.0	125	5.0
Total	5099	4837	94.9	262	5.1

Table 4: Development of Post-HAART TB in relation to base level CD4 cell count

observed involving the lungs and other organs . Sputum smear microscopy for AFB was found to be positive in 91 (41%) of 220 patients with pulmonary tuberculosis (including 16 with disseminated TB). Extra-pulmonary TB was detected in 42 (16%) patients, of whom 34 had single or multiple groups of lymph nodal involvement. While four patients had brain and or meningeal TB, two patients each had bone / joint TB and abdominal TB. 34 (81%) patients with Extra-pulmonary TB had either FNAC or Tissue biopsy confirmation. Overall clinical picture along with the ultrasonography and radiological imaging techniques (CT / MRI scans) contributed to the diagnosis of extra-pulmonary TB in the remaining patients.

Time of Occurrence

Development of Post-HAART TB was noticed during first six months of follow-up in 144(54.9%) patients (Table 2), of whom 109 (41.6%) had tuberculosis within three months. 202 (77%) patients were detected to have TB within one year of initiation of HAART (Table 2).Even after one year follow-up, Post-HAART TB continued to occur, though with reducing frequencies. 60 patients of the total 262 developed post-HAART TB (22.9%) 12 months after initiation of Anti-Retroviral Therapy. Significantly, six patients were also found to have immunological failure to first line Anti-Retroviral Therapy.

Post-HAART Tuberculosis in relation to prior anti-TB treatment

The breakdown rate of tuberculosis among previously treated tuberculosis patients after initiating HAART was analysed (Table 3). Among 2484 such already treated TB patients, development of TB after HAART was identified in 125 (5%) patients, which was almost the identical figure(5.2%) witnessed among 2615 TB treatment-naïve patients. The difference between the two groups is statistically insignificant (p=.786).

Table 5: Prognostic factors and risk of developing Tuberculosis after initiating HAART in HIV patients

Prognostic Factor	Variable	Total Number of ART Patients Assessed	Total Person Year Follow-up	Post-ART TB		100-Person Year Risk for Post ART TB
				Patients	%	
Age Group (in years)	15 – 29	981	1767.6	49	5.0	2.77
	30 – 44	3511	6454.8	183	5.2	2.83
	45+	607	1028.3	30	4.9	2.91
Gender	Male	3036	5433.1	177	5.8	3.26
	Female	2063	3828. 6	85	4.1	2.22
Overall		5099	9250.8	262	100	2.83

Post-HAART Tuberculosis in relation to base level CD4 cell count

Table 4 shows the frequency distribution of Post-HAART Tuberculosis in relation to the prevailing immunogenicity, measured as base level CD4 cell count, at the time of HAART initiation. 155 (5.9%) of 2608 patients, who had base level CD4 cell cont < 100 developed TB. Risk of developing TB was found to extend up to CD4 cell count level of 200. 230 (5.6%) of 4091 patients with CD4 cells up to 200 had post-HAART tuberculosis. The risk was significantly reduced to 3.2 for those 881 patients with CD4 cell count.

100-Person Year Risk for Post-HAART Tuberculosis

Five thousand ninety nine patients with total of 9250.8 person-year follow-up were assessed for developing post-HAART tuberculosis. Two hundred sixty two patients developed post- HAART tuberculosis and the overall risk was found to be 2.83 per 100-person year (Table 5). Two prognostic factors, age group and gender, were analysed for the risk of contributing to the development of post-HAART TB. The age groups, 15 – 29 years, 30 – 44 years and >44 years had almost equal risk (2.77, 2.83 and 2.91 per 100-person year respectively). However, in the gender analysis, men had an higher risk of post-HAART TB (3.26 per 100-person year) as against in women (2.22 per 100-person year), which is statistically significant ($p<.05$).

DISCUSSION

Tuberculosis and the emerging multi-drug-resistant TB epidemic represent major challenges to National TB and AIDS Control Programmes in resource-limited settings[2]. HAART benefits the individual patients from the occurrence of repeated opportunistic infections and also in most effectively treating them when occurred. For instance, evidences are available in Indian published literature for the need of HAART in effectively managing HIV-TB co infections. In spite of clinical and bacteriological improvement during treatment for TB in patients with HIV, immunologic deterioration may continue in the absence of HAART[8], highlighting the need for anti-retroviral treatment in addition to anti-tuberculosis treatment to improve the long term outcome[9].

Post-HAART TB had the similar manifestations of tuberculosis occurring in advanced immune suppression, witnessed in other Indian studies[10,11]. Higher Sputum smear negative pulmonary TB (59%), higher proportion of extra-pulmonary TB (16%) and increased frequency of disseminated TB (6.1%) were observed in this study , as most (59%) of the patients had <100 CD4cell count at the time of initiation of HAART and most (55%) of Post-HAART TB having developed within the first six months. The level of immuno-deficiency at which HAART is initiated and the response to HAART are important determinants of the risk of TB. In a multivariate analysis, a low baseline CD4+ count was found to be significantly associated with the risk of acquiring TB after the initiation of HAART[12].

Immune Reconstitution Inflammatory Syndrome (IRIS) had been implicated in the development of post-HAART TB, varying between 11% and 47%, particularly in developing countries where HIV and tuberculosis (TB) co-infection is very common[13]. IRIS occurred more frequently in ART-naïve patients with low baseline CD4 cell counts and high viral load[1,13,14,15] with lymph-node enlargement and extra-pulmonary and disseminated TB. Most of the 144 (55%) developed post-HAART TB in this study within the first six months of initiation of HAART are likely to be the resultant of immune reconstitution. However, it is also a matter of fact that 118 patients developed TB after six months, who are unlikely to fall in this category.

Incidence and clinical manifestations of post-HAART TB are likely to vary according to varying follow-up periods. Among 3151 patients analysed, with median follow-up time ranged from 3.7 months in Thailand or Kenya to 11.1 months in Cambodia, Incidence rates were 7.6, 10.4, 17.6, 14.3 and 4.8/100 person-years for pulmonary TB and 12.7, 4.3, 6.9, 2.1 and 0/100 person-years for extra-pulmonary TB in the programmes in Cambodia,

Thailand, Kenya, Malawi and Cameroon, respectively[6]. However, in the present study the overall incidence was relatively lower (2.83/100 person-years), as it had the longer follow-up period ranging from one to four years. As against the reported clinical manifestations of tuberculosis in the meta analysis[6], 62.3% of pulmonary TB and 54.9% of extra-pulmonary TB were diagnosed within three months after HAART initiation, the present study demonstrated lower extra pulmonary TB (16%) possibly because of longer follow-up.

Long term follow-up is necessary for the realistic assessment of Post-HAART TB incidence. Though the level of immunodeficiency at which HAART is initiated and the response to HAART are important determinants of TB, this risk remains appreciable even among those with a good response to HAART[12]. It should be realized that full restoration of circulating CD4 cell numbers occurs only among a minority of patients. Suboptimal restoration of *M. tuberculosis*-specific immune responses may greatly reduce the extent to which HAART is able to contribute to TB control at the community level because patients receiving HAART live much longer and yet would maintain a chronically heightened risk of TB[4]. However, it is also true that HAART is the major factor in prolonging survival in these patients[12].

It is not only the occurrence of post-HAART TB, but also the high mortality, even before completing the prescribed course of ATT, attributable to low immunity and complicating opportunistic infections[16], especially within first six months calls for suitable interventional strategies. This study specifically identified men (2.83/100 person-years) and <200 CD4 cell count (2.83/100 person-years) as the prominent risk factors. Further, early identification of HIV-TB co-infection is vital and linking both the programmes for effective earlier management of Tuberculosis and AIDS is the key for reducing the morbidity and mortality resulting from tuberculosis[17].

In conclusion, it is the long term follow-up of adult patients with HIV initiated on HAART provided the incidence of post-HAART TB as 2.83/100 person-years in India. The vital finding of this study, post-HAART tuberculosis occurred predominantly in male patients during the first year after the initiation of HAART, had brought out at least two important operational research questions to pursue, namely, the value of early institution of HAART, preferably when CD4 count is between 201 and 350 and the feasibility and efficacy of six month TB chemoprophylaxis to reduce incidence of post-HAART TB.

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CHANCHAL SINGH MEMORIAL AWARD - 2009

The Tuberculosis Association of India awards every year a cash prize of Rs.1000/- to a medical graduate (non-medical scientists working as bacteriologists, biochemists, etc, in the field of tuberculosis included) who is below 45 years of age and is working in the field of tuberculosis, for an original article not exceeding 30 double spaced foolscap size pages (approximately 6,000 words, excluding charts and diagrams) on tuberculosis. Articles already published or based on work of more than one author will not be considered. Papers may be sent, in quadruplicate, to reach the Secretary-General, Tuberculosis Association of India, 3, Red Cross Road, New Delhi-110001, before 30th June, 2009.

Original Article

TUBERCULOSIS MORTALITY TRENDS IN DELHI AFTER IMPLEMENTATION OF RNTCP

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Summary

Objectives: To study the impact of Revised National TB Control Programme on mortality among tuberculosis patients in Delhi and to correlate mortality trends with programme indicators.

Methods: Record based evaluation of mortality trends from TB registers of all chest clinics of Delhi after implementation of Revised National TB Control Programme.

Results: The study showed a statistically significant decline in tuberculosis mortality among new smear positive cases after the implementation of Revised National TB Control Programme ($z=4.478$ $p<0.05$). The mortality among new smear negative and extra pulmonary cases also showed reduction, though not statistically significant.

Conclusion: Mortality due to tuberculosis has been considerably reduced in Delhi over the years with the Revised National TB Control Programme implementation since 1997. ***[Indian J Tuberc 2009: 56:77-81]***

Key words **:** RNTCP, Mortality, Impact, Trends

INTRODUCTION

Tuberculosis, a disease caused by *Mycobacterium tuberculosis* is an important cause of morbidity and mortality worldwide. Globally 9.2 million new cases were reported and 1.7 million deaths occurred in 2006[1]. Reported incidence of TB in India, as per WHO, is 168/100000 and there are about 28 deaths/lac population in India[1]. These deaths due to tuberculosis are preventable by adequate treatment strategy. The millennium development goal aims to halve the prevalence and mortality due to TB by 2015 as compared to 1990 figures by halting and reversing the incidence[2]. DOTS strategy is a right step in this direction.

Delhi has an area of 1483 sq km.[3] and presently with a population of 17 million, the density of population is about 9390/sq km. Before the launch of the Revised National TB Control Programme in 1997, the State had 14 chest clinics which increased to 24 chest clinics alongwith a network of 180 DMCs each covering a population of one lac and 580 DOT centres where the facility of drug distribution is available. These measures of augmentation of infrastructure are expected to yield better results. It is expected that the mortality of patients due to tuberculosis will come down alongwith morbidity, since successful TB programme reduces both the prevalence and disease specific mortality.

The vital registration data regarding mortality rates, is often not available or is incomplete. The mortality surveys using verbal autopsy reports may provide reliable estimates but require collecting information in detailed forms by interviews with the intimate family members regarding deaths and may not be taken in a positive way by the family at times.

WHO defines the TB mortality as number of TB cases dying during the treatment regardless of the cause[4]. It has been calculated that total mortality due to tuberculosis has been reduced to about 4%[5] as compared to earlier figure of 29% for sputum positive cases[6] and 10-12% for sputum negative cases[5]. The differential mortality is about 18% which means that 18 lives are saved per 100 persons treated under the programme[7].

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OBJECTIVES

A retrospective record analysis for the State of Delhi was undertaken with following objectives :

- To study the impact of RNTCP on mortality among Tuberculosis patients in Delhi.
- To correlate mortality trends with the programme indicators.

MATERIAL AND METHODS

Study area comprised whole of Delhi State having a population of 170 lacs with a total case detection rate of 257/lac. Study design was a record based evaluation of mortality trends for past nine years. For data analysis, records on mortality were scrutinized from TB registers of all the chest clinics of Delhi State. Data was analysed with a statistical significance of $p<0.05$ using proportion test (z-test).

RESULTS

Revised National TB Control Programme of Delhi has been adjudged as the one of the best programmes in the country for all the programme indicators namely case detection rate, success rate, mortality rate and default rates. Table shows the mortality for the year 1999, 2002 and 2006.

*Annual case detection rate for Delhi was 296/lac as compared to national average of 130/lac and annual new smear positive case detection rate also was 87% well above national average of 70% in 2007[8]. Figure 1 shows the programme performance indicators contributing to mortality trends since 1999 to 2006.

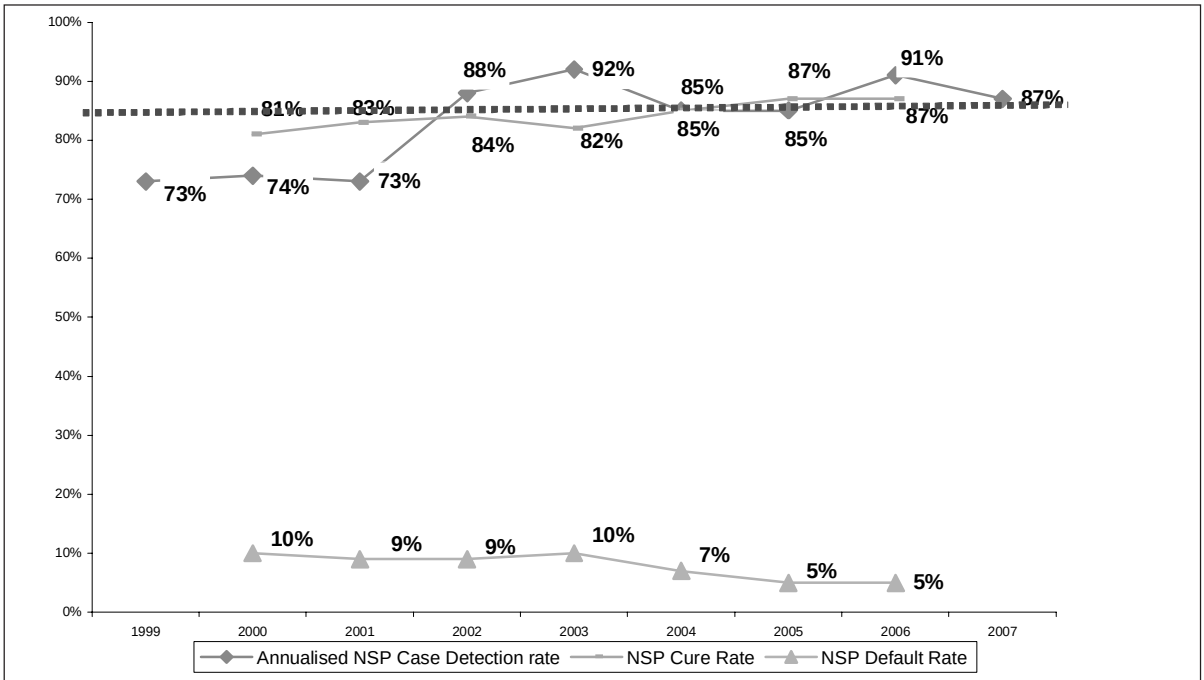
Trend of mortality is a significant and vital parameter for the evaluation of RNTCP. Figure 2 shows the mortality trend for different types of cases treated under the programme over the years since 1999 onwards.

The various programme factors which probably may have contributed to declining trend in mortality among sputum positive cases over the years since the start of RNTCP in 1997 are infrastructure increase (in 1999, there were in all 51 DOT Centres which increased to 540 by the end of 2006), ACSM (Advocacy, Communication and Social Mobilization) activities which had also been increased from 1997 onward and medical colleges involvement in 2003. Active participation of private sector and NGOs was also augmented under the programme. On the whole, in every way the

Table: Mortality among tuberculosis patients registered under RNTCP in Delhi

Year	Treatment outcome results (at the end of prescribed treatment) for mortality among									
	New smear positive		New smear negative		New extra pulmonary		Re-treatment cases		Total cases	
	No. Regd.	No. Died	No. Regd.	No. Died	No. Regd.	No. Died	No. Regd.	No. Died	No. Regd.	No. Died
1999	7928	282 3.55%*	4450	82 1.84%	3284	27 0.82%	4951	265 5.01%	20613	656 3.18%
2002	10615	272 2.56%	7262	108 1.48%	6606	57 0.86%	7640	366 4.90%	32123	803 2.49%
2006	13864	350 2.52%*	9457	181 1.91%	13626	122 0.89%	10661	544 5.1%	47608	1197 2.5%

*$p<0.05$ significant decline in mortality among NSP cases.



* z = 14.28, p<0.05 significant decline over the period in default rates (1999-2006)
* z = 25.92, p<0.05 significant increase in case detection rates (1999-2006)
* z =12.00, p<0.05 significant increase in success rates (1999-2006)

Figure 1 : Programme performance indicators contributing to mortality trends

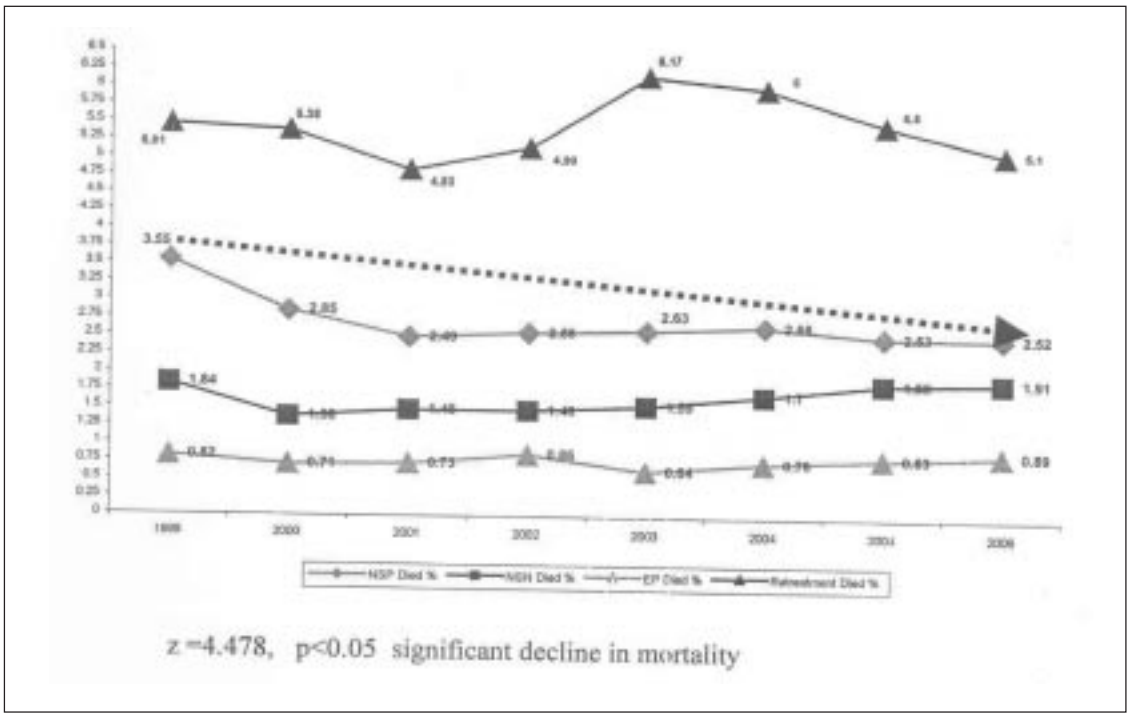


Figure 2 : Mortality trends in tuberculosis cases (1999-2006)

emphasis was to strengthen DOTS in the Delhi state to achieve RNTCP targets.

DISCUSSION

Kolappan et al[9] in their retrospective cohort study followed up 2674 patients for average period of 60 days and found that excess mortality was six times more than general population in their cohort and suggested that mortality rate and excess mortality be routinely used as a monitoring tool for evaluating the efficiency of national control programme.

A five-year epidemiological study in south India[10] estimated that specific death rate due to tuberculosis in 1978 was 84/lakh population >5 years and it represented 9% of mortality due to all the causes in the same population which were much lower than the estimates of TB deaths in India i.e. 250/lakh in 1949.

WHO defines tuberculosis mortality as the number of tuberculosis cases dying during the treatment regardless of the cause[4]. By this definition the death rate calculation will include patients who die during the course of treatment from causes unrelated to tuberculosis from different co-morbidities or even accidents. Besides this will not include the cases who are not registered under the programme and are being treated by private set up. Some of the cases who default from treatment may also die because of tuberculosis. Moreover, the patients may die due to tuberculosis after their outcome has been recorded in the TB register.

In a study in Iran by Alvi and Salami[11]; analysis of medical records of 3960 patients showed that 125 died during treatment; of which 67.2% were directly attributed to tuberculosis while 32.8% died due to medical problems unrelated to tuberculosis like cardiovascular diseases, AIDS, bacterial superinfection and sepsis, COPD, diabetes, renal failure, cancer or drug induced hepatitis. A study from Russia by Mathew et al[12] also found that 74.7% of 183 deaths were due to tuberculosis and rest were due to cardio-vascular diseases, carcinoma, alcohol related or indeterminate causes. Similar results were given by other workers Olle Goig JE[13] and Davies et al[14] in their studies.

In the cohort reported by Kolappan et al[9] from Chennai, the mortality continued to occur even after the completion of treatment and they observed therefore, that the mortality restricted to treatment period may result in underestimation of mortality and they followed up their patients for 20 months from the start of treatment. In our study we analysed the data of deaths under the programme and restricted ourselves to the mortality of tuberculosis during treatment period as per WHO definition.

The term mortality rate which for this article actually pertains to fatality rate i.e. number of deaths during treatment period divided by the total number of patients registered during the period was analysed for the cohorts for different years and further detailed analysis was made according to the type of cases. The case fatality among smear positive patients was observed to be higher compared to smear negative patients, the findings similar to Kolapan et al[9]. A significant falling trend of mortality among new smear positive patients over the years was also observed in this study.

Another method of observation may be to calculate excess mortality among tuberculosis patients compared to mortality in standard population. In general population, the mortality rate during 2006 was 5.99/1000 for Delhi[3]; whereas the mortality rate among newly registered TB cases for Delhi region during 2006 (Table) was 17.67/1000 (653/36947). Thus, the mortality among tuberculosis patients was three times higher than the mortality rate in general population. The standard mortality ratio (SMR) according to WHO is a better measure of the mortality than the general mortality among tuberculosis patients during treatment. So, for the present study, the SMR was found as 2.94 (95% Confidence Interval (CI;2.2 – 3.7) for the cohort of 2006. The standardized mortality ratio (SMR) was six times higher in the cohorts of 2002-2003 from Chennai[7].

The HIV status of the patients was not assessed being retrospective study and it is not a national policy for testing of HIV in all cases of tuberculosis, since the cases of tuberculosis may

have approximately 5% or less of HIV positivity; only cases of tuberculosis among whom there is a high index of suspicion or those who voluntarily disclose their HIV status or high risk behaviour are examined for HIV status.

The study has shown that in the state of Delhi, mortality due to tuberculosis has been considerably reduced, at least among new sputum positive cases, over the years with the implementation of RNTCP, since 1997. The mortality among smear negative and new extra pulmonary and retreatment cases, however, did not show any significant decline, presumably because the emphasis during phase I of RNTCP had been mainly on new smear positive cases. If such a successful programme is continued and quality care is provided as per International Standards of TB Care of Stop TB Strategy, the attainment of millennium development goal and control of 'problem of tuberculosis will be an achievable target.

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Original article

COMPARISON OF PHENOTYPIC AND GENOTYPIC METHODS FOR PYRAZINAMIDE SUSCEPTIBILITY TESTING

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***Objectives*:** To evaluate Pyrazinamide (PZA) susceptibility results obtained by phenotypic MGIT 960 TB system against enzymatic Pyrazinamidase assay and genotypic *pncA* gene sequencing. To find the prevalence of infections caused by *M. bovis* in PZA resistant *M. tuberculosis* complex isolates.

***Methods*:** 33 consecutive PZA resistant and 30 consecutive PZA susceptible isolates reported for PZA susceptibility testing by MGIT 960 TB system were included in this study. Presence of active pyrazinamidase enzyme was sought by using the Wayne assay. The *pncA* gene was amplified by PCR and then sequenced to screen mutations. All the PZA resistant isolates were further spoligotyped to identify *M. bovis,* if present.

***Results*:** Of 33 PZA resistant strains by MGIT 960, 31 were Wayne assay negative and two were positive. Of the 30 susceptible PZA strains six were Wayne assay negative reporting false resistance. *PncA* gene sequencing revealed that 32 of the 33 MGIT PZA resistant isolates had diverse nucleotide changes scattered throughout the *pncA* gene (one isolate did not show any mutation). Of the 30 phenotypically susceptible isolates, 21 were wild types whilst nine isolates showed the presence of a silent mutation C-T at codon 195. Fifteen mutations found in this study has not been described earlier. Not a single isolate of *M. bovis* was detected among PZA resistant *M. tuberculosis* complex isolates.

***Conclusion*:** MGIT 960 showed better concordance with sequencing results in comparison with Wayne assay. In present study, a high proportion (85%) of MDR-TB isolates from patients receiving anti-TB treatment were found to be resistant to PZA. **[*Indian J Tuberc 2009; 56:82-90*]**

***Key Words*:** Pyrazinamide susceptibility, *M. tuberculosis*, *pncA* gene mutations

INTRODUCTION

Pyrazinamide (PZA) is an important drug in the short-course tuberculosis (TB) chemotherapy. It is active against semi-dormant, persisting and non-dividing TB bacilli, even against those residing intracellularly.[1,2] PZA, a nicotinamide analog is a pro-drug that is activated by bacterial pyrazinamidase (PZAase) enzyme to the active moiety, pyrazinoic acid (POA) which kills *M. tuberculosis*.[3] PZAase enzyme is encoded by *pncA* gene. Many studies have reported that mutations in *pncA* gene result in loss of PZAase activity causing PZA resistance in *M. tuberculosis*.[3-6] The mechanism of natural PZA resistance in other mycobacteria is more complex. Many species of mycobacteria like *M. bovis*, *M. bovis* BCG are naturally resistant to PZA due to alterations in *pncA* gene.[7] In *M. bovis*, a single point mutation (C to G) at nucleotide position 169 in *pncA* gene, causes substitution of amino acid histidine to aspartic acid producing a non-effective PZAse.

With increasing incidence of Multi-Drug Resistant (MDR) and Extensively Drug Resistant (XDR) TB, rapid and accurate prediction of PZA resistance is essential. The conventional culture tests used for PZA susceptibility testing are time-consuming and difficult to perform as the drug is active only in a relatively low pH environment which causes nearly 50% inhibition in the colony count of *M. tuberculosis* and a considerable reduction in colony size compared with a neutral pH environment. Other important factors like inoculum size, drug concentration can also affect the susceptibility results. Various rapid liquid culture methods such as BACTEC 460, MGIT 960 BacT/ALERT 3D system has been reported to be useful for rapid and reliable susceptibility testing of MTB isolates. In several studies, a lack of PZAase activity has been observed to be correlated with PZA resistance. This made the detection of PZAase activity an interesting alternative to the conventional methods[7,8] This assay detects the presence of active

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PZAase enzyme by the hydrolysis of PZA to POA as evidence by a colour change.[9]

At our tertiary care centre, PZA susceptibility is routinely performed using MGIT 960 TB system. On analyzing the data of 236 total requests for PZA susceptibility by MGIT 960 TB system during a period of Jan to May 2007, 141 (59%) were found to be resistance to PZA. As MGIT 960 TB system is very senstitive to a inoculation procedure, the present study was undertaken to evaluate PZA susceptibility results obtained by this system with enzymatic PZAase assay (Wayne's method) and genotypic *pncA* gene sequencing. As *M. bovis* is intrinsically resistant to PZA, another objective of this study was to find the prevalence of infections caused by *M. bovis* in PZA resistant isolates by spoligotyping.

MATERIAL AND METHODS

Setting: A tertiary care centre located in Central Mumbai with a referral bias towards non-responding cases.

Subjects: Thirty-three consecutive PZA resistant and 30 PZA susceptible MTB complex isolates processed by MGIT 960 TB system over a period of May - July 2007 were further evaluated by PZAase assay, *pncA* gene sequencing and fingerprinting (spoligotyping).

PZA Susceptibility using BACTEC MGIT 960 TB system:

All the isolates were tested for PZA susceptibility by the BACTEC MGIT 960 TB system.(Beckton Dickinson, Sparks, MD) at PZA concentrations of 100 ìg/ml, as recommended by the manufacturer.

Procedure

1. Prior to inoculating the PZA set tubes, 0.8 ml of BACTEC MGIT 960 PZA supplement was added to both growth control and PZA tubes and 100 µl of PZA solution was added to the PZA tubes.
2. Inocula were prepared following instructions of the manufacturer:

   (i) MGIT cultures were used for PZA susceptibility testing no sooner than the day following instrument positivity (day 1) and no later than five days following the day of instrument positivity. On days one and two following positivity, an undiluted inoculum was used, while on days three through five suspensions were diluted 1:5 with sterile saline then 0.5 ml inoculated into the MGIT PZA tubes by using syringe and needle. The growth control tube was inoculated with 0.5 ml of 1:10 dilution of the *M.tuberculosis* suspension.

   (ii) Cultures grown on Lowenstein-Jensen (LJ) medium were used for PZA susceptibility testing no later than 14 days after the first appearance of colonies on the slant. Colonies were scraped from the medium with a sterile loop. A suspension adjusted to be equivalent to a 0.5 McFarland standard was prepared by using glass beads to ensure homogeneity and then diluted 1:5 prior to inoculating 0.5 ml of the suspension into the MGIT PZA set. All inoculated PZA sets were loaded into the BACTEC MGIT 960 instrument within eight hours of inoculation.

3. Interpretation of results

Using pre-defined algorithms, readings were automatically interpreted by the BACTEC MGIT 960 instrument and reported as either susceptible or resistant. The "unloaded PZA set report" listed growth units, time to result, and susceptible, resistant, or invalid results.

Table 1: Analysis of PZA susceptible isolates by three different methods

No	Strain	Pyrazinamidase Assay	MGIT 960	Sequencing	Sensitivity	Spoligo Pattern
1	3119	Susceptible	Susceptible	Wild type	S to all	26
2	3147	Susceptible	Susceptible	Wild type	S to all	U
3	3168	Susceptible	Susceptible	Wild type	R-SI. S-REKEtPOZ	50
4	3200	Susceptible	Susceptible	Wild type	R-SIR,S-EKEtPOz	37
5	3259	Susceptible	Susceptible	195 C→T	S to all	288
6	4450	Susceptible	Susceptible	Wild type	S to all	U
7	4492	Resistant	Susceptible	Wild type	S to all	1(B)
8	4515	Susceptible	Susceptible	Wild type	R-IEt,I-P, S-SREKOZ	48
9	4607	Susceptible	Susceptible	Wild type	S to all	U
10	4666	Susceptible	Susceptible	Wild type	S-SIREZ	42
11	4753	Susceptible	Susceptible	195C→T	S to all	U
12	4755	Susceptible	Susceptible	Wild type	S to all	26
13	4760	Susceptible	Susceptible	195 C→T	S to all	U
14	15	Resistant	Susceptible	Wild type	R-IREEtPO,S-SKZ	53
15	30	Susceptible	Susceptible	Wild type	S to all	340
16	147	Susceptible	Susceptible	Wild type	S to all	334
17	158	Resistant	Susceptible	195 C→T	R-I,S-SREKEtPOZ	U
18	171	Susceptible	Susceptible	Wild type	R-SIRE,S-KEtPOZ	U
19	174	Resistant	Susceptible	195 C→T	S-SIREZ	U
20	281	Susceptible	Susceptible	Wild type	S to all	U
21	342	Susceptible	Susceptible	Wild type	R-SIRE,S-KEtPOZ	U
22	424	Susceptible	Susceptible	Wild type	S to all	340
23	437	Susceptible	Susceptible	195 C→T	S to all	U
24	482	Resistant	Susceptible	195 C→T	R-I,S-SREKEtPOZ	288
25	710	Susceptible	Susceptible	Wild type	S to all	53
26	759	Susceptible	Susceptible	Wild type	S to all	126
27	1070	Susceptible	Susceptible	Wild type	R-SIP,I-Et,S-REKOZ	26
28	956	Susceptible	Susceptible	195 C→T	S to all	U
29	1152	Susceptible	Susceptible	Wild type	S to all	U
30	4631	Resistant	Susceptible	195 C→T	S to all	357

Key: S- Streptomycin K -Kanamycin
I – INH Et - Ethionamide
R – Rifampicin P - PAS
E – Ethambutol O - Ofloxacin

Table 2: Analysis of PZA resistant isolates by three different methods and mutations identified in PZA resistant isolates

No	Strain	Pyrazinamidase Assay	MGIT 960	Sequencing	Sensitivity	Spoligo Pattern
1	3005	Resistant	Resistant	29 A→G, 195 C→T	R-SIRZ,S-E	356
2	3042	Resistant	Resistant	-11 A→G, 483 G→A	R-SIREKEtOZ,I-P	1(B)
3	3139	Resistant	Resistant	42 C→A, 535 Deletion A	R-SIREZ,S-KEtPO	655
4	3181	Resistant	Resistant	195 C→T, 535Deletion A	R-SZ,S-IREKEtPO	U
5	3232	Resistant	Resistant	-11 A→G, 535 Deletion A	R-SIROZ,S-EKEtP	1(B)
6	4031	Resistant	Resistant	195 C→T, 406 G→A	R-SIRPZ,S-EKEtO	U
7	4477	Resistant	Resistant	389 Insertion GG	R-SIREZ,S-KEtPO	1(B)
8	4480	Resistant	Resistant	395 G→C	R-SIREtOZ,I-E, S-KP	1(B)
9	4518	Resistant	Resistant	359 T→G	R-SIRZ,S-EKEtPO	1(B)
0	4649	Resistant	Resistant	389-90 Insertion G	R-IREZ,S-SKEtPO	429
11	4721	Resistant	Resistant	488 T→C	R-SIROZ, I- Et, S-EKP	11
12	O28	Resistant	Resistant	195 C→T, 314 G→A, 535 DA	R-SIREKEtOZ,S-P	126
13	44	Resistant	Resistant	347 T→G	R-IREPZ,S-SKEtO	U
14	47	Susceptible	Resistant	226 A→C	R-SIREKOZ, S-EtP	1(B)
15	95	Resistant	Resistant	289 G→T	R-SIRZ,S-EKEtPO	1(B)
16	115	Resistant	Resistant	226 A→C	R-SIREtZ, I-P, S-EKO	U
17	120	Resistant	Resistant	559 T→G	R-SIREPOZ,I-Et, S-K	1(B)
18	138	Resistant	Resistant	Deletion 382-90	R-Z,S-SIREKEtPO	138
19	149	Resistant	Resistant	200 insertion 8 NA	R-SIREZ,S-KEtPO	U
20	159	Resistant	Resistant	-11 A→G	R-SIRZ, I-P, S-EKEtO	1(B)
21	180	Susceptible	Resistant	195 C→T, 515 T→C	R-SIRZ,S-EKEtPO	357
22	193	Resistant	Resistant	156 C→A, 546 G→T	R-SIREKEtZ, S-PO	48
23	201	Resistant	Resistant	286 A→G	R-IREOZ, I-EtP, S-SK	U
24	206	Resistant	Resistant	14 T→G	R-SIREPOZ,S-Ket	1(B)
25	227	Resistant	Resistant	418C-T,415 Del T	R-IRZEtOS-SKP	48
26	246	Resistant	Resistant	195 C→T,328 G→T, 535DA	R-IREZ,S-SKEtPO	22
27	365	Resistant	Resistant	14 T→G	R-SIREPOZ,S-KEt	1(B)
28	386	Resistant	Resistant	538 G→T	R-IREZ,I-Et,S-KP	340
29	405	Resistant	Resistant	-11 A →G	R-SIRZ,S-KEtPO	1(B)
30	414	Resistant	Resistant	Wild type	R-IREOZ,S-SKEtP	1(B)
31	425	Resistant	Resistant	152 A → G	R-SIREOZ,S-KEtPP	1(B)
32	836	Resistant	Resistant	195 C→T, -11 A→G	R-IREZ	26
33	1017	Resistant	Resistant	80 T→C	R-SIREOZ,S-KEtPP	1(B)

Key: S- Streptomycin K -Kanamycin
I – INH Et - Ethionamide
R – Rifampicin P - PAS
E – Ethambutol O - Ofloxacin
DA – Deletion of A
(1B) - Beijing genotype

Quality control

M. tuberculosis (H37Rv, ATCC strain 27294) was used for each lot of BACTEC MGIT 960 PZA medium and BACTEC MGIT 960 PZA drug used in this study.

Enzymatic Pyrazinamidase Assay (Wayne's method):

Briefly a heavy a bacterial inoculum (2-3 weeks old) was overlaid on the surface of Dubos agar in a test tube containing 100 µg PZA per ml. The inoculated tubes were incubated at 37^0 C for four days and seven days. Then 1ml of freshly prepared 1% ferrous ammonium sulphate was added. The tubes were allowed to stand at room temperature for 30 minutes and then examined for a pink band in the agar medium. If the four-day tube was negative or doubtful, the test was repeated at seventh day using the second tube. Interpretation of PZAase assay was done independently by two observers. Development of pink colour band due to enzymatic hydrolysis of PZA to free POA was considered as sensitive whereas absence of pink band was considered as resistant.

Screening of *pncA* gene for pyrazinamide (PZA) resistance

DNA extraction was done from culture media using cetyltrimethylammonium bromide (CTAB) - NACl method. *PncA* gene PCR was performed as described previously.[3,10] The *pncA* forward primer 5'GTCGGTCATGTTCGCGATCG3' (105 bp upstream of *pncA* putative promoter region) and the reverse primer 5'GCTTTGCGGCGAGCGCTCCA3' [60 bp downstream of stop codon of the *M. tuberculosis pncA* gene (U59967)]. Cycling parameters were 95^0C – 5 mins followed by 95^0C – 1min, 62^0C – 1 min, 72^0C – 1 min, 30 cycles were performed and were followed by final elongation of 72^0C – 5 mins. The expected size of the *pncA* PCR products was 720 bp. The *pncA* PCR products were run on 2% agarose gel. Then the PCR products were later sent for commercial DNA sequencing to validate the results. Mutations in the sequences of the *pncA* gene from pyrazinamide-resistant strains were identified by comparison with the wild-type *M.*

Table 3: Comparison of PZAase assay with culture

N = 63	MGIT 960 TB system	
	Susceptible (30)	Resistant (33)
Wayne's PZAase Positive (26)	24	02
Wayne's PZAase Negative (37)	06	31
Sensitivity	94%	
Specificity	80%	

Table 4: Comparison of MGIT 960 and PZAase assay with sequencing

N = 63	MGIT 960 TB system		Wayne's PZAase Assay	
	Susceptible (30)	Resistant (33)	Susceptible (26)	Resistant (37)
Sequencing Wild type/ Silent mutation* (31)	21 / 9*	01	19 / 5*	3 / 4*
Sequencing Mutants (32)	00	32	02	30
Sensitivity	97%		92%	
Specificity	100%		81%	

Key: * - Silent mutations

tuberculosis pncA gene sequence using BLAST (www.ncbi.nlm.nih.gov).

Finger printing - Spoligotyping

All the 63 culture isolates were subjected to spoligotyping using a commercially available *Isogen kit, Netherlands* as described by Kamerbeek *et al.*[11] Briefly, the entire Direct Repeat (DR) region of these isolates was amplified using specific biotynylated primers; followed by hybridization to a set of 43 known spacer sequences (probes) covalently linked to a nylon membrane (*Isogen, Netherlands*). Detection was done by using Enhanced Chemiluminescence (ECL) based detection system (Amersham, UK).

RESULTS

Analysis of 30 PZA susceptible and 33 PZA resistant isolates by four different methods viz BACTEC 960 TB, Wayne's PZAase assay, sequencing and spoligotyping are shown in Tables 1 and 2 respectively, Among 33 PZA resistant isolates, 31 were Wayne assay negative (PZA resistant) and two were positive (PZA sensitive). Whereas among 30 PZA susceptible isolates six were Wayne assay negative (PZA resistant) and 24 were positive (PZA sensitive) [Table 3].

Of the 30 phenotypically susceptible isolates (Table 1), 21 were identified as wild types on sequencing. Nine isolates showed the presence of a silent mutation C→ T at codon 195, which probably represents the natural polymorphism of the gene and has been reported before. Sequencing of 33 PZA resistant isolates (by MGIT 960) confirmed *pncA* gene mutations like nucleotide substitutions (missense mutations), insertions and small deletions causing amino acid substitutions or frame shifts leading to nonsense polypeptides in 32 cases. These mutations were dispersed along the *pncA* gene. Four isolates carried mutations at -11(A→G), the upstream region of the gene. Double mutations were noticed in 11 isolates. Fifteen mutations found in this study had not been described earlier. Mutation on *pncA* gene was not detected only in one PZA resistant isolate. As described in Table 4, considering sequencing as a gold standard the sensitivity and specificity of MGIT 960 TB system for PZA susceptibility testing was found to be 97% and 100% whereas for Waynes PZAase assay it was found to be 92% and 81% respectively.

As shown in the Table 2, of the 33 PZA resistant isolates by MGIT 960, 31 were MDR-TB cases, one was resistant to SM and PZA and the remaining one was PZA mono-resistant. In the present study, PZA resistance was found to be strongly associated with MDR-TB. Further screening of these PZA resistant isolates by spoligotyping (Table 2) showed the prevalence of Beijing genotype (15/33 i.e 45%). *M. bovis* was not detected among PZA resistant *M. tuberculosis* complex isolates by spoligotyping and sequencing.

DISCUSSION

Rapid and reliable susceptibility testing for PZA is vital as it is administered in the early intensive phase of anti-TB treatment. In vitro PZA susceptibility testing of mycobacteria is difficult as it requires an acid environment (pH 5.5).[12,13] A heavy inoculum may result in false resistance because the bacterial mass locally neutralizes the acidic pH in the media. On the other hand, with a low inoculum, the mycobacteria may not grow at a low pH.

Various studies have reported the performance of the automated MGIT 960 system for testing of susceptibility to PZA at pH 5.9.[14-18] At our tertiary care centre, BACTEC MGIT 960 system is being used for routine PZA susceptibility testing from January 2007. Over all 59% PZA resistance was observed by MGIT 960 System over a period of four months (Jan – May 2007). In the present study, the results of 30 PZA susceptible and 33 PZA resistant isolates obtained by MGIT 960 were evaluated against pyrazinamidase assay and *pncA* gene sequencing (Table 3).

Lack of PZAase activity has been observed to be correlated with PZA resistance in several studies.[3,5,9,12] Therefore, the detection of PZAase

activity by Wayne's PZAase has been used for PZA susceptibility testing. The Wayne assay is simple to perform, cost effective and results are available within seven days. In the present study, discrepancy between MGIT 960 and PZAase assay was noticed in eight isolates, As shown in Table 3, two of the 33 MGIT PZA resistant isolates were classified as PZA susceptible by Wayne's method. PZA resistant isolates with a positive PZAase test have been reported in previous studies by several authors[13,23,24] confirming that PZA resistant isolates are not always PZAase negative. Similarly six MGIT PZA susceptible isolates were identified as PZA resistant by Wayne's assay. Various factors like pH of the medium, inoculum size, growth phase of the organisms, subjectivity in the result interpretation etc. can contribute to the false resistance by PZAase assay. Often, development of faint colour band can lead to the possibility of a manual error whilst interpreting the results. Previous studies have also commented on the difficulty of interpretation of this test.[24] Another disadvantage of PZAase assay is that it cannot be performed directly from liquid culture broth. Generally at least three week old heavy inoculum from L.J. is required, for the assay to be positive. Tests for PZAase have shown agreements with culture methods of between 89% and 99%.[8,12,19] In the present study sensitivity and specificity of PZAase assay was found to be 92% and 81% respectively compared to the gold standard i.e sequencing (Table 4). Singh et al reported increased PZAase assay specificity (96%) on prolonged incubation upto ten days.[24] So additional incubation from seven to ten days may help to increase the specificity of PZAase assay.

The sequencing results obtained from this study are important as there is very limited data on *pncA* gene mutations from India. As shown in the Table 2, 31 types of variations including 15 variations not reported previously were identified in 33 PZA resistant isolates. All novel mutant sequences and representative sensitive *pncA* sequences were deposited in Genbank (accession nos. FJ151173 to FJ151196). Secondly the types of mutations found were diverse: Nucleotide substitutions (77%), deletions (12%), insertions (9%) were observed. Such characteristics are also observed in previous studies.[5,9,10,20-23] Lastly, the mutations were dispersed throughout the *pncA* gene including the upstream region. This scattered pattern of mutation is more obvious when all reported data are combined. To date, mutations have been found to alter 44% of the codons (81/186) with no specific hotspots for variation. This is very unusual compared to other drug related genes, such as *rpoB* and *katG* where several restricted sites are involved in the conversion to drug resistance. Only one PZA resistant strain did not show any alteration in the coding region or the promoter of the *pncA* gene. For this strain, it has been postulated that the PZA resistance could be due to mutations in an unknown *pncA* regulatory gene.[9]

None of the sensitive strains revealed mutations observed in the resistant isolates. Nine PZA susceptible isolates showed presence of a silent mutation C→T at codon 195 which probably represents the natural polymorphism of the gene and has been reported earlier.[25] By using sequencing as a means of early resistance detection, the inconsistency of phenotypic pyrazinamide assays can be circumvented. Unique mutation CAC→GAC at nucleotide position 169 (HIS57ASP) in the *pncA* gene defined originally in *M. bovis*, (member of *M. tuberculosis* complex family) was not observed in any of the PZA resistant strains.

As shown in Table 2, 31 of the 33 PZA resistant strains were found to be resistant to INH and RIF i.e MDR whereas among 30 PZA susceptible strains only four were resistant to INH and RIF (MDR). All the 35 MDRTB patients were receiving anti-TB treatment for various periods of time. In the present study, PZA resistance is found to be associated with MDRTB (30/35 i.e 85%). Only one PZA mono-resistant strain was observed. In a study from Peru, 47 of 80 (59%) MDR-TB patients had *M. tuberculosis* resistant to PZA.[26] The high rate of PZA resistance in this study could be attributed to the fact that ours is a tertiary referral center and most of the patients are chronic cases and more likely to be unresponsive to therapy. Further analysis of all the PZA resistant strains by spoligotyping showed that among 31 MDR isolates 15 belonged to Beijing

genotype. Among PZA resistant strains *M. bovis* was not detected by spoligotyping.

To summarize, PZA resistance was found to be associated with MDR-TB and was more common in patients receiving anti-TB treatment. It is important to test for pyrazinamide resistance especially in all suspected MDR-TB, re-treatment and relapse cases, since only four of the 35 MDRTB strains (15%) were susceptible to PZA. PZA susceptibility by MGIT 960 showed better concordance with sequencing (gold standard). The fully automated non-radiometric MGIT 960 system is less labour-intensive for PZA susceptibility testing and results are available between 8-12 days. However, cost and feasibility remain the main constraint. Qualitative measurement of PZAase activity can be a cost effective method for the rapid screening of PZA resistance however there is subjectivity in interpreting the results. Fifteen ovel mutations were identified among PZA resistant isolates by sequencing. *M. bovis* was not detected among PZA resistant strains by spoligotyping.

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I, S.C. Goyal, Secretary General of the Tuberculosis Association of India, 3, Red Cross Road, New Delhi-110 001, hereby declare that the particulars given above are true to the best of my knowledge and belief.

S.C. Goyal
On behalf of the Tuberculosis Association of India

Indian J Tuberc 2009; 56: 91-94

STATUS REPORT ON RNTCP*

The Revised National Tuberculosis Control Programme (RNTCP) has achieved the annualized NSP case detection rate of 67% and success rate of 87% at the national level as per the reports submitted at the end of fourth quarter, 2008. With this, the annualized NSP CDR for the year 2008 is 72% and the programme has once again achieved at the national level the objective of at-least 70% NSP case detection and NSP treatment success of at-least 85%.

RNTCP performance in fourth quarter 2008

During the fourth quarter, over 1.65 million suspects were examined, 207,144 sputum positive cases were diagnosed, and 351,593 TB cases were registered for treatment. The annualized total case detection rate is 123 cases per 100,000 population. The new smear positive PTB case detection rate (annualized) for the fourth quarter 2008 was 67 % of the estimated cases, with a total of 143, 540 new smear positive PTB cases being registered for treatment. In addition, 90,272 new smear negative PTB cases, 49,531 new extra pulmonary TB cases, 46,316 smear positive re-treatment PTB cases and 21,514 'Others' were also initiated on treatment during this quarter. The treatment success rate amongst the new smear positive PTB cases registered in the fourth quarter 2007 is 87%. The sputum conversion rate and cure rate among the new sputum positive PTB cases were 90% and 84% respectively.

The default rates among the NSP (5.6%), NSN (7.2%) and re-treatment cases (14.1%) are showing declining trends. Though this is encouraging, the current default rates still remain a cause of concern.

The Central TB Division has conducted a nationally representative case control study on Cat-II defaulters which had shown that in about one

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

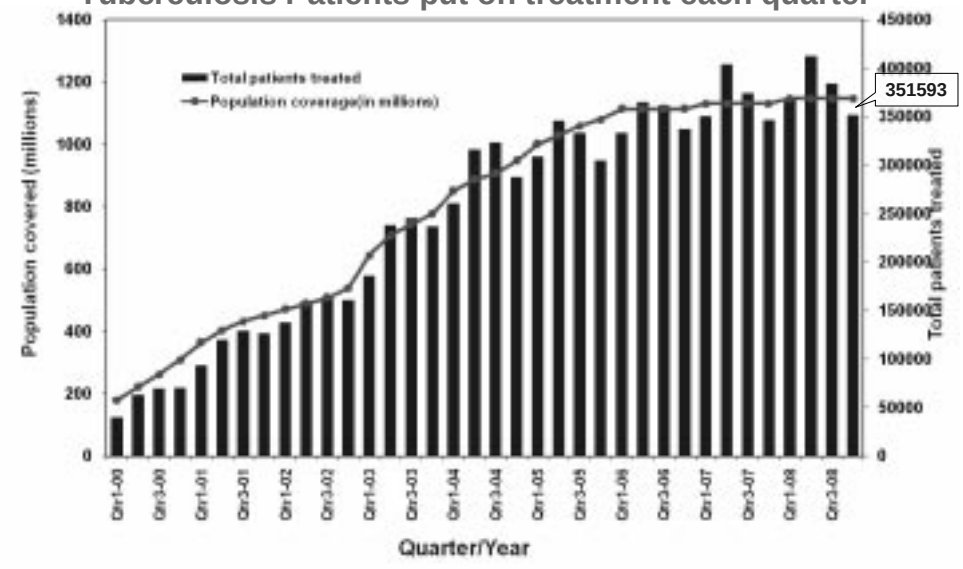

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Table: Performance of RNTCP Case Detection (2008, fourth quarter), Smear Conversion (2008, third quarter), and Treatment Outcomes (2007, fourth quarter)

State	Population (in lakh) covered by RNTCP[1]	Suspects examined per lakh population	No of Smear positive patients diagnosed[2]	Total patients registered for treatment[3]	Annualized total case detection rate	New smear positive patients registered for treatment	Annualized new smear positive case detection rate (%)		No of new smear negative cases registered for treatment	No of new EP cases registered for treament	No. of smear positive retreatment cases registered for treatment	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	257	97	226	220	71	69	92%	86	43	21	95%	82%	84%
Andhra Pradesh	822	163	18597	28498	139	12282	60	80%	7926	2878	3952	91%	87%	89%
Arunachal Pradesh	12	200	264	569	190	188	63	84%	139	102	81	89%	87%	88%
Assam	299	116	4749	8698	116	3686	49	66%	2581	1054	773	91%	86%	88%
Bihar	938	82	10369	20005	85	8481	36	48%	6321	1259	1844	89%	81%	88%
Chandigarh	11	263	367	497	187	178	67	71%	84	142	69	91%	87%	88%
Chhatisgarh	236	108	2858	6407	108	2408	41	51%	2573	799	316	89%	83%	87%
D & N Haveli	3	213	85	137	209	39	60	74%	31	29	13	93%	76%	76%
Daman & Diu	2	312	44	43	91	14	30	37%	18	7	1	92%	54%	58%
Delhi	171	212	5190	9638	226	2780	65	69%	1538	2849	1501	90%	88%	88%
Goa	16	178	242	527	129	157	39	48%	112	153	58	93%	81%	84%
Gujarat	564	173	14097	19051	135	8547	61	76%	2637	2258	4077	92%	86%	87%
Haryana	238	145	4836	7608	128	2683	45	48%	1589	1081	1664	90%	84%	85%
Himachal Pradesh	66	195	1728	2845	174	1110	68	71%	450	646	463	94%	87%	89%
Jammu & Kashmir	124	138	1760	2861	93	1309	42	45%	467	613	390	92%	89%	90%
Jharkhand	300	111	4980	9161	122	4087	54	73%	2919	643	715	90%	85%	91%
Karnataka	574	180	9709	15699	109	5936	41	55%	3614	3021	2090	87%	78%	79%
Kerala	342	194	3507	6226	73	2721	32	64%	1236	1506	574	84%	81%	83%
Lakshadweep	1	91	2	4	23	2	12	15%	2	0	0	-	100%	100%
Madhya Pradesh	693	102	10075	18645	108	6462	37	47%	6383	2162	2379	89%	83%	86%
Maharashtra	1069	146	18581	34455	129	13005	49	61%	8644	5849	3801	90%	84%	86%
Manipur	26	110	280	990	151	228	35	46%	342	213	70	88%	86%	86%
Meghalaya	25	132	458	1025	162	311	49	65%	206	303	108	86%	86%	87%
Mizoram	10	198	235	621	253	182	74	99%	167	188	39	94%	88%	88%
Nagaland	22	93	420	763	140	285	52	70%	178	148	108	91%	91%	91%
Orissa	399	129	6647	12040	121	5198	52	61%	3085	2145	939	89%	83%	87%
Puducherry	11	268	418	283	105	145	54	72%	38	54	39	88%	84%	84%
Punjab	266	142	4813	7897	119	3133	47	50%	1427	1521	1428	89%	84%	88%
Rajasthan	646	132	14711	24614	152	8855	55	68%	7326	2838	4570	92%	88%	90%
Sikkim	6	263	157	380	256	95	64	85%	77	127	58	90%	86%	86%
Tamil Nadu	664	201	9911	19284	116	7468	45	60%	5068	4068	2037	90%	84%	86%
Tripura	35	164	433	674	77	386	44	59%	142	92	46	91%	87%	89%
Uttar Pradesh	1909	143	40026	64104	134	28826	60	64%	17304	6556	8691	91%	85%	88%
Uttarakhand	95	146	1977	2961	125	1117	47	50%	711	433	522	90%	79%	85%
West Bengal	879	145	14521	24157	110	11165	51	68%	4851	3751	2879	89%	84%	86%
Grand Total	**11477**	**144**	**207144**	**351593**	**123**	**143540**	**50**	**67%**	**90272**	**49531**	**46316**	**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

third of the cases, the cause of default was patient migration from one area to another. To further strengthen the 'referral for treatment' and 'transfer out' mechanisms to check the default rate, Central TB Division has compiled the "national contact details directory". This directory has been placed on the programme's website (www.tbcindia.org) and contains the contact details of State TB Officers, District TB Officers, Medical Officers-Tuberculosis Control, Senior Treatment Supervisors and Senior TB Laboratory Supervisors.

Other major Activities

National Laboratory Committee Meeting

The 15th meeting of National Laboratory Committee was held in October 2008. The committee has recommended that RNTCP may change the diagnostic criteria of smear positive TB in accordance with new International guidelines which are as follows:

- A pulmonary TB suspect is any person with cough for two weeks, or more,
- The number of sputum specimens required for diagnosis is two, with one of them being morning sputum, and even one specimen positive out of the two is enough to declare a patient as smear positive TB.

Meeting of the National Task Force of Medical Colleges

The National Task Force (NTF) CME and workshop for the involvement of medical colleges was held at the All India Institute of Medical Sciences, New Delhi from 22nd to 24th Oct, 2008. More than 200 delegates from various medical colleges across the country participated in the CME and more than 80 medical college faculty, who are the members of the National, Zonal and State Force, participated in the workshop. As of now, 267 medical colleges are providing RNTCP diagnostic and treatment services for patients seeking health care from the medical colleges. The NTF also endorsed the proposed changes in the RNTCP definitions of TB suspect and smear positive TB and the RNTCP recommendations on the number of sputum smear examinations required to diagnose smear positive TB. The other important recommendations made by the NTF will also further strengthen the involvement of medical colleges under the programme.

Progress in accreditation of Intermediate Reference Laboratories (IRL)

The IRL of Kerala was accredited under RNTCP in December 2008 and it is currently undertaking culture and DST services for the MDR TB suspects and patients from the State. Another eight IRLs (Haryana, Rajasthan, West Bengal, Tamil Nadu, Uttarakhand, Chhattisgarh, Jharkhand and Orissa) are under the accreditation process. They are expected to be accredited in 2009.

During the quarter, the NRLs visited IRLs of Rajasthan, Pondicherry, Mizoram, Meghalaya and Tripura States as part of NRL EQA OSE visits.

Progress in DOTS-Plus services for MDR-TB cases

The States of Andhra Pradesh, Haryana, West Bengal, Kerala and Delhi have started the Cat-IV treatment services during the quarter. By the end of this quarter, 252 MDR-TB cases were on RNTCP Category IV treatment in the country. Gujarat and Maharashtra, which began the Category IV services in August 2007, are making encouraging progress. Both these States have expanded their DOTS-Plus services to a few more districts.

Progress in the involvement of NGOs and PPs

The involvement of health institutions under the Catholic Bishop's Conference of India (CBCI) which began during the third quarter 2008, have increased their participation in RNTCP during the current quarter by conducting a national level review, four State level workshops, more than 50 sensitization programmes for over 1600 persons, five training workshops and had signed four MoUs in the new NGO/PP schemes.

A meeting of the IMA National Working Group (TB) was held at the IMA headquarters in

the month of November to review the progress of the activities that were undertaken by IMA to promote the involvement of private practitioners under RNTCP. During the quarter, the Indian Medical Association (IMA) has sensitized about 2700 private practitioners through 66 CMEs and have trained about 130 private practitioners through the seven district level training programmes. In doing so, they have created 228 DOT centres and 11 DMCs in the private sector.

Progress in ACSM

ACSM capacity building training workshops were held for the State TB Officers, State IEC Officers and Communication Facilitators with the collaboration of National Institute of Health and Family Welfare (NIHFW). These five days' training workshops have provided opportunity for key functionaries to plan and implement locally appropriate ACSM activities. These workshops highlighted the importance of ACSM to enhance the programme performance and also to ensure availability of TB services in patient friendly environment.

Besides these, with the support of media agency, hands on training workshops have been planned in six states. The focus of these workshops is to demonstrate effective use of communication material in the field. New TV and radio spots have been prepared by the media agency. These also highlight "Cough more than two weeks" as symptom of TB.

Second bi-annual meeting of the STOs and RNTCP Medical Consultants

The second bi-annual national review meeting of the STOs and RNTCP medical consultants was held at Agra, Uttar Pradesh, in November, 2008. The focus of this meeting was on the qualitative aspects of RNTCP diagnostic and treatment services. Accordingly, the issues pertaining to the quality of diagnosis and treatment, and the issues pertaining to creating an enabling environment for early and increased case detection, reducing default, expanding reach and community involvement/ empowerment that were highlighted during the meeting needs to be implemented and monitored routinely.

Case Reports

TWO CASE REPORTS OF ULTRASONOGRAPHY FEATURES IN MALE GENITAL TUBERCULOSIS

Vinod Mehta[1]*, Amit Mittal[2]*, Permeet Bagga[2] and Mamta Singla[1]*****

(Received on 16.7.2008. Accepted on 6.1.2009)

***Summary*:** Genitourinary tuberculosis is the common manifestation of extra-pulmonary tuberculosis. In the male genital tract, the epididymis followed by seminal vesicle, prostate, vas deferens and testis are commonly affected sites. Ultrasonography (USG) is the best imaging modality for the diagnosis of the diseases of male genital tract. We are presenting USG findings in two cases of male genital tuberculosis with involvement of the prostate, seminal vesicle, epididymis and vas deferens. **[*Indian J Tuberc 2009; 56:95-99*]**

Key Words: Ultrasound, Male genital Tuberculosis

INTRODUCTION

Urogenital tuberculosis is primarily a disease of adults and approximately 30% of cases of extra-pulmonary tuberculosis involve the urogenital tract[1].The clinical and radiological features of genitourinary tuberculosis may mimic those of many diseases. A high index of suspicion is required, especially in high risk population. Recognition and understanding the spectrum of imaging features of extra-pulmonary tuberculosis can aid in the diagnosis of genitourinary tuberculosis and high resolution USG being non-invasive, non-ionizing, cheap, and real time imaging modality is excellent for the diagnosis of diseases of the male genital tract.

Case No. 1:

A 38-year-old male presented with history of pain and swelling in the left side of the scrotum for the last three months. There was also history of pain during sexual intercourse. On local examination, there was left inguino-scrotal swelling, which was tender on palpation. Systemic examination and laboratory investigations were normal.

Patient was referred for ultra-sonography. On USG of the scrotum, left epididymis was enlarged and heterogeneously hypoechoic with focal localized collection with thick internal echoes in the tail of the epididymis, suggestive of abscess formation (Fig.1). The head of the epididymis was normal. The vas deferens on the left side was thickened and hypoechoic and was dilated proximally, full of collection with internal echoes in it, suggestive of infective material (Fig.-2). The left testis was normal. The right sided scrotum along with its spermatic cord was normal. Based on the history, clinical examination and the USG findings, the diagnosis of chronic infective pathology was kept, with tuberculosis as one of the possibilities. FNAC was performed from the left vas deferens which showed many caseating granulomas composed of epithelioid cells, multi-nucleated giant cells and caseous necrosis in the background of pus, which was diagnostic of tuberculosis. Patient was put on conservative anti-tubercular treatment regimen consisting of Isoniazid, Rifampicin, Ethambutol and Pyrazinamide and patient improved clinically on follow-up visits.

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Case No 2:

Another young 32-year male patient came to the Department of Surgery with complaints of pain during urination, urgency and frequency of micturition for last one month. On clinical examination, there was swelling with mild tenderness in the right side scrotum. His general physical examination and laboratory investigations were normal. USG revealed that the prostate was enlarged and hypoechoic with evidence of abscess formation, more towards right side. The abscess was extending into the right seminal vesicle with enlargement and thickening of the seminal vesicle and collection with internal echoes seen in the seminal vesicle (Fig.-3). The abscess was extending directly from the prostate into the right seminal vesicle. The left seminal vesicle was normal. On scrotal USG, tail of the epididymis on right side was enlarged and heterogeneously hypoechoic with focal abscess formation and there were few calcified specks in it and there was also evidence of scrotal skin thickening (Fig. 4). However, the right testis and spermatic cord were normal. The left sided scrotum was normal. In view of the history, clinical examination and USG findings, diagnosis of pyogenic infection of the prostate, right seminal vesicle and right epididymis was made with abscess formation in the prostate and the right seminal vesicle. The patient already had a course of antibiotics without any response. Subsequently the patient was subjected to per-rectal examination of the prostate, which was diffusely enlarged and tender. On prostatic massage, per-urethral purulent secretions were collected and subjected to microscopic examination. Smear from purulent material showed degenerated acute and chronic inflammatory cells in the background of caseous necrosis. Subsequently, with the possibility of tubercular pathology in mind, ZN (Ziehl-Neelsen) staining for acid fast bacilli was done which was positive. So, the prostatic abscess

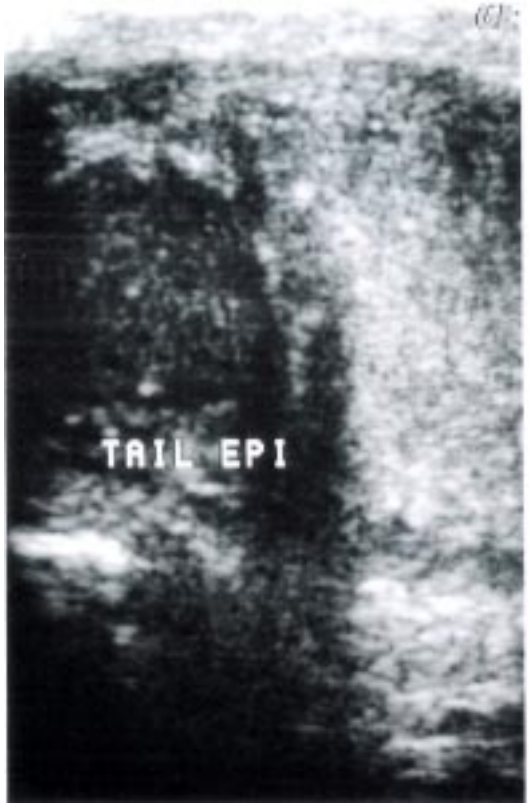

Fig. 1: USG showing enlarged and hypoechoic left tail of the epididymis with abscess formation.

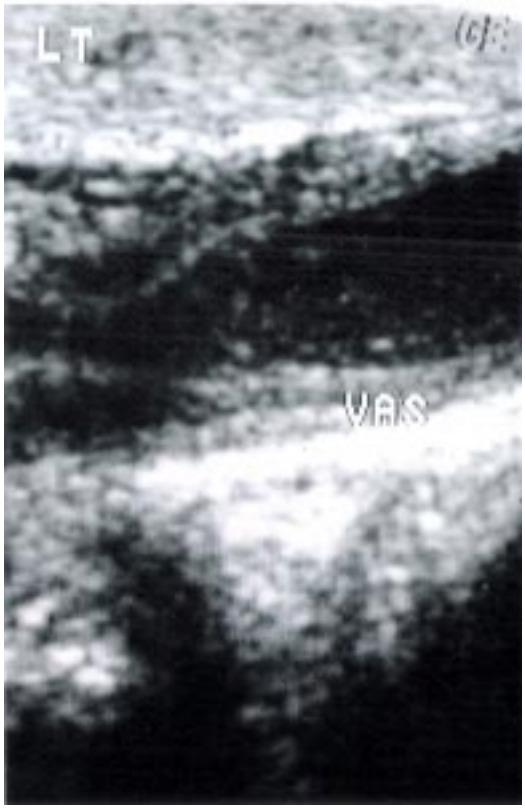

Fig. 2: USG showing hypoechoic and thick walled vas deferens with proximally dilated lumen filled with purulent secretions.

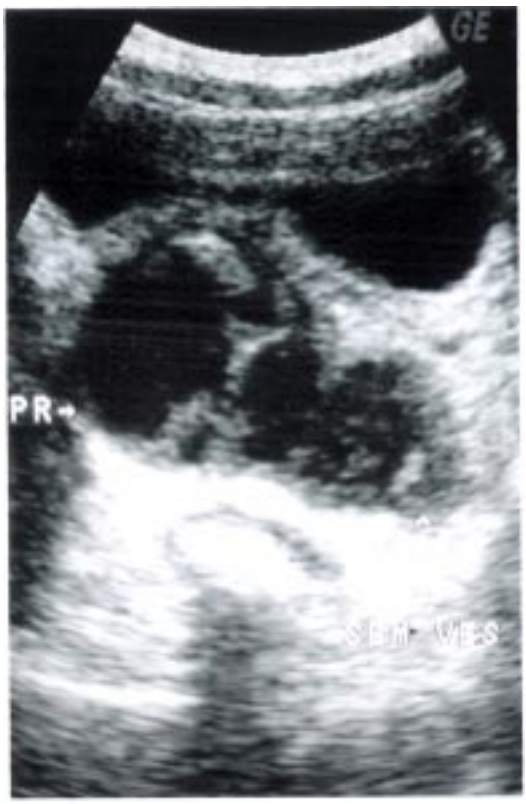

Fig. 3: USG image showing enlargement of the prostate with abscess formation in it and extension of abscess into the seminal vesicle with enlargement and hypoechogenicity of the right seminal vesicle.

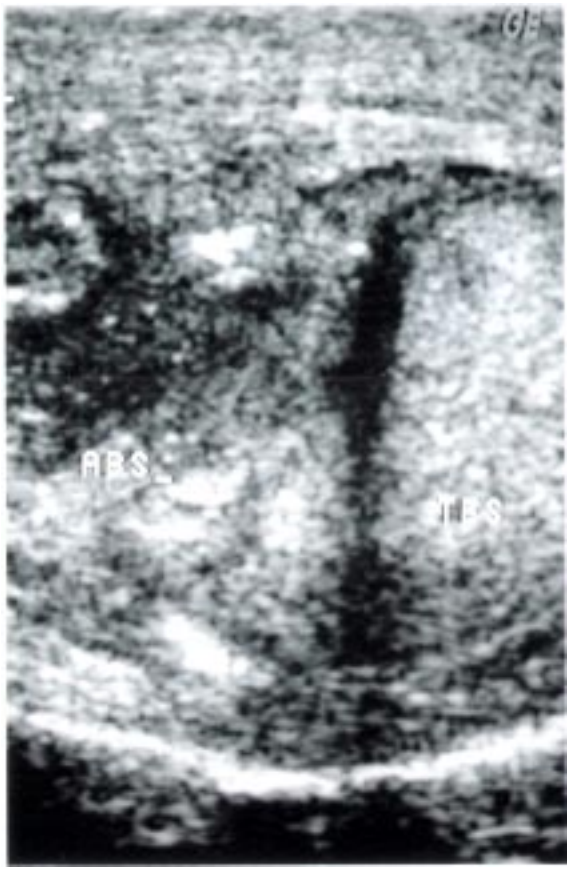

Fig. 4: USG showing enlarged heterogeneously hypoechoic right epididymis with focal abscess in formation.

was drained and the ATT was started with four drugs. Patient improved clinically on follow-up with ATT and abscess drainage.

DISCUSSION

The prevalence of tuberculosis has been increasing over the past decade, due to the rising number of people with Acquired Immuno Deficiency Syndrome (AIDS) and the emergence of the drug-resistant strains of *Mycobacterium tuberculosis*[2]. Before the HIV epidemic, approximately 15% of newly reported cases of tuberculosis had extra-pulmonary involvement. In the years since, reported cases of extra-pulmonary tuberculosis infection have increased. Genitourinary tuberculosis still presents a diagnostic and therapeutic challenge[3]. Now genital tuberculosis has also been reported with intravesical Bacillus Calmette-Guérin (BCG) therapy used for urinary bladder carcinoma[4,5]. The kidneys are often the primary site of urogenital tuberculosis; however in our cases kidneys were normal. In males, the epididymis is one of the most common sites of genital tuberculosis which usually starts in the tail of the epididymis and can propagate to the entire duct. Tubercle bacilli reach the epididymis by haematogenous spread, lymphatic route, or descent from the kidney with retrograde canalicular spread via the vas deferens. Genital tuberculosis commonly presents as scrotal swelling, pain, discharge and pain during urination[1]. In our cases, one of the patients presented with inguino-scrotal swelling and second patient presented with pain during urination.

Ultrasonography is currently the best technique for imaging the male genital system. The three gray-scale sonographic appearances of tuberculous epididymitis include diffusely enlarged heterogeneously hypoechoic, diffusely enlarged homogeneously hypoechoic and nodular enlarged heterogeneously hypoechoic lesions. Other associated sonographic findings include intra-scrotal extra-testicular calcification which was seen in our second case[6]. Epididymal infection can extend to the vas deferens and shows thickening with hypoechogenicity of its wall. Purulent secretion causes dilatation of its lumen as was also seen in our first case[7]. The tubercular epididymitis may have an abscess cavity that contains caseous material. The presence of an abscess in the epididymis indicates an advanced disease. On gray scale sonography, the echogenicity of an abscess is not useful in differentiating tuberculous epididymal abscess from pyogenic epididymal abscess[8]. However, some clinical features like long term scrotal swelling with minimal or without tenderness and colour doppler feature of lower degree of blood flow in the peripheral portion of the abscess may suggest tubercular pathology as suggested by Yang *et al*[8]. Additional useful information for differentiation includes intrascrotal calcifications and sinus tract, clinical evidence of tuberculosis elsewhere in the body, immuno compromised state, and failure to respond to conventional antibiotics[9].

TB involvement of the prostate and seminal vesicles is usually secondary to infection from the upper genito-urinary tract. Tuberculosis can involve the prostate as a form of prostatitis or abscess[10]. On sonography, tuberculous prostatitis reveals hypoechogenicity and increased vascularity, similar to that of prostate cancer[11]. The prostatic abscess is seen as hypoechoic lesion in the prostate with posterior enhancement[7]. Tubercular infection of the seminal vesicle leading to abscess formation is rarest form of extrapulmonary tuberculosis[12].

In our second case, there was formation of the prostatic abscess which was extending into the right seminal vesicle along with epididymitis, which might be the source of infection for prostate and seminal vesicle.

FNAC as a minimally invasive technique plays a prime role in the diagnosis of male genital tuberculosis. Tuberculosis can be diagnosed on FNAC by detection of acid fast bacilli (AFB) or caseating granulomas in smears[13].

According to Viswaroop et al, the sensitivity and specificity of FNAC for the diagnosis of tuberculous epididymitis were 87% and 93%, respectively. The positive predictive value was 87% for the diagnosis of both tuberculous and non-specific epididymitis[14]. In our first case diagnosis was made by finding caseating granulomas in the vas deferens smears and in the second case AFB were seen in the prostatic secretions.

Patients with genital tuberculosis usually respond to the antituberculous therapy; however surgery may be required in severe cases to deal with abscesses, obstructive symptoms, or failure of chemotherapy[9,15]. Our first case responded with ATT and in second case prostatic abscess was drained along with ATT.

In conclusion, high resolution sonography is excellent modality for the male genital system pathology. If the patient presents with clinical history of long duration along with mild clinical symptoms and the ultrasonography is suggestive of inflammatory pathology with abscess formation in the genital organs, possibility of tuberculosis is to be kept in mind especially in the endemic areas and patient should be investigated for the same.

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Case Report

SPINA VENTOSA DISCHARGING TUBERCLE BACILLI – A CASE REPORT

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Summary: Tubercular dactylitis is an unusual form of skeletal tuberculosis. Radiographic features of cystic expansion of the short tubular bones have led to the name of "Spina Ventosa" for tuberculosis dactylitis of the short bones. We report here a case of a Spina Ventosa in a 15 year-old female, who presented with complaint of draining sinus on the dorsum of the third digit of the left foot. ***[Indian J Tuberc 2009; 56: 100-103]***

Key Words: Tuberculous dactylitis, Spina Ventosa, Acid Fast Bacilli

INTRODUCTION

Tuberculous infection of metacarpals, metatarsal and phalanges of hands and feet is known as tubercular dactylitis and it is uncommon after the age of five years[1]. Although there is extensive literature on osteoarticular tuberculosis, there have been few studies on the involvement of the foot which is rare and accounts for only about 10% of all cases of skeletal tuberculosis[2]. Radiographic features of cystic expansion of the short tubular bones in tubercular dactylitis have led to the name of "Spina Ventosa" for this (Spina = short bone; Ventosa = inflated with air)[3].

CASE REPORT

A 15 year-old female was brought with complaint of draining sinus from the dorsal aspect of third toe of the left foot. Initially she had pain in left foot five months back for which symptomatic treatment was given by a general practitioner, following which she developed a swelling on dorsum of third toe of left foot. After a trivial trauma pus started discharging from the involved site. Despite broad spectrum antibiotic therapy by her general practitioner, there was no clinical improvement. She had no systemic symptoms like fever, loss of weight and loss of appetite.

There was no past history of anti-tubercular treatment or any family history of tuberculosis

On examination, she had a resting pulse rate of 90/min, blood pressure of 106/62 mmHg and there was no significant peripheral lymphadenopathy.

Examination of left foot revealed swelling over the dorsum of third middle phalanx of left foot and pus discharging sinus (Figure 1). Chest examination was normal. Examination of other systems was unremarkable.

Her hemoglobin was 13 gm%; Total Leucocyte count was 8,600/cmm with Differential Leucocyte count of Neutrophils 65%, Lymphocytes 30%, Monocytes 3%, Eosinophils 2% and ESR of 2 mm/hour. Her random blood sugar was 86 mg/dl. Her HIV, VDRL test, sickling and blood culture tests were negative. Her ultrasonography of abdomen revealed no abnormality and Chest X-ray was normal. Mantoux test showed 14 mm induration at 48 hours. X-ray left foot AP and lateral view revealed osteolytic lesions with radiolucent marrow space in third middle phalanx of left foot and thinning of cortex surrounded by soft tissue swelling (Figure 1). Pus from discharging sinus showed acid fast bacilli in direct smear (Figure 2) and was positive for *Mycobacterium Tuberculosis* by Bactec method.

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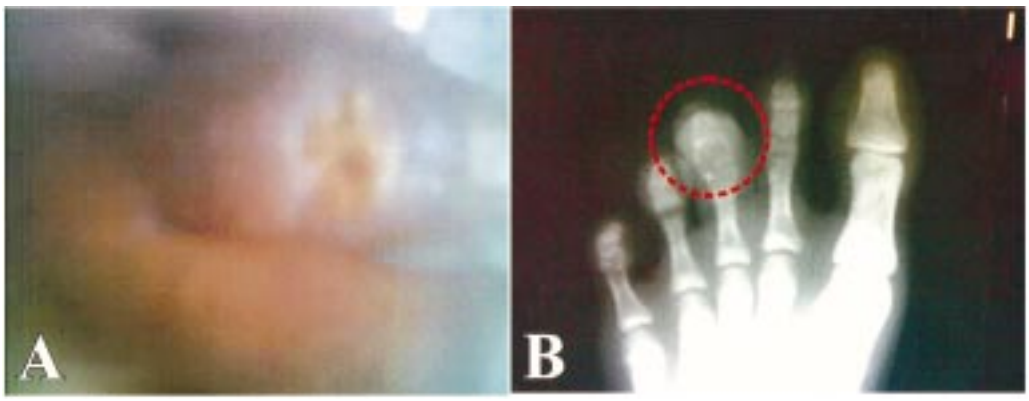

Figure 1: Prior to institution of anti-tubercular treatment. A. External appearance of the dorsal aspect of the third toe of the left foot showing discharging sinus at initial presentation. B. X-ray AP view of the left foot (showing lytic lesion of the 3rd digit).

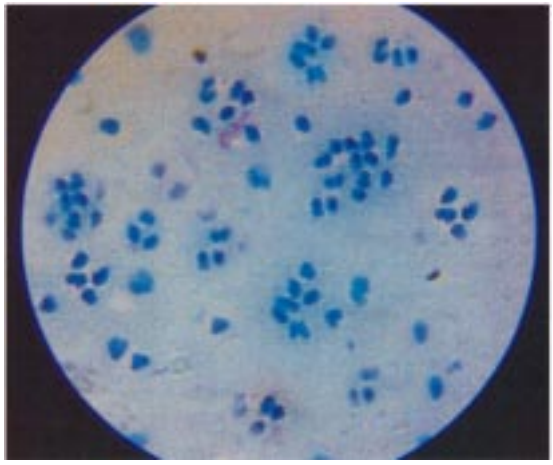

Figure 2: ZN Stain of pus specimen showing Acid Fast Bacilli along with presence of many pus cells.

So the diagnosis of tubercular dactylitis was established and her treatment was begun with four drugs (Rifampicin, Ethambutol, Isoniazid and Pyrazinamide) for two months, followed by two drugs (Rifampicin, Isoniazid) for four months. Four months after the initiation of anti-tubercular treatment, the discharging sinus healed and swelling disappeared along with decrement of shadow in radiology (Figure 3).

DISCUSSION

Skeletal tuberculosis is relatively uncommon compared to the pulmonary form of tuberculosis.

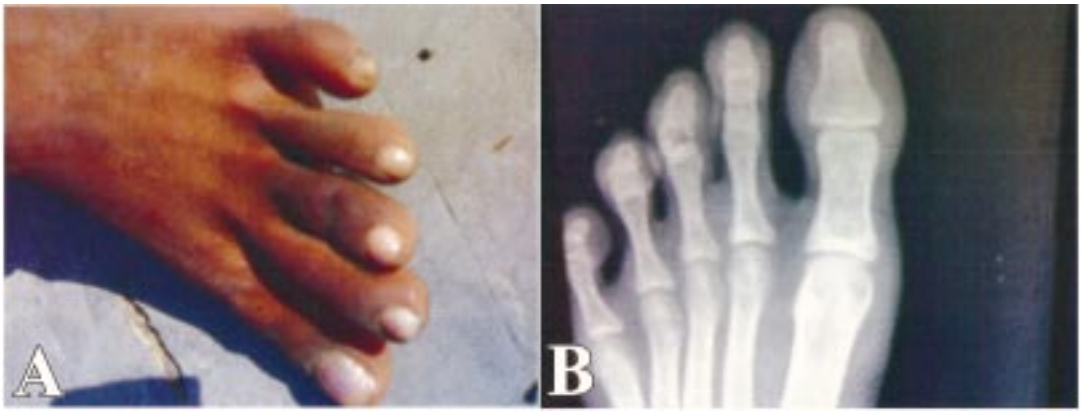

Figure 3: Four months after institution of anti-tubercular treatment. A. External appearance of foot showing clinical resolution of discharging sinus of 3rd digit of the left foot. B. X-ray AP view of the left foot showing radiological resolution of the lytic lesion of the 3rd digit.

Only 1/3rd of patients with bone tuberculosis are diagnosed with concomitant active pulmonary disease[4]. In fact, bones and joints are affected in 1%–3% of all cases and the spine and the hip are most commonly involved[1]. It occurs in 1–5% of children who have untreated initial pulmonary TB[4]. The incidence of tubercular dactylitis among children with TB is reported to be 0.65-6.9%. The bones of the hands are more frequently affected than bones of the feet[5]. In the feet, the calcaneus is the bone most commonly involved[1].

In infancy and childhood before the epiphyseal centres are well established, the hematopoietic marrow in the short bones offers a fertile field for hematogenous bacterial implants. The infection rapidly involves the entire marrow space. Tuberculous granulation tissue expands the relatively soft cortex as it is resorbed or infarcted by the underlying process. The resultant fusiform expansion of the bone with thinned cortex and relatively radiolucent marrow space due to trabecular destruction resembles an inflated balloon. Typically, there is no periosteal layering or thickening, and sequestration ordinarily does not occur. Sclerosis may be seen in long standing cases[6].

In natural course, the disease heals with shortening of the involved bone and deformity of the neighbouring joint[1]. The condition usually presents as a painless swelling of a digit of few months' duration.

The main differential diagnoses include sickle cell dactylitis which exhibits features similar to that of tubercular dactylitis but is characteristically bilateral and dissolution of the sickle cell lesions is typically followed by irregularly sclerotic new bone formation. Other differential diagnoses are congenital syphilis, pyogenic osteomyelitis, fungal infections and tumors[1]. In syphilis, the bone is thickened by periosteal reaction. Clinically, pyogenic osteomyelitis tends to be acutely painful, swollen, and hot, with generalized fever. Tuberculous osteomyelitis is more often only mildly painful, pyrexia is minimal, and the whole condition is relatively benign[2].

Diagnosis of tubercular dactylitis is made on radiographic features as explained above and culture of *Mycobacterium tuberculosis* .The non-specific nature of the radiographic findings may delay the diagnosis. As it is a paucibacillary lesion, it becomes difficult to demonstrate or culture acid-fast mycobacteria from these lesions. However, the gold standard for the diagnosis of osseous tuberculosis is culture of *Mycobacterium tuberculosis* from bone tissue[7].

The significance of a history of trauma[8], reported by one third of patients, is unknown. Pulmonary tubercular involvement is uncommon in cases of tubercular dactylitis[9]. In endemic regions, the clinical features, radiological appearance and elevated ESR are sufficient to diagnose tuberculosis and begin treatment[5].

Management is essentially by antitubercular drugs, rest to the involved part in functioning position and early active exercise[1]. Current recommendations for the treatment include a two month initial phase of Isoniazid, Rifampicin, Pyrazinamide, and Ethambutol followed by a six to 12 month regimen of Isoniazid and Rifampicin[9]. Few studies argue that six month of antitubercular treatment is appropriate for tubercular dactylitis because of its paucibacillary nature[10].

The interesting aspects of this case were: firstly the discharging pus showed acid fast bacilli in direct smear, secondly there was no evidence of tubercular involvement elsewhere in the body, a normal ESR and the adolescent age group as the condition is quiet rare after five years of age. Also the bones of the hands are more frequently affected than bones of the feet[5] in such cases, contrary to the presentation in this patient. Thus this case has been reported to highlight this unusual manifestation of a common disease - Tuberculosis.

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ANDHRA PRADESH STATE TB & CHEST DISEASES WORKERS' CONFERENCE

The 31st Andhra Pradesh TB and Chest Diseases Workers' Conference was organized at Narayana Medical College and General Hospital, Chintareddy, Palem, Sri Pottisriramulu, Nellore on 29th and 30th November, 2008 under the joint auspices of the Department of Pulmonology, Narayana Medical College and General Hospital, Nellore, the Tuberculosis Association of Andhra Pradesh and the District Tuberculosis Association of Sri Pottisriramulu. The theme of the conference was "Prevent HIV and Cure TB".

Shri K. Ramgopal, Collector and District Magistrate, Nellore, inaugurated the conference. Dr. Premanand Raya, formerly Prof. and HOD, and Emeritus Professor of TB and Chest Diseases, S.V. Medical College, Tirupati was President of the Conference. Dr. T.V. Venkateswarulu, Honorary General Secretary of the TB Association of Andhra Pradesh, presented the report on the activities of the Association.

Gold medal awards and prizes were presented to the orators and others. Senior TB workers were honoured on the occasion. A souvenir was also brought out on the occasion. About 300 delegates from all over the State and eminent speakers from Chennai and other places, attended the Conference.

Case report

BCG INDUCED MYCOBACTERIAL SPINDLE CELL PSEUDOTUMOR IN AN INFANT

S. Suchitha[1], C.S. Sheeladevi[2], G.V. Manjunath[3] and R. Sunila[4]

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***Summary*:** Mycobacterial Spindle cell Pseudotumor (MSP) is a rare complication of mycobacterial infection, especially the atypical variety. It is characterized by an exuberant spindle cell proliferation. This has been reported in the lymph nodes, skin, spleen, lungs, brain, etc. The incidence is higher in immuno-compromised patients, especially those with acquired immunodeficiency syndrome. It is rare to encounter this lesion in infants. We report a case of MSP in the axillary lymph node of a 7-month-old infant, following Bacillus Calmette Guerin (BCG) vaccination due to *Mycobacterium tuberculosis* complex, which was proved by PCR. **[*Indian J Tuberc 2009; 56:104-107*]**

Key words: Spindle cell pseudotumor, Mycobacteria, BCG vaccination.

INTRODUCTION

Spindle cell pseudotumors due to mycobacteria are characterized by an exuberant proliferation of spindle cells, mimicking a neoplasm. They have been described in various sites like lymph nodes, brain, spleen, lung, skin, bone marrow and appendix. These lesions are more common in immuno-compromised patients, including HIV positive individuals. The lesion may mimic spindle cell tumors in lymphnodes such as primary Kaposi sarcoma, inflammatory pseudotumor and palisaded myofibroblastoma of lymph nodes[1]. MSP of lymph nodes may rarely occur in infants after BCG vaccination[1]. Thus it is very important to recognize these lesions.

CLINICAL RECORD

A seven month-old infant who had received BCG vaccination, when three days' old, presented with swelling in the left axilla and an ulcer at the site of BCG inoculation of three months duration. Routine investigations were within normal limits and he was HIV negative. A biopsy was undertaken and revealed a matted group of lymph nodes adherent to axillary vessels, which were excised.

Pathological findings

Gross examination revealed two gray white tissue masses, larger measuring 5x 3.5x2cm and the smaller measuring 5x2x1 cm. Cut surface of the masses were gray white and nodular. No area of necrosis was noted.

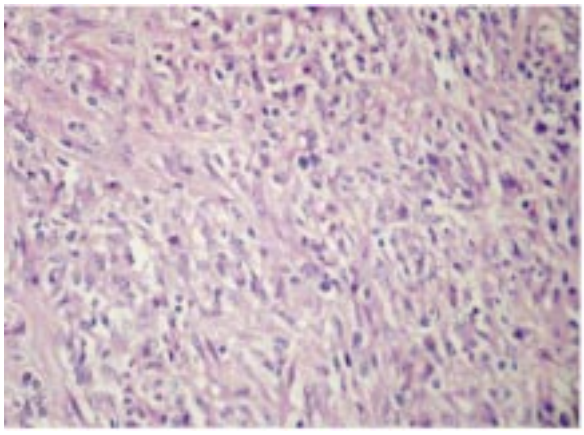

Fig. 1: Photomicrograph showing spindle cells in sheets and fascicles admixed with inflammatory cells (H&E stain x 40)

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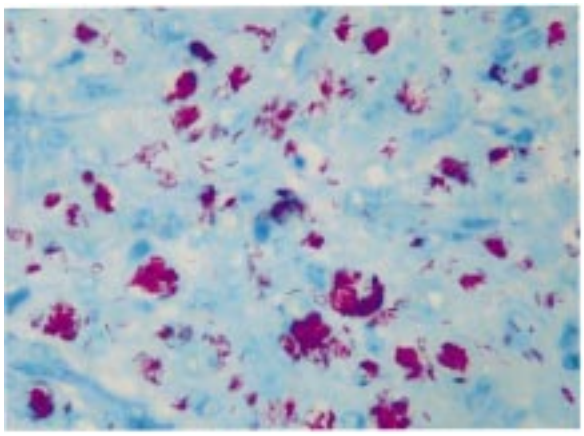

Fig. 2: Photomicrograph showing numerous acid-fast bacilli (ZN stain x 100)

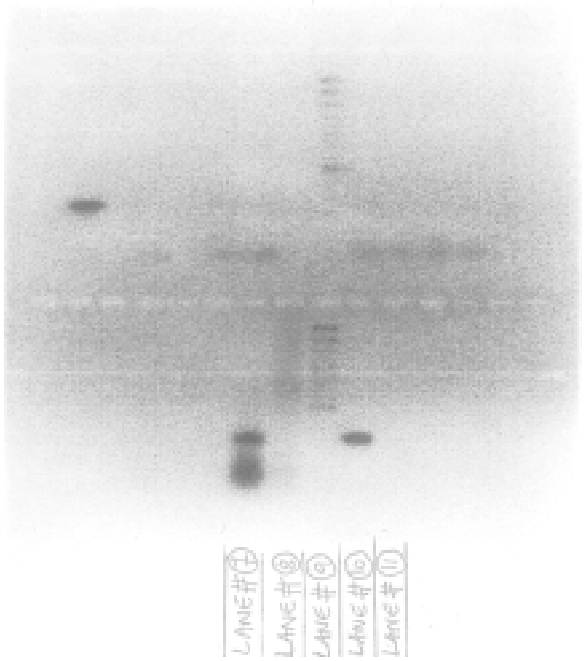

Fig. 3: Photographs of the PCR gel:
Lane 7 = Phenol-Cholorofom extract (PCR Positive)
Lane 8 = Kit extract (PCR Negative)
Lane 9 = Molecular wight marker
Lane 10 = Positive control DNA
Lane 11 = Negative control

On light microscopy, the lymph node showed effacement of architecture with proliferation of spindle cells arranged in sheets and fascicles (Fig. 1). The cells had indistinct cell borders with eosinophilic granular cytoplasm and round to oval nuclei. These cells were admixed with capillaries, inflammatory cells including small lymphocytes, plasma cells and neutrophils. No multinucleated giant cells, mitotic figures, pleomorphism or necrosis were observed. The Ziehl Neelsen stain revealed numerous elongated acid-fast bacilli both within and outside the macrophages (Fig. 2). The spindle cells were immunoreactive to CD 68.They were negative for desmin and showed focal positivity for S-100 protein. PCR was done on paraffin embedded blocks with two sets of extracts for the IS6110 gene of *Mycobacterium tuberculosis* complex (includes *M.tuberculosis* hominis and *M. tuberculosis* bovis strains). One of the tubes (Phenol-Chloroform extract) was positive by PCR (Fig 3), while the second tube (kit extract) was negative, which was attributed to improper protocol for DNA extraction using the kit.

DISCUSSION

Mycobacterial spindle cell pseudotumor (MSP) is an exuberant spindle cell lesion induced by Mycobacteria. This phenomenon occurs most commonly in immuno-compromised hosts, particularly those with acquired immuno-deficiency syndrome. One of the atypical presentations of mycobacterial infection is MSP. Wood et al. first described this lesion, in 1985. Since then, very few cases have been reported, mostly in adults, arising in lymph nodes and frequently associated with atypical mycobacterial species, especially *Mycobacterium avium intracellulare* (MAI)[3]. Only one report of two infants with MSP in lymph nodes associated with BCG vaccination could be accessed in published literature to date[1].

Histopathologically, in lymph nodes there is partial or complete obliteration of architecture by proliferation of cytologically bland spindle cells lymphocytes, plasma cells and neutrophils[4,5]. Epithelioid cells, areas of necrosis, mild to moderate

nuclear pleomorphism in both spindle cells and epithelioid cells have been noted[3].

Mycobacterial spindle cell pseudotumor has been described in the lymph nodes of two infants after receiving BCG vaccination[1]. Vaccination of all newborns with BCG is a standard practice in India. Although this practice generally is considered safe and effective, rarely complications have occurred. The associated disease is called 'BCGitis'[6].

The pattern of post vaccination BCG infection differs clinically and pathologically, based on the immunologic status of the patients. Patients with normal immunity have enlarged regional lymph nodes and abscess at the vaccination site. The microscopic appearance is similar to tubercular lymphadenitis, showing multiple epithelioid granulomas with or without caseous necrosis. Patients with disseminated BCG infection associated with immuno-deficiency usually have generalized skin rashes and skin nodules. Biopsies reveal diffuse (lepromatous-like) infiltrate of histiocytes and, often, polymorphonuclear cells. The histiocytes have abundant gray cytoplasm, which is packed with acid-fast bacilli. Another pathologic pattern has ill-defined epithelioid granulomas and giant cells. AFB are absent or rare in this type[6]. In this case, the child had axillary lymphadenopathy. No granulomas, giant cells or lepra-like cells were seen. There was proliferation of spindle cells arranged in sheets and fascicles mimicking a spindle cell neoplasm.

The startling ability of this proliferative lesion to mimic spindle cell neoplasm suggests that acid-fast stains should be a part of evaluation of any spindle cell lesion lacking nuclear atypia, particularly in immunodeficient patients[4]. In this case, the spindle cells revealed numerous acid-fast bacilli by Ziehl Neelsen staining.

The immuno-histochemical profile of MSP has been variable in different reports. It has been amply demonstrated by immuno-histochemistry and electron microscopy that the proliferating spindle cells of mycobacterial pseudotumor are histiocytic in origin. Positive staining with desmin and S-100 protein has been reported[8,2]. In the present case, the spindle cells were positive for CD 68, focally positive for S-100 protein and negative for desmin.

The pathogenesis of this phenomenon is not clear. Some authors have contended that a complex host microorganism interaction rather than HIV infection may be responsible for the formation of pseudotumor. The tissue response is probably related to the interaction of the organism with the host's defense capabilities[9].

The mycobacterial spindle cell pseudotumor of lymph nodes occurs in immuno-deficient patients such as with AIDS, steroid use, chemotherapy, immuno-suppressant therapy and in patients with idiopathic lymphocytopenia (CD4+Tcells)[3]. This was a seven month-old infant with prior history of BCG vaccination and was HIV negative. Although the lesion mimicked a spindle cell neoplasm, an infectious etiology was pursued on the basis of bland nuclear morphology, inflammatory cells and clinical settings. The immune status of the patient could not be assessed, as the child died 10 days after the surgery. Among the two reported instances, one of the infants did not respond to antitubercular treatment (isoniazid, rifampicin and streptomycin), deteriorated and died. The outcome in the other instance is not known[1].

The primary surgical treatment (incisional drainage or biopsy) is not considered an ideal form of management in BCG lymphadenitis because of high fistulisation and poor wound healing. Surgery should, therefore, be confined to the unusual event of real doubt about the underlying diagnosis with needle aspiration and anti-tubercular drugs being preferred as initial approach [10,11]. All cases of idiopathic BCG infection (unknown immunodeficiency type) respond well to antimycobacterial drugs with immunomodulators (gamma interferons)[11].

In summary, mycobacterial spindle cell pseudotumors are rare complications of mycobacterial infection. While MSP associated with atypical mycobacterial infection has been reported, its occurrence in infants following BCG vaccination is very rare. The role of immune

deficiency in the poor outcome needs to be delineated. The mortality in our report and in the other report highlights the need for greater awareness of this condition. The patients could then receive early and appropriate care.

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FORUM

Outcome of Directly Observed Treatment Short course (DOTS) in a case of pulmonary tuberculosis with Hyperuricemia

During treatment of tuberculosis (TB), hyperuricemia commonly occurs as a side effect of pyrazinamide (PZA) administration[1]. Ethambutol (EMB) may also raise the serum uric acid level[2]. Hyperuricemia has been correlated with hypertension, cardiovascular disease and renal disease and treatment strategies include use of allopurinol[3]. The association of TB and hyperuricemia is uncommon and the safety aspects of using intermittent treatment regimen containing PZA and EMB in such cases are not known.

A-25 year old unmarried male was admitted in July 2007 for chronic cough and recent onset of hoarseness of voice. In May 2005, he was admitted with renal failure and detected to have hyperuricemia, hypercalcemia and hypercalciuria. He recovered from the renal failure and had been on oral allopurinol for hyperuricemia. His urinalysis was normal. Three sputum smears were positive for acid fast bacilli. HIV serology was negative. His blood chemistry revealed normal serum electrolytes, blood urea nitrogen of 18mg/dl, serum creatinine of 0.9mg/dl and serum uric acid of 8mg/dl. Category I treatment regimen under Revised National Tuberculosis Control Programme (RNTCP)[4] was started with Isoniazid (600mg), Rifampicin (450mg), EMB (1200mg) and PZA (1500mg). Allopurinol was continued at 100mg per day. However, serum uric acid increased to 10.3mg/dl after two doses of anti tubercular treatment. The dose of allopurinol was increased to 200mg per day and subsequently serum uric acid decreased to 6 mg/dl and remained within normal range throughout the intensive phase. Allopurinol was reduced to 100 mg per day once the serum uric acid level was 3.8 mg/dl after PZA and EMB was stopped.

We are not aware of any previous case report of TB with pre-existing hyperuricemia having received DOTS. Successful outcome in our case suggests that pre-existing hyperuricemia on treatment without any organ dysfunction may not be a contraindication for starting Category I DOTS regimen but close monitoring of serum uric acid is necessary.

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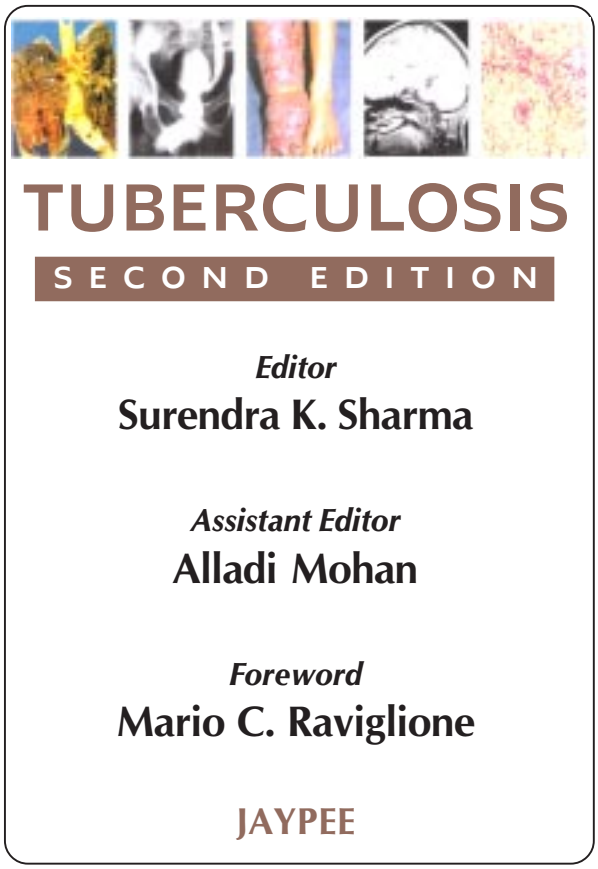
BOOK REVIEW

Tuberculosis - Second Edition, 2009; (Editor) Surender K. Sharma (Assistant Editor) Alladi Mohan; Published by Jaypee Brothers Medical Publishers (P) Ltd., B-3, EMCA House, 23/23B, Ansari Road, Darya Ganj, New Delhi-110 002; 1040 pages, 67 Chapters with black and white and multi-coloured figures, tables, X-rays, etc; ISBN 978-81-8448-514-1; Price not mentioned.



The publication of the second edition of the Book "Tuberculosis" edited by Prof. S. K. Sharma is a welcome addition as a valuable literature on the dreaded disease of TB. This is extensively revised, thoroughly updated with noted contributions from the leading TB experts from all parts of the world. The book is well comprehended with latest references and rich illustrations of medical and pathology sections. This book has a perfect amalgamation between laboratory diagnosis, pathogenesis, surgical procedures, global network of TB control etc. Special credit goes for the helpful internet links for getting the current information on the international guidelines as released from time to time. The under-graduate and post-graduate medical students, medical practitioners, accompanied staff, health care authorities of the patients would be immensely guided and helped by this useful companion. The second edition has covered in detail on the developments of all the aspects of TB care by addition of various new chapters regarding immunogenetics, drug and vaccine development, partnership of the public private bodies and the RNTCP. Dealing and management suggestions of pediatric TB have a special relevance. The new tables in separate coloured boxes in all the chapters, figures have highlighted the facts and important take home messages needed by all the users. High quality self-explanatory radio images for TB as well as HIV TB co-infection are extremely important. By detailed description of the host pathogen interaction, use of liquid culture and molecular diagnostic, recent guidelines by WHO for the disease management and the International TB care standards has added special meaning to it. The following areas of silico-tuberculosis, ocular, genitourinary TB have essentially opened up new horizons of bad effects of TB spreading. The chapters on reactivation and re-infection of TB, anti TB drug resistance surveillance, tackling of M&XDR TB are the current topics to deal and eliminate the dreaded disease. Recent developmental details in various sub-areas of TB have added special attraction to the clinical researchers and also for the budding researchers. The second edition, indeed in all aspect has a vivid coverage for all sorts of practical utility and I wish to recommend it for all the users.

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Indian J Tuberc 2009; 56: 110-112

ABSTRACTS

Increasing incidence of fluoroquinolone-resistant *Mycobacterium tuberculosis* in Mumbai, India

D. Agrawal, Z.F. Udwadia, C. Rodriguez and A. Mehta. *Int J Tuberc Lung Dis* 2009; **13(1):** 79-83

Objective was to analyse the incidence of fluoroquinolone (FQ) resistant *Mycobacterium tuberculosis* (TB) in our laboratory from 1995 to 2004. It was a retrospective review and analysis of the drug susceptibility test records of all *M. tuberculosis* culture-positive samples from our Microbiology Department from 1995 to 2004 was done. FQ resistance has increased exponentially in our laboratory, from 3% in 1996 to 35% in 2004. The incidence of multi-drug-resistant tuberculosis has also increased during the same period, from 33% in 1995 to 56% in 2004. The incidence of FQ-resistant *M. tuberculosis* is gradually increasing to alarming levels. This may be due to widespread use of this vital group of drugs in the treatment of community-acquired infections. We urge that these broad spectrum antibiotics be used judiciously, and ideally be reserved for treatment of resistant TB in TB-endemic areas.

Pharmacokinetics of pyrazinamide in children with primary progressive disease of lungs

D.S. Arya, S.K. Ojha, O.P. Semwal and M. Nandave. *Indian J Med Res* 2008; 128; 611-615.

As the dosages recommended for children are based on weight, empirical and derived by extrapolation from the studies in adults, pyrazinamide (PZA) pharmacokinetics in children is likely to be different from adults. Limited information exists regarding the pharmacokinetics of PZA in paediatric patients of primary progressive disease (PPD) of lungs. This study aims to look at the changed pharmacokinetics of pyrazinamide in children with PPD of lungs by using reverse phase high-pressure liquid chromatography (HPLC). A total of 40 children (age range 5 to 13 years) of PPD were receiving pyrazinamide (30 mg/kg/day). On 11th day of short course anti-tubercular therapy, blood samples (two per day from 11th to 13th day) were collected at 0 hour (pre-dose), 1, 2, 3, 4, 8 and 24 hours after pyrazinamide administration and concentration of pyrazinamide was estimated by reverse phase high-pressure liquid chromatography. The mean peak serum concentration, the time to reach mean peak serum concentration, total clearance, concentration at time zero, volume of distribution, terminal elimination rate constant, elimination half-life, total area under serum concentration- time curve were measured. The mean serum concentrations of pyrazinamide were found higher than its minimum inhibitory concentration (20 µg/ml) required to inhibit the growth of tubercle bacilli from one to eight hours continuously. Our results suggest that a dose of 30 mg/kg/day achieves much higher concentration of pyrazinamide as compared to its minimum inhibitory concentration (20 µg/ml). Therefore, lowering of pyrazinamide dosage is suggested in children for better patient compliance along with reduction in cost, side-effects and toxicity without compromising its efficacy.

Novel mutations in *emb B* gene of ethambutol resistant isolates of *Mycobacterium tuberculosis*

Amita Jain, Rajesh Mondal, Shashikant Srivastava, Rajendra Prasad, Kamlesh Singh and R.C. Ahuja. *Indian J Med Res* 2008; **128**; 634-639.

Ethambutol (EMB) resistance, thought to be occurring due to mutations in *embB* gene of *Mycobacterium tuberculosis* on the rise is a cause of grave concern. The present study was planned to investigate the presence of EMB resistance in *M. tuberculosis* isolates and to look for prevalent mutations in *embB* gene. A total of 591(283 from new and 308 from previously treated cases) sputum samples from the same number of pulmonary

tuberculosis cases were cultured. Isolates were tested by one per cent proportion method for resistance to isoniazid, rifampicin, streptomycin and ethambutol. Minimum inhibitory concentration (MIC) of EMB was measured by absolute concentration method. Ten randomly selected isolates were subjected to single strand conformational polymorphism (SSCP) and direct DNA sequencing to look for mutation in 364 bp segments of *embB* gene. Of 353 isolates of *M. tuberculosis* from 591 sputum samples, 62 (17.58%) were resistant to EMB, of which, 16 (25.8%) showed initial resistance and 46 (74.2%) acquired. Mono resistance to EMB was rare. Only two isolates showed resistance to EMB alone. From 62 EMB resistant isolates, 88.7 per cent (55) were resistant to INH, 82.2 per cent (51) to rifampicin and 61.2 per cent (38) were resistant to streptomycin. Co-resistance to isoniazid and rifampicin (multidrug resistant, MDR- TB) with EMB resistance was seen in 41(66.1 %) isolates. High level of EMB resistance was seen in 16.5 per cent isolates. SSCP showed altered mobility in eight of ten isolates tested. Among the eight mutants, four had known mutations at codon Met 306 being replaced by Val/ Leu. The second most frequent mutation encountered was at codon Phe 287 being replaced by Val, Cys or Leu (novel mutations). Sequence analysis revealed 10 novel mutations in codon 221, 225, 227, 271, 272, 281, 282, 287, 293 and 294 within *embB* gene. Presence of high frequency of EMB resistance, occurrence of high level EMB resistance, co-existence of MDR- TB with EMB resistance and novel mutations in *emb B* gene of *M. tuberculosis* clinical isolates reported highlight the need to work on larger samples to identify the diagnostic marker of EMB resistance in mycobacteria.

A simple screening tool for active tuberculosis in HIV-infected adults receiving anti-retroviral treatment

W. Were, D. Moore, P. Ekwaru *et al*. *Int J Tuberc Lung Dis* 2009; **13(1):** 47-53.

Reliable clinical algorithms that screen for active tuberculosis (TB) in human immunodeficiency virus (HIV) infected people initiating or receiving anti-retroviral treatment (ART) in sub-Saharan Africa could reduce the need for diagnostic procedures. We estimated the utility of six TB-related signs and symptoms, alone or in combination, compared with the Uganda Ministry of Health diagnostic guidelines for participants with prevalent (baseline), early ART (≥3 months on ART) and incident TB (>3 months on ART). Of 1995 participants screened for ART eligibility, 71 (3.6%) had prevalent TB. The presence of any one of the following: cough ≥3 weeks, fever ≥4 weeks, lymphadenopathy or baseline body mass index ≤18 kg/m^2 had a sensitivity of 99% (95% CI 96-100), a specificity of 66% (95% CI 64-68) and a negative predictive value (NPV) of 100% (95% CI 99-100) for predicting active TB. During ART follow-up, TB incidence was 2.4 (95% CI 1.6-3.4)/100 person-years. The presence of cough ≥3 weeks or general weakness was 100% sensitive (95% CI 99-100), 66% specific (95% CI 59-74) and had an NPV of 100% (95% CI 99-100). Use of a simple TB screening algorithm can accurately identify, in a resource-poor African setting, HIV-infected individuals who require further procedures to diagnose active TB.

Prevalence of anti-tuberculosis drug resistance in an HIV/AIDS reference hospital

F. Aguir M.A. Vieira, A Staviack *et al*. *Int J Tuberc Lung Dis* 2009; **13(1):** 54-61

. We conducted a study to estimate the prevalence of resistance to anti-tuberculosis drugs and to identify associated factors. In a cross-sectional study, clinical and laboratory data were collected retrospectively from 2001 to 2005. Patients with isolation of *Mycobacterium tuberculosis* and available drug susceptibility tests were considered eligible. Data on demographic characteristics, risk factors for resistance, HIV serology and past TB history were collected and analysed by ÷^{2} Mann-Whitney test and Poisson regression. We analysed 350 treatments, of which 62 were for patients with previous TB. HIV status was positive in 31.2% of cases. Resistance was found in 15.7% and multi-drug resistance (MDR) in 4.3% of cases. Previous treatment ($P < 0.001$) and relapse within two years were associated with

resistance ($P < 0.03$). Pulmonary cavities were associated with MDR ($P < 0.001$). Homelessness was associated with any resistance in newly diagnosed patients ($P < 0.01$). Working in a hospital was not associated with resistance.

T-cell assay conversions and reversions among household contacts of tuberculosis patients in rural India

M. Pai, R. Joshi, S. Dogra *et al*. *Int J Tuberc Lung Dis* 2009; **13(1):** 84-92

Interferon-gamma assays (IGRAs) are alternatives to the tuberculin skin test (TST), but IGRA conversions and reversions are not well understood. In a pilot study, we determined conversions and reversions using QuantiFERON-TB Gold In-Tube® (QFT) among household contacts of TB cases, and evaluated the effect of using various definitions and criteria for conversions. In a cohort of 250 contacts in India, 46% were TST-positive at baseline and 54% were QFT-positive. We re-tested this cohort after 12 months. Conversion rates were estimated using several definitions. Of the 250 contacts, 205 (82%) underwent repeat testing. Among 85 contacts with baseline TST-negative/QFT-negative results, TST conversion rates ranged between 7.5% and 13.8%, and QFT conversion rates ranged between 11.8% and 21.2%, depending on the definitions used. Among 109 contacts who were QFT-positive at baseline, seven (6.4%) had QFT reversions. QFT reversions were most likely when the baseline TST was negative and QFT results were just above the diagnostic cut-off. QFT conversions and reversions occurred among contacts of TB cases. Conversion rates seemed to vary, depending on the test and definitions used for conversions. These findings need to be verified in larger studies in various settings.

59th TB SEAL CAMPAIGN – ANDHRA PRADESH

The 59th TB Seal Campaign was inaugurated on 2nd October, 2008 at Raj Bhavan, Hyderabad (Andhra Pradesh), by Shri N.D. Tiwari, Governor of Andhra Pradesh. Dr. D. Rameshchandra, Director of Health, presided over the function. Dr. T.V. Venkateswarulu, Honorary General Secretary of the Tuberculosis Association of Andhran Pradesh, read the report on the TB Seal Campaign and activities of the State Association. The Governor of Andhra Pradesh gave away the TB seal shields and awards to the recipients. The function was attended by about 250 persons.

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