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Editorial

TUBERCULOSIS PREVENTION: AN ENIGMA WORTH UNRAVELLING

[*Indian J Tuberc 2012; 59: 65-67*]

"Never too old, never too bad, never too late, never too sick to start from scratch once again." - Bikram Choudhury.

Prevention is better than cure, is a clichéd age-old adage but it has epitomical significance in rendering a better and healthy society. A lot has been said about Tuberculosis detection, treatment, etc., but, surprisingly, not much is being done about prevention of tuberculosis infection.

Tuberculosis (TB) is an infectious disease of epidemic proportions caused by the bacillus *M. tuberculosis.* It typically affects the lungs (pulmonary TB) but can also affect other sites as well (extra pulmonary TB).[1] In 2010, there were 8.8 million (range, 8.5–9.2 million) incident cases of TB, 1.1 million (range, 0.9–1.2 million) deaths from TB among HIV-negative people and an additional 0.35 million (range, 0.32–0.39 million) deaths from HIV-associated TB.[1] In order to implement various measures to prevent tuberculosis infection, it is essential to know the process of transmission and pathogenesis of the disease. *M. tuberculosis* is carried as droplet nuclei which are airborne particles that can be generated by persons who have pulmonary or laryngeal TB disease when they cough, sneeze, shout, or sing.[2] The particles are approximately 1–5 μ*m.* These droplet nuclei can be airborne for prolonged periods.[3] The probability that a person exposed to *M. tuberculosis* will become infected depends primarily on factors like concentration of infectious droplet nuclei in the air, the number of organisms generated by the TB patient, the amount of ventilation in the area of exposure and the duration of exposure. The risk of transmission is mainly dependant on the factors linked to the source (sputum positivity), environment (duration of exposure, inadequate ventilation, infectious droplet nuclei) and the recipient (immuno-compromised status). Infection occurs when a susceptible person inhales droplet nuclei containing *M. tuberculosis.* After droplet nuclei reach the alveoli, local infection might be established, followed by dissemination to draining lymphatics and hematogenous spread throughout the body.

Usually within 2–12 weeks after initial infection with *M. tuberculosis,* the immune response limits additional multiplication of the tubercle bacilli. However, certain bacilli remain in the body in dormant state. This condition is referred to as latent tuberculosis infection (LTBI). Persons with LTBI are asymptomatic (they have no symptoms of TB disease) and are not infectious. Approximately, 5%-10% of persons who become infected with *M. tuberculosis* and who are not treated for LTBI will develop TB disease during their lifetimes. The risk of progression of LTBI to TB disease is the highest during the first several years after infection. *Mycobacterium tuberculosis* disease is generally through reactivation of the existing dormant bacilli when there is suppression of immune status. Bacillus Calmette-Guérin (BCG) vaccination does not reduce the risk of infection. It does decrease the risk of progression from latent TB infection to active TB, especially disseminated or central nervous system disease in children.

Although early diagnosis and initiation of treatment of infectious cases is the best measure to reduce transmission of infection, preventive measures for TB are directed towards reducing the risk of

infection (infection control strategies/ primary chemoprophylaxis), preventing the breakdown of infection to disease (secondary chemoprophylaxis) and control in the community (BCG vaccination). There are three categories of infection control measures: administrative, environmental, and personal respiratory protection.[4] Administrative controls are the most important since environmental controls and personal respiratory protection have limited impact by themselves in the absence of solid administrative control measures. Each of these measures operate at a different point in the transmission process. Administrative measures such as fast tracking the TB patients in OPDs and segregating in IPDs minimize the chance of the exposure of helath care workers (HCWs) and other patients to droplet nuclei of *M. tuberculosis.* Early identification of TB suspects without having to make multiple visits to health care facility, sputum collection in open spaces, reducing unnecessary stay in the hospital premises or hospitalization and early discharge are the main steps. Other important measures include a risk assessment in the facility, development of an Air borne Infection Control plan and adequate training of HCWs to implement the plan. Personal respiratory protection helps HCWs in areas where the concentration of droplet nuclei cannot be adequately reduced by administrative and environmental controls.[6] Patients should also be taught cough hygiene that is to turn their heads and cover their mouths and nose with hand or cloth while coughing.

Environmental or engineering measures reduce the concentration of infectious droplet nuclei in the air where contamination of air is likely. They prevent the spread and reduce the concentration of infectious droplet nuclei in ambient air. Primary environmental controls consist of controlling the source of infection by having well spaced wards, minimum six feet distance between two beds, using local exhaust ventilation (e.g.hoods,tents,booths) and diluting and removing contaminated air by using natural ventilation. Secondary environmental controls consist of controlling the airflow to prevent contamination of air in areas adjacent to the source (airborne infection isolation rooms), having proper air exchanges and cleaning the air by using high efficiency particulate air (HEPA), filtration, or ultraviolet germicidal irradiation.[5] Best option is the natural ventilation followed by the mechanical ventilation in which the movement of air is facilitated by the use of fans. Natural ventilation can occur when a room has adequate openings in the form of windows and doors in opposite directions with free flow of ambient air in and out. Additional windows or other openings may be considered that would allow for more ventilation.

Personal respiratory protection is the last measure, which may serve as a complement to administrative and environmental control measures. Since personal respiratory protection may not always be felt convenient or affordable in routine settings, it is most appropriate for use in high-risk areas in referral hospital setting. The type of surgical masks (cloth, paper) commonly used by HCWs does not filter out infectious droplet nuclei, but are of use when patients wear them to prevent the generation of aerosols and droplet nuclei. Personal respiratory protective devices for HCWs that are capable of adequately filtering out infectious particles are more expensive than surgical masks. To protect HCWs from *M.Tuberculosis* airborne droplet nuclei, a respiratory protective device with the capacity to filter 1-micron particle is needed. Respirators are special type of masks, which provide filtration of droplet nuclei and have to be closely fitted to the face to prevent leakage around the edges. The N-95 masks can be used by HCWs and are fibrous in nature- constructed from flat, non-woven mats of fine fibers. Respirator filters that collect at least 95% of the challenge aerosol are given a 95 rating and are rated “N” as they are not resistant to oil. Three “mechanical” collection mechanisms operate to capture particles: inertial impaction, interception, and diffusion. Inertial impaction and interception are the mechanisms responsible for collecting larger particles, while diffusion is the mechanism responsible for collecting smaller particles. In some fibrous filters constructed from charged fibers, an additional mechanism of electrostatic attraction also operates. This mechanism aids in the collection of both larger and smaller particle sizes. Respirator filters must meet stringent certification tests established by National Institute for Occupational Safety and Health (NIOSH). The most important aspect of a NIOSH-certified respirator’s performance will be how well it

fits to the face and minimizes the degree of leakage around the face piece. This must be measured for each individual and their selected respirator. Each respirator wearer should receive initial fit test.[5] Performing hand hygiene before and after touching the respirator is mandatory.

The second component in reducing the risk of infection is primary chemoprophylaxis. Primary chemoprophylaxis is intended to prevent occurrence of TB infection, for example in a newborn child of a sputum positive mother. Usually isoniazid (INH) is given in the dose of 5mg/kg. It is given till the mother remains sputum positive or for three months. At the end of three months, if Mantoux test is positive, chemoprophylaxis is continued for six months after ruling out active disease. If negative, BCG vaccine is given. Secondary chemoprophylaxis refers to treatment of sub-clinical infection. It is indicated in tuberculin test positive individuals. Based on the sensitivity and specificity of the tuberculin skin test and the prevalence of TB in different groups, three cut-off levels have been recommended for defining a positive tuberculin reaction: ≥5 mm, ≥10 mm, and ≥15 mm of induration.[6] Mantoux test (MT) >5 mm is considered positive in patients with human immune deficiency (HIV), fibrotic lesions on chest radiograph and close contacts of infectious TB, >10 mm reaction is positive in recent converters. More than 10 mm increase over two years seen in intravenous drug users known to be HIV negative, patients with predisposing medical conditions like diabetes, immunosuppressive drugs, patients from high prevalence countries. For all others, a reaction of > 15 mm is considered positive. It has been demonstrated that the administration of INH daily for six to 12 months reduces the risk of reactivation by up to 80%.

Finally, the last aspect in the prevention of TB in the community is BCG vaccine. The effectiveness of the vaccine is expressed in terms of its protective efficacy. Variable results from nil to 80% have been observed in various trials. It provides a high level of protection against serious forms of childhood TB such as miliary and meningeal TB (greater than 80%). However, its protective efficacy for preventing pulmonary TB in adolescents and adults is variable. WHO recommends BCG under Expanded Programme of Immunization. Around ten vaccine candidates either aimed at replacing the present vaccine, BCG, or at enhancing immunity induced by BCG have left the laboratory stage and entered clinical trials. Further research and development activities backed by a steady financial support are required.[7] Thus, let treatment alone not occupy our minds all the time. A fair share of our crusade against TB should be given to the prevention as well!

Shailly Saxena*, Vinaya Karkhanis and J.M.Joshi****

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CONTRIBUTIONS OF THE TUBERCULOSIS RESEARCH CENTRE, CHENNAI IN THE FIELD OF EPIDEMIOLOGY OF TUBERCULOSIS (A REVIEW OVER 50 YEARS)*

S. Radhakrishna**

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I deem it a great honour to give an invited talk on the contributions of the Tuberculosis Research Centre in the domain of the epidemiology of tuberculosis. The Centre's mammoth contributions in the treatment of the disease are very well-known, and have tended to overshadow its equally impressive record in epidemiology. I should hasten to point out that what goes by the name of the Tuberculosis Research Centre today (in fact, recently renamed National Institute for Research in Tuberculosis - NIRT) is really an amalgam of two ICMR projects – namely, the originally established Tuberculosis Chemotherapy Centre in 1956 in Madras City and the subsequently initiated Tuberculosis Prevention trial in 1966 in Chingleput district.

All epidemiological studies conducted by NIRT were undertaken in a rural population in Tiruvallur district, about 40 km from Madras, except the one in Madras City that compared the risk to close family contacts of patients treated at home with those treated in sanatorium.

To obtain information on time trends in the prevalence of tuberculosis, total population surveys were done at 2½ year intervals for 15 years.[1] At intake, all subjects aged ≥ 10 years had a 70 mm x-ray, and if it showed any abnormality, two sputum specimens were collected and examined by smear and culture, and drug sensitivity was determined (where applicable). At subsequent surveys, the same procedure was repeated but extending the eligible age to ≥ 5 years, and including new entrants (new-borns, settlers, persons missed at intake).

Estimation of Prevalence

Denoting by Ni the number of subjects eligible for x-ray in the i^{th} age-group, by n_i the number actually x-rayed, by S_i the number eligible for sputum examination, by s_i the number with sputum examined, and by f_i the number with a positive smear/culture, the number of positives in the i^{th} age-group is usually estimated from the expression $Ei = (f_i / s_i) \times (S_i / n_i) \times N_i$. The tacit assumption here is that the outcome of an x-ray or a sputum examination is the same in those missed as in those actually investigated. As this assumption is not necessarily tenable, new procedures were evolved.

(1) From those with both an x-ray and a sputum result, the probability of a positive smear/culture was determined for each of the x-ray groups, separately for males and females (Table 1). For subjects with an x-ray outcome but no sputum examination, the appropriate probability was applied to estimate the likely number of positive cases.

(2) From subjects with an x-ray and known symptom history, the relative risk of a subject with no x-ray having chest symptoms compared to a subject with x-ray was estimated, separately for males and females, in the age-groups 10-24, 25-44 and ≥ 45 years (Table 2). This was regarded as a proxy for the relative risk of a subject with no x-ray having smear-positive/culture-positive tuberculosis compared to a subject with

* This paper is based on an invited talk delivered at the 29th Annual Conference of the Indian Society of Medical Statistics in Chennai on 3rd November 2011.

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Table 1: Association between x-ray appearance and result of sputum examination

X-ray appearance	Probability of Positive Culture		Probability of Positive Smear	
	Male	Female	Male	Female
Probably Active TB	0.6345	0.4497	0.5601	0.3787
Possibly Active TB	0.2016	0.0815	0.1188	0.0392
Inactive TB	0.0315	0.0151	0.0083	0.0019
Other lung pathology	0.0053	0.0036	0.0012	0.0008
Normal	0.0003	0.0001	0.0001	0.0000

x-ray. Using the appropriate relative risk, the number of sputum-positive cases in those with no x-ray was estimated.

(3) Adding the estimated cases from (1) an (2) above to the observed number of sputum-positive cases, a consolidated total was derived, from which the prevalence in the i^{th} age-group was determined.

(4) Since the profile by age and sex varied over the years, the prevalence in each period was standardized to the baseline survey population profile, using the 'Direct' Method of Standardization.[2]

Trends in prevalence

The time trends in the prevalence of tuberculosis are shown in Table 3, for 7 population surveys undertaken over 15 years at 2½-year intervals on approximately 100,000 subjects in each period (commencing 1968-1970)[1], as well as for another undertaken at 30 years (1999-2001).[3] The culture-positive prevalence decreased from 1017 at intake to 688 per 100,000 at 15 years, a rather modest decrease of 2.1% per annum. The figures for smear-positive prevalence were 630 and 519 per 100,000, and the annual decline was 0.9%.[1] During this period, it was the usual practice to prescribe 12 months of daily self-administered chemotherapy, as stipulated in the National Tuberculosis Programme initiated in 1962. In 1985, short-course regimens of chemotherapy were introduced in this area, and the prevalence decreased from 688 to 510 per 100,000 in the next 15 years, an annual decline of 2.1% for culture-positive tuberculosis which was the same as that observed earlier. However, smear-positive tuberculosis decreased substantially from 519 to 276 per 100,000 over a period of 15 years, an annual decline of 4.1%, compared to 0.9% earlier (Table 3).

Table 2: Association between history of chest symptoms and x-ray availability

Age (years)	Relative Risk (RR)*	
	Male	Female
10 -- 24	0.05	0.05
25 -- 44	0.06	0.11
> 45	0.14	0.16

* of symptoms in subjects with no x-ray in relation to subject with x-ray. This RR was taken as a proxy for a 'No x-ray ' subject having TB, compared to a subject with x-ray.

In 1999, the Directly Observed Treatment Strategy (DOTS) was incorporated in a Revised National Tuberculosis Control Programme (RNTCP). A model DOTS project was established in the Centre's project area and total population surveys undertaken at baseline (1999-2001)[3], and at 2½, 5 and 7½ years in all subjects aged ≥ 15 years.[4-6] These showed a halving of the culture-positive prevalence in the first 5 years from 607 to

309 per 100,000, an annual decrease of 12.4% (Table 4)[5]. A similar pattern was seen for smear-positive prevalence, the corresponding figures being 326 and 168 per 100.00, representing an annual decrease of 12.2%. There was, however, a significant increase at 7½ years, to 388 per 100,000 for culture-positive tuberculosis and 180 for smear-positive tuberculosis.[6] Various hypotheses were considered for this finding (e.g. changes in clinical, x-ray or laboratory standards, changes in the gender-age profile) and ruled out, and no information was available on changes in the magnitude of incidence cases. A decrease in the routine case-finding efficiency in the Programme was, however, seen and this might have been the cause, as the 'missed' cases would have continued to be sputum-positive (due to lack of treatment) and swelled the numbers at the next survey (at 7½ years).

Estimation of incidence

At intake (1968-70), the at-risk population (i.e. after excluding those with a positive smear or culture) was admitted to a BCG prophylaxis trial,[7] with randomization of individual subjects on a 1:1:1 basis to BCG in a high dose, BCG in a low dose or a Placebo. All subjects were followed for 15 years, during which new cases were detected by:

(a) the periodic population surveys at 2½, 5, 7½, 10, 12½ and 15 years,
(b) selective case-finding once every 10 months in high-risk subjects (those with abnormal x-ray, symptomatics, absentees at routine surveys), and
(c) all persons with chronic cough attending Government public health institutions (at any time).

These *multiple* ascertainment methods ensured detection of most of the 'incidence' cases. Estimates of cases 'missed' due to failure to take an x-ray or failure to collect sputum specimens were made in the same manner as that described above. The entire estimation procedure was, however, restricted to subjects with no BCG scar at intake who received Placebo, and other eligible persons at subsequent rounds, including new entrants.

If N_k and n_k denote the population sizes of the Placebo group and new entrants in the k^{th} round ($k = 1, 2, 3, 4, 5, 6$), and I_k and i_k the incidences, the overall incidence in the community was estimated

Table 3: Time trends in the prevalence of pulmonary tuberculosis*

Year	Period	Number of subjects	Prevalence of P.TB (per 100,000)**	
			Culture-positive	Smear-positive
0	1968 - 1970	77089	1017	630
2.5	1971 - 1973	84760	894	576
5	1973 - 1975	88213	942	603
7.5	1976 - 1978	94837	894	498
10	1979 - 1981	98816	820	550
12.5	1981 -19 83	102999	808	602
15	1984 - 1986	104611	688	519
30	1999 - 2001	83431	510	276
	Annual decrease in:			
	Pre SCC period (0-15)		2.1%	0.9%
	SCC period (15-30)		2.1%	4.1%

* in subjects aged 15 years or more
** Standardized with reference to 1968-1970 Survey Population.

Table 4: Impact of DOTS strategy on the prevalence of tuberculosis*

Year	Survey Period	Population	Prevalence of TB (per 100,000)	
			Culture-positive	Smear-positive
0	1999 - 2001	83425	607	326
2.5	2001 - 2003	85474	454	259
5	2004 - 2006	89413	309	168
	Decrease per annum		12.4%	12.2%
7.5	2006 - 2008	92255	388	180
	Incrase per annum		9.5%	2.8%

* in subjects aged 15 years or more

by the expression $(3 N_k I_k + n_k i_k)/(3 N_k + n_k)$ [1], *assuming that all trial subjects had received Placebo.* Since the profile by age and sex of the population at risk varied over the 6 periods, the incidence in each period was standardized to the population at risk in the period 1971 - 1973, using the 'Direct' Method of Standardization.[2]

Trends in incidence

The annual incidence of culture-positive tuberculosis declined from 352 in 1971-1973 to 189 per 100,000 by 1984-1986, representing a decline of 4.3% per year (Table 5); correspondingly, the smear-positive incidence decreased by 2.3% per annum from 157 to 113 per 100,000.[1] These findings were surprising in view of the fact that the decline in prevalence (which would be expected to precede the decrease in incidence) was very modest. Detailed analyses showed that there was little change in the incidence in those with a normal x-ray or a nontuberculous abnormality (Table 6). As a matter of fact, the decrease was almost entirely due to subjects with a TB abnormality on x-ray (Table 6), 4.7% per annum for culture-positive tuberculosis (7001 to 3215 per 100,000) and 3.8% per annum for smear-positive tuberculosis (3357 to 1551 per 100,000).[1] As there was no evidence of a temporal trend in the classification of x-rays as abnormal, the explanation for the decreased incidence (which was not associated with a decrease in the ARTI – see below) might be that many patients might have received antituberculosis drugs, as treatment facilities became more wide-spread in the area. Alternatively, there could have been greater resistance to breakdown with tuberculosis due to overall improvement in socio-economic conditions.

Table 5: Time trends in the incidence of pulmonary tuberculosis*

Year	Period	Number of subjects	Annual Incidence of P.TB (per 100,000)**	
			Culture-positive	Smear-positive
0 - 2.5	1971 - 1973	26986	352	157
2.5 - 5	1973 - 1975	38461	250	142
5 - 7.5	1976 - 1978	44091	251	106
7.5 - 10	1979 - 1981	50794	207	104
10 -12.5	1981 -1983	56067	209	127
12.5 -15	1984 - 1986	65526	189	113
	Annual decrease		4.3%	2.3%

* in subjects aged 10 years or more.
** Standardized with reference to 1971-1973 Survey Population.

Annual risk of tuberculous infection (ARTI)

The annual risk of tuberculous infection is a valuable epidemiological index that is computed

Table 6: Incidence of TB related to x-ray status at the start of the period

Status of New case	X-ray status at start of period	Standardized annual incidence of tuberculosis (per 100,000)							
		1971-73	1973-75	1975-78	1979-81	1981-83	1984-86	Decline	P
Culture-positive	Normal	132	146	113	100	137	104	1.7%	> 0.2
	Non TB abnormality	1639	1915	1013	1455	1937	1468	0.2%	> 0.2
	TB Abnormality*	7001	4986	4347	3889	4649	3215	4.7%	0.03
Smear-positive	Normal	62	90	48	52	90	71	Nil	
	Non TB abnormality	628	982	366	694	1137	679	Nil	
	TB Abnormality*	3357	2279	1292	1717	2447	1551	3.8%	> 0.2

* Probable or possible active tuberculosis but all cultures negative

from the prevalence of tuberculous infection in unvaccinated young children as follows:

Prevalence of tuberculous infection = P
(in unvaccinated subjects)

Age of subjects tested (midpoint) = n

Let Annual risk of tuberculous infection be denoted by r

Then, r is determined from the equation $(1 - r)^n = (1 - P)$, and expressed as a percentage.

From the results in children aged < 10 years, the ARTI was estimated to be 2.0% in 1968-1970.[1] Relating this to the annual incidence estimate of 157 per 100,000 in 1971-1973 in subjects aged > 10 years, i.e. 116 per 100,000 in the total population of all ages, it is deduced that an ARTI of 1% corresponds to an incidence of 57 new smear-positive cases per 100,000 population. This values is within the 95% confidence limits of 39 -59 for the estimate of 49 derived by Styblo.[8]

There was no change in the Annual Risk of tuberculous infection over 15 years, the estimate of ARTI being 1.7%, 1.9% and 1.7% from 4889 children aged 1-9 years (belonging to a subset of eight Panchayats) who were tested initially, at 10 years and at 15 years.

Trends in drug resistance

Drug resistance affects the outcome of chemotherapy, and trends in this parameter are therefore of considerable clinical and epidemiological interest. In general, in prevalence cases, there was an increasing trend during the 15-year period, from 12.5% to 20.7% for Isoniazid, 6.4% to 12.1% for Streptomycin, and from 4.6% to 9.4% for both drugs (Table 7);[9] regression analysis of log proportion resistant on time showed that the increase per annum was 3.1% for Isoniazid, 4.9% for Streptomycin and 5.3% for both drugs. For new cases emerging during follow-up (incidence cases), the same patterns were seen, the annual increase (by regression analysis) being 3.8% for Isoniazid, 7.4% for Streptomycin and 8.0% for both drugs (Table 8).[9]

Risk of tuberculosis in close family contacts

Contacts of infectious tuberculosis patients constitute a particularly vulnerable group, and so it was necessary to establish that they were not at a

Table 7: Trends in drug resistance in prevalence cases of TB

Survey period	No. of patients	Percentage of patients with resistance to		
		Isoniazid	Streptomycin	Both drugs
1968 - 70	689	12.5	6.4	4.6
1971 - 73	693	18.5	7.2	5.9
1973 - 75	755	21.1	6.8	5.3
1976 - 78	855	15.3	7.7	5.0
1979 - 81	790	19.9	10.1	8.1
1981 - 83	832	21.4	10.9	7.9
1984 - 86	733	20.7	12.1	9.4
Annual increase		3.1%	4.9%	5.3%
95% CI		0.6% - 5.1%	3.6% - 6.2%	3.0% - 7.7%

higher risk than corresponding family contacts of infectious patients, who were quarantined (and treated) in sanatorium. A spin-off from the famous 'Madras Classic'[10] was a comparison of the risks to 'home' contacts and 'sanatorium' contacts (Table 9). In initially uninfected contacts, the incidence of tuberculosis over a 5-year period was 10.5% in 'home' contacts and 11.5% in 'sanatorium' contacts.[11] In all contacts, initially uninfected and infected, the corresponding incidences were 9.8% and 14.4%. Thus, there was no evidence that treating the infectious patient at home posed an extra risk to family contacts. However, there was little doubt that the family contacts themselves were at a higher risk than noncontacts (i.e. household members in homes with no TB case). Thus, the annualized incidence was 526 per 100,000 in contacts of very infectious (smear-positive) patients and 271 per 100,000 in smear-negative, culture-positive patients, as compared with 198 per 100,000 in noncontacts.[12] The Relative risks were 2.7 and 1.4, respectively, for the two contact series. A multivariate analysis, using Cox's proportionate hazards model, yielded an Adjusted Hazards ratio of 3.4 for contacts of smear-positive patients, and 1.7 for smear-negative patients

Table 8: Trends in drug resistance in incidence cases of TB.

Survey period	No. of patients	Percentage of patients with resistance to		
		Isoniazid	Streptomycin	Both drugs
1971 - 73	709	6.3	3.1	1.7
1973 - 75	621	9.2	4.2	2.7
1976 - 78	577	5.4	4.5	2.8
1979 - 81	520	10.0	6.5	4.4
1981 - 83	531	10.5	9.6	5.3
1984 - 86	530	10.0	6.0	3.8
Annual increase		3.8%	7.4%	8.0%
95% CI		minus 0.7% - 8.6%	2.9% -12.2%	3.4% - 12.7%

Table 9: Risk to family contacts from patients treated at home or isolated in sanatorium

Induration to PPD-S at intake	Infectious patient treated at	Number of contacts	Year of emerging case of tuberculosis					Total cases in 5-year period	
			First	Second	Third	Fourth	Fifth	Total	%
0 - 4 mm (uninfected)	Home	86	7	0	1	1	0	9	10.5
	Sanatorium	87	7	1	2	0	0	10	11.5
5 mm or more (infected)	Home	159	5	4	4	1	1	15	9.4
	Sanatorium	177	13	4	7	2	2	28	15.8
Total	Home	245	12	4	5	2	1	24	9.8
	Sanatorium	264	20	5	9	2	2	38	14.4

(Table 10). In the mid 1950s, there was a hypothesis, based on experimental evidence in animals, that isoniazid-resistant strains might be less virulent than isoniazid-sensitive strains in man.[13] The evidence from this community trial did not support the hypothesis. Thus, the annualized incidence was 295 in contacts of isoniazid-resistant patients and 311 for contacts of isoniazid-sensitive patients, as compared with 162 per 100,000 in noncontacts (Table 11).[14] The relative risks were 1.8 and 1.9 for the two series of contacts, and the corresponding Adjusted Hazards ratios were 2.4 and 2.0, respectively.

Prevention of tuberculosis

Since early diagnosis and effective treatment are not easy to organize in a mass domiciliary programme, prevention of tuberculosis, especially in those uninfected, is an attractive proposition. BCG vaccination had been reported as highly effective in the UK and in American Indians. Trials in Puerto Rico and Andhra Pradesh showed only a moderate degree of protection, while those in Alabama and Georgia showed no protection. In view of this confusing picture, the world's largest BCG trial was mounted in Tiruvallur district.[7] All persons aged ≥ 1 month in 209 Panchayats and 9 town blocks (N = 281,161), irrespective of their tuberculin status, were allocated at random to receive BCG vaccine (high dose) or BCG (low dose) or a placebo. Of these, 109863 had a normal x-ray and were uninfected at intake; 37000 of these received BCG high dose, 36459 received BCG low dose and 36404 received a placebo. There were several remarkable features about this trial:

(1) Size of the Trial Population was extremely large (N = 110,000)

Table 10: Additional risk to family contacts from patients treated at home*

Type of exposure at home	No. of contacts	Person-years	Annual incience (per 100,000)	Relative Risk	Adjusted Hazard ratio**	(95% CI)
Smear-positive Culture-positive (S+C+)	3506 - 7766	57516	526	2.7	3.4	(3.0 - 3.9)
Smear-negative Culture-positive (S-C+)	2910 - 6383	50901	271	1.4	1.7	(1.4 - 2.0)
No TB patient at home	246845	2555334	198	1.0	1.0	

* As per guidelines of the National TB Programme
** Adjusted by logistic regression for differences between the series in age, sex and infection status at intake.
Note: Attributable Risk was 52% for all contacts, 62% for S+C+ contacts and 27% for S-C+ contacts .

Table 11: Risk to family contacts from isoniazid-resistant and isoniazid-sensitive patients treated at home*

Type of exposure at home	No. of contacts	Person-years	Annual incience (per 100,000)	Relative Risk	Adjusted Hazard ratio**	(95% CI)
Isoniazid-resistant patient (H-res)	1305	7430	295	1.8	2.4	(1.7 - 3.4)
Isoniazid-sensitive patient (H-sens)	12650	55076	311	1.9	2.0	(1.7 - 2.4)
No TB patient at home	246845	2599459	162	1.0	1.0	

*As per guidelines of the National TB Programme
** Adjusted by Cox's propotionate hazards model with exposure group, sex and PPD-S at intake as variables, and age and
'reassignment' as time-dependent covariates, and allowing for clustering within households

Table 12: BCG double-blind community trial in Chingleput (15-year follow-up)

Age (years)	Vaccine series	No. of subjects*	Person-years	No. of cases**	Annual incidence (per 100,000)	Protection
ALL	BCG 0.1 mg	37000	380455	189	50	Nil
	BCG 0.01 mg	36459	374273	191	51	Nil
	Placebo	36404	373978	180	48	
0 - 9	BCG 0.1 mg	20537	219575	44	20	27%
	BCG 0.01 mg	20237	218330	47	22	21%
	Placebo	20207	219253	60	27	

* uninfected at the start of the trial

** At least one pofitive culture identified as *Mycobacterium tuberculosis*.

(2) Randomization was at the level of individual, and not cluster
(3) The duration of follow-up was very long (15 years), especially for a study in a developing country
(4) Computer aids (e.g. advance printed lists of field tasks to be undertaken), were employed for facilitating adherence to protocol
(5) Very high coverage was attained for X-ray (81%) and Sputum (95%) examinations
(6) Multiple ascertainment methods of netting incidence cases – Population surveys at intervals of 2½ years; more frequent follow-up (once in 10 months) of high risk subjects, continuous passive surveillance through Govt. PHIs.
(7) 'Double-blind' trial to eliminate bias (conscious or subconscious) in the conduct of the study.
(8) Quality of X-ray and bacteriological standards was carefully monitored throughout.
(9) Fingerprinting of all study subjects was undertaken at intake and on diagnosis, to ensure correct identification of subject. This was especially important as the trial involved very young children and a 15-year follow-up.

The outcome of this trial is summarized in Table 12. In general, BCG offered no protection against tuberculosis.[15] In children aged < 10 years, however, there was a suggestion of protection of the order of 25% but it failed to achieve statistical significance; in any case, the magnitude of the protection was of no public health importance. There are several possible hypotheses to explain the lack of protection from BCG. One of these is that protection offered by infection with nontuberculous mycobacteria[16] (a highly prevalent phenomenon in tropical countries such as India) might have masked the possible efficacy of BCG. Some evidence in support of this hypothesis came from this trial (Table 13). Thus, in

Table 13: BCG protection related to infection with nontuberculous mycobacteria

Characteristic	Series	No TB infection No nonTB infection*	No TB infection but nonTB infection present**
Annual incidence (per 100,000)	Placebo	42	61
	BGG low dose	33	68
	BCG high dose	27	74
Protection (univariate)	BGG low dose	21	Nil
	BCG high dose	37	Nil
Protection (multivariate)	BGG low dose	23	Nil
	BCG high dose	38	Nil

* PPD-S =0-7 mm, PPD-B < 10 mm (constitued only 40 % of subjects with no tuberculous infection).

** PPD-S = 0-7 mm, PPD-B ≥ 10 mm (constituted 60 % of subjects with no tuberculous infection)

subjects who had neither a tuberculous nor a nontuberculous mycobacterial infection, the protective efficacy of BCG low dose was 21% and that of BCG high dose was 37%. When adjustment was made for the effect of other concomitant variables, these estimates became 23% and 38%, respectively.[17]

To sum up, the following important questions in the epidemiology of tuberculosis have been unequivocally answered by the Tuberculosis Research Centre:

(a) What is the magnitude of the TB disease burden in Tiruvallur district?

(b) Were there any time trends with respect to the prevalence or incidence of tuberculosis in the last four decades?

(c) How was the prevalence of tuberculosis dented by the National Tuberculosis Programme - the initial one, the one with short-course regimens of chemotherapy and the current DOTS strategy?

(d) Did drug resistance show any time trends during this period?

(e) What was the risk to contacts from treating the TB patient at home?

  (i) in relation to isolating patient and treating him/her in sanatorium
  (ii) in relation to the general population in the study area.
  (iii) did the isoniazid sensitivity status of patient affect the risk ?

(f) How effective was BCG in preventing tuberculosis in un-infected subjects in the general population?

(g) Did concomitant non-tuberculous mycobacterial infections affect the efficacy of BCG?

(h) Is the Annual risk of tuberculous infection a good measure of the incidence of new cases in the community?

The vast amount of knowledge that has been generated by the statistically well-planned, meticulously executed and thoroughly analyzed epidemiological studies of the Tuberculosis Research Centre illustrates the immense value of imaginative epidemiologists working closely with skilled statisticians on public health issues. The sum of their individual contributions is supplemented by the beneficial effect of their interaction, which is my reason for saying that the Tuberculosis Research Centre has evolved a synergistic model that other research groups would do well to emulate. To epidemiologists in the audience, I would say "A statistician is like a wife. You can't live with one, for fear of too many awkward questions about design, sample size, analytical methods etc. But you can't live without one either, for he prevents you from wasting time on 'chance' red herrings, enables you to make valid generalizations, and provides you with the P values that often serve as visas for entry into prestigious research journals". To statisticians in the audience, I would exhort them to fully understand the practical problems faced by the researcher and field worker, and not be too theoretical or dogmatic in their approach. For example, 100% coverage for investigations is most desirable but often unattainable, nor is successful follow-up of all study subjects possible, in developing countries such as India, and these inadequacies invariably lead to biased estimates. If standard methods are not applicable, newer techniques must be evolved by the statistician to deal with these problems. By doing so, he will endear himself to the investigator and ensure that his advice is willingly sought at the planning stage itself for all future studies. In the ultimate analysis, a good doctor-statistician relationship is as important in medical research as a good doctor-patient relationship is in clinical medicine.

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The findings reported here cover five decades and are the handiwork of over 600 staff members whose sense of commitment and untiring zeal must be admired. The senior scientists involved are named below, project by project:

1. Risk to contacts of home and sanatorium patients

Wallace Fox**,** R.H. Andrews, S.Velu, C.V. Ramakrishnan, S.Devadatta, S.R. Kamat, J.J.Y. Dawson, S.Radhakrishna, P.R. Somasundaram

2. BCG trial

Raj Narain, J.Guld. R.S.Vallishayee, G.V.J.Baily,G.D.Gothi,P.Chandrasekhar, S. Mayurnath, M. Datta, M. Radhamani, P. Gopi, J. Frimodt-Moller, S. P. Tripathi, R. Prabhakar, C. Alexander, P. Venkataraman, A. M. Diwakara

3. Epidemiological data-mining reports

S.Radhakrishna, T.R.Frieden, R.Subramani

4. Model DOTS project

T.R.Frieden, P.R.Narayanan, T.Santha, R. Subramani, C.Kolappan, S.Radhakrishna, P.Gopi, R.C.N.Paramasivan, P.Venkataraman, F.Wares, N.Selvakumar

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Original Article

MULTI-ANTIGEN AND ANTIBODY ASSAYS (SEVA TB ELISA) FOR THE DIAGNOSIS OF TUBERCULOUS PLEURAL EFFUSION*

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Summary

Objective: Prospective evaluation of inhouse developed SEVA TB ELISA using cocktail of Mycobacterial antigens ES-31 and EST-6(containing ES-38 and ES-41) and their specific antibodies in the diagnosis of Tuberculous pleural effusion was done in a tertiary care hospital.

***Methods*:** Detection of circulating free and immune-complexed (IC) antigens and antibody by sandwich and indirect peroxidase ELISA respectively was done in pleural fluid and sera specimens. Total 33 patients with pleural effusion, including 24 patients diagnosed as tuberculous pleural effusion based on clinico-radiological, microbiological and biochemical profile (protein, LDH and ADA) of pleural effusion and nine patients with non-tuberculous pleural effusion, were studied.

Results: Pleural fluid showing either antigen or immune-complexed antigen or antibody positive was considered as ELISA positive for tuberculous pleural effusion. Multi antigen and antibody assay (SEVATB ELISA) showed 100% specificity and 83% sensitivity in pleural fluid while 78% specificity and 92% sensitivity in serum of tuberculous pleuritis patients.

Conclusion: This study showed usefulness of SEVATB ELISA, using cocktail of ES-31 and EST-6 antigens and their antibodies for antibody and antigen detection respectively in analysis of either sera or pleural fluid samples of suspected tuberculous pleuritis patients as an adjunct test to clinical diagnosis. **[*Indian J Tuberc 2012; 59:* 78-82]**

Key words: Mycobacterial ES antigens, ES-31, EST-6, SEVA TB ELISA, Tuberculous pleuritis, Multi antigen and antibody assay, Pleural effusion.

INTRODUCTION

Tuberculosis (TB) is a common cause of pleural effusion in countries like India in particular young adults and children. Detection of TB by ELISA in serum is widely explored all over the world but only few studies have been reported in diagnosis of tuberculous pleural effusion. Keertan Dheda *et al*[1] explored IP-10 and LAM antigen levels in pleural effusion and concluded that IP-10 may help in ruling out TB, while anti-BCG peroxidase ELISA was observed to be not sensitive in tuberculous pleurisy by Banchuin *et al*[2]. In earlier studies from our laboratory, we have observed usefulness of SEVA TB ELISA using Mycobacterial ES-31and EST-6 antigens and their specific antibodies in detection of antibody and antigen in the diagnosis of suspected patients of pulmonary and extrapulmonary tuberculosis attending a tertiary care Hospital, at Sevagram[3]. This study explores usefulness of inhouse developed SEVA TB ELISA assay for the detection of tuberculous antibody, circulating free and immunecomplexed antigen in pleural fluid and serum of suspected tuberculous pleural effusions.

MATERIAL AND METHODS

Patient's sera and pleural effusion

In this prospective study, pleural fluid and serum samples were collected from patients who underwent diagnostic evaluation between December 2010 and June 2011 at tertiary care Kasturba Hospital, Sevagram. Twenty four patients were diagnosed as having tuberculous pleural effusion based on clinical symptoms and biochemical parameters [pleural fluid

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to serum protein and Lactate Dehydrogenase (PLDH/SLDH) ratios, pleural fluid Adenosine Deaminase (ADA) level], Acid Fast Bacilli (AFB) positivity. Except for five transudative pleural effusion cases, all the 24 cases of exudative effusion had either increased PLDH/SLDH ratio(>0.6) or pleural fluid to serum protein ratio (>0.5) elevated ADA(>40 IU/L) suggestive of tuberculous pleural effusion.

Non-tuberculous pleuritis included patients having transudative pleural effusion due to congestive cardiac failure, hypoproteinemia or kidney disease.

Isolation of mycobacterial ES-31 and EST-6 antigens and their antibodies

Mycobacterial detergent-soluble sonicate antigen (DSS Ag) was prepared from *M. tuberculosis* H_{37}Ra bacilli[4]. Briefly, ten loopful bacilli were phenol(5%) inactivated for 1 h at 48^{0}C then suspended in 4 ml of 0.05 M phosphate-buffered saline (PBS) (pH 7.2). The bacilli were sonicated for 30 min with 30sec bursts at 1min intervals. The sonicate was incubated in 2 ml of sodium dodecyl sulfate (SDS) extraction buffer (5% SDS, 5% 2-mercaptoethanol, and 8 M urea in 0.01 M PBS, pH 7.2) in boiling water bath for five minutes, then incubated at 48^{0}C for 24 h. The supernatant was separated, dialyzed against 0.01M PBS, pH 7.2, for 48h, and labelled as DSS Ag and used for immunization. Anti-DSS-IgG antibodies were raised in goat by immunizing intramuscularly with 500 µg protein/mL DSS antigen and 1mL of Freund's incomplete adjuvant on days 0, 20, 33, and 45. Immune sera were collected on days 32, 44, 57, 60, and thereafter fortnightly. Anti-DSS IgG was isolated by 33% saturation with ammonium sulfate followed by diethylaminoethyl-cellulose ion exchange column chromatography as described earlier[5].

Mycobacterial ES-31 antigen was isolated from *M. tuberculosis* H_{37}Ra culture filtrate (ES) antigen by affinity chromatography using anti–ES-31 antibody-coupled Sepharose-4B column as described earlier[6]. EST-6 antigen was obtained by 6% trichloroacetic acid (TCA) solubilisation of *M. tuberculosis* ES antigen, followed by SDS-PAGE fractionation and elution of the 6th gel fraction[7]. Anti-ES-31 antibody was isolated from anti-DSS IgG by affinity chromatography[4]. Briefly, Anti–DSS IgG was passed through the cyanogen bromide-activated Sepharose-4B column and anti-ES-31 antibody was eluted by glycine–HCl buffer (0.01 mol/L, pH 2.5) and collected in Tris-HCl buffer (0.01M, pH 8.6). Similarly, anti-EST-6 antibodies were isolated from anti-DSS IgG by affinity chromatography using EST-6 antigen-coupled Sepharose-4B beads. Cocktail antigen (ES-31 and EST-6) and antibody (anti-ES-31, and anti-EST-6) were prepared by mixing the antigens and antibodies in equal proportion.

Enzyme Linked Immunosorbent Assay for cocktail antibody, circulating free and IC-antigen

Indirect peroxidase ELISA was performed for detection of antibody using cocktail antigen (ES-31 and EST-6)[3]. Briefly, the wells of ELISA plates (NUNC) were sensitised with cocktail antigen 2µg/well in 0.06M carbonate buffer pH9.6 overnight at 4^{0}C, followed by blocking with 2% BSA for 1hr at 37^{0}C. Plate was washed twice with PBS containing 0.05% Tween20 (PBS/T) followed by addition of sera or pleural fluid (1:50 dilution) in PBS/T for one hour at 37^{0}C, then washed three times. After that the wells were incubated in 1:15000 diluted rabbit-anti-human-IgG peroxidase conjugate for 1h at 37^{0}C. The wells were washed three times with PBS/T. The color was developed using TMB substrate (20X concentration). The reaction was stopped using 50µl 2N H_2SO_4. Then mean optical density at 450nm was read with ELISA reader.

Detection of circulating free cocktail antigen (ES-31 and EST-6) using affinity purified anti-cocktail antibody (anti-ES-31 and anti-EST-6) was performed by sandwich plate peroxidase ELISA[3]. Briefly, the plates were sensitised with anti-cocktail antibody 100µg/well, and the wells were finally exposed to 1:1000 diluted goat anti-cocktail antibody IgG peroxidase conjugate.

For the detection of immunecomplexed antigen, serum and pleural fluid samples were treated with Glycine-HCl buffer (0.1M) followed by heating at 65^{0}C for 15 minutes. To obtain cell free supernatant, serum or pleural fluid was centrifuged at 1000 rpm for five minutes at 4^{0}C[3].

RESULTS

In this prospective study, diagnostically useful ES-31 and EST-6 antigens and their specific antibodies were explored by peroxidase enzyme immunoassay for the detection of tuberculosis in pleural fluid and serum specimens of suspected tuberculous pleuritis patients. The pleural fluid samples of nine non-tuberculous pleurisy patients were screened for free Ag, IC-Ag and antibodies by microtitre plate peroxidase ELISA and the cut off OD was observed to be 0.41, 0.32 and 0.98 respectively. Pleural fluid was considered as positive if either free or IC antigen or antibody is positive. All the nine non-tuberculuos pleural fluid samples were negative by ELISA for the detection of Ab, Ag as well as IC-Ag while 20 out of 24 tuberculous pleural fluids were ELISA positive on combining Ab/Ag/IC-Ag detection result. Based on the observed absence of antibody, free antigen and IC-Ag, the pleural fluid ELISA showed 100% specificity. Further, detection of antibody (42%), free antigen (42%) and IC antigen (40%) was observed (Figure1) in tuberculous pleural fluid. However, combined (Ab/Ag/IC-Ag) positive result of pleural fluid ELISA showed 83% sensitivity, based on response to Anti Tubercular Therapy (ATT). Based on clinical diagnosis and biochemical parameters, 23 of 24 patients with TB pleurisy were given ATT. Serum from same patients were also analysed by ELISA which showed 92% sensitivity and 78% specificity (Table 2).

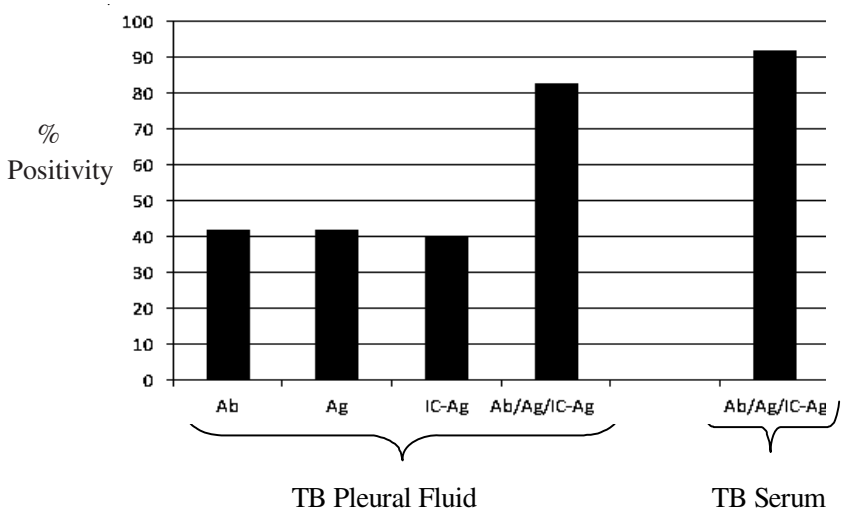

Figure 1: Percentage positivity of Ab, Ag and IC-Ag detection in pleural fluid and serum by SEVA TB ELISA.

Table 2: Analysis of ELISA results in serum and pleural fluid.

	Serum ELISA			Pleural fluid ELISA								
Study group	**-ve**	**+ve**	**% Positivity**	**Ag**		**Ab**		**IC-Ag**		**Ag/Ab/IC-**		**% Positivity**
				-ve	**+ve**	**-ve**	**+ve**	**-ve**	**+ve**	**-ve**	**+ve**	
Non-tuberculous (9)	7	2	**22**	9	-	9	-	9	-	9	**-**	**0**
TB pleural effusion samples (24)	2	22	**92**	-	10	-	11	-	8	4	20	**83**

OD cut off fo free antigen, antibody and IC-antigen positivity in pleural fluid are 0.41, 0.98 and 0.31 respectively

DISCUSSION

In clinical settings, tuberculous pleural effusion is diagnosed by exudative effusion with elevated levels of total ADA[8]. Detection of specific IgG to antigen-60 was found to be useful with 53% sensitivity and 100% specificity in the detection of tuberculous pleural effusion by ELISA[9]. A double antibody sandwich ELISA using anti-BCG and peroxidase labelled anti-BCG showed promise in detection of pulmonary tuberculosis. However, only three out of 26 pleural fluid specimens with tuberculous pleurisy were shown ELISA positive whereas 26 sera and urine samples of tuberculous pleurisy and all control specimens were negative by double antibody sandwich ELISA[10]. In a prospective study by Richter *et al*[11] of 118 HIV seropositive patients with pleural effusion, 84 patients showed presence of mycobacterium by Auramine, ZN staining or culture in pleural fluid or pleural tissue in referral hospital. TB was the underlying cause in 95% of patients who presented with pleural effusion. They further reported usefulness of two biochemical markers for the diagnosis of tuberculous pleural effusion namely pleural fluid protein(>50 g/L) and ADA(>10 U/L) in high prevalence of tuberculous pleurisy in patients. Detection of pleural fluid anti-A60 IgM was observed to be useful with 77% sensitivity and 94% specificity in the diagnosis of tuberculous pleurisy[12].

Our laboratory has reported usefulness of mycobacterial excretory secretory antigens in the diagnosis of different forms of TB by SEVATB ELISA in serum. Cocktail of antigens (ES-31, ES-43 and EST-6) was found to be useful in the detection of pulmonary tuberculosis cases with 91% sensitivity and 97% specificity[4].

In the present study, we tried to explore whether pleural fluid of pulmonary tuberculosis patients contains mycobacterial ES antigens and their specific antibodies similar to serum of patients attending a tertiary care Kasturba Hospital. As antigens used in the present assays are isolated from *M. Tuberculosis* H_{37}Ra, this assay is specifically imperative in detecting *M. tb* infection. Mycobatcterial ES-31 and EST-6 antigens and their specific antibodies

Table 1: Analysis of circulating free and immunecomplexed antigen and antibody ELISA in serum and pleural fluid.

Sr No.	Sample number	Serum Ab/Ag/IC-Ag result	pleural fluid			
			Ag	Ab	IC-Ag	Ag/Ab/IC-Ag
Non-TB pleural effusion						
1	P-4001	-	-	-	-	-
2	P-4004	+	-	-	-	-
3	P-4005	+	-	-	-	-
4	P-4012	-	-	-	-	-
5	P-4021	-	-	-	-	-
6	P-4022	-	-	-	-	-
7	P-4025	-	-	-	-	-
8	P-4026	-	-	-	-	-
9	P-4030	-	-	-	-	-
Tuberculous pleural effusion						
1	P-4002*	+	+	+	ND	+
2	P-4003	+	+	+	ND	+
3	P-4006	+	+	+	ND	+
4	P-4007	+	+	+	ND	+
5	P-4008	+	+	-	+	+
6	P-4009	+	-	+	-	+
7	P-4010	+	-	-	-	-
8	P-4011	+	-	-	+	+
9	P-4013	+	+	-	+	+
10	P-4014	+	-	-	+	+
11	P-4015	+	-	+	-	+
12	P-4016	+	+	-	+	+
13	P-4017	+	-	+	-	+
14	P-4018	-	+	-	-	+
15	P-4019	+	-	-	-	-
16	P-4020	+	-	-	-	-
17	P-4023	+	+	-	-	+
18	P-4024	-	-	-	-	-
19	P-4027	+	-	+	-	+
20	P-4028	+	-	-	+	+
21	P-4029	+	-	-	+	+
22	P-4031	+	+	-	+	+
23	P-4032	+	-	+	-	+
24	P-4033	+	-	+	-	+

ND – Not Done

*excepting for one (P-4002), all the 24 patients of TB pleurisy received anti tuberculosis therapy.

were used for the detection of free circulating antigen, immune-complexed antigen and antibody by sandwich and indirect peroxidase ELISA respectively. Of total 33 patients studied, nine were non-tuberculous pleural effusion and 24 were of TB patients. Two sera out of nine non-tuberculous and 22 out of 24 tuberculous pleural effusion patients showed ELISA positivity, showing 78% specificity and 92% sensitivity using serum as test sample. Individual Ab, free Ag and IC-Ag showed 42%, 42% and 40% positivity respectively in pleural fluid while on combining all the three (Ab/Ag/IC-Ag), 83% positivity was observed (Table 2). None of the Non-Tuberculuos pleural effusions showed positivity showing 100% specificity. Based on clinical and biochemical profile, 23 out of 24 patients of tuberculous pleurisy received ATT. Retrospective analysis showed that, one tuberculous patient did not get ATT though he was ELISA positive and showed decreased PLDH/SLDH in pleural fluid. Three patients showed ELISA negativity in pleural fluid but positive in serum and also received ATT (Table 1). One patient showing ELISA negativity in pleural fluid and serum but has shown elevated level of PLDH/SLDH and received ATT. Consideration of positivity of both serum and pleural fluid improves sensitivity at the cost of specificity. **This study shows usefulness of SEVA TB ELISA in the diagnosis of tuberculous pleurisy. Further extensive study with more number of patients is required to confirm the findings.**

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Original Article

MORBIDITY AND MORTALITY AT FIVE YEARS AFTER INITIATING CATEGORY I TREATMENT AMONG PATIENTS WITH NEW SPUTUM SMEAR POSITIVE PULMONARY TUBERCULOSIS

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Summary

***Background*:** Evaluation of disease outcome is central to the assessment of tuberculosis control programmes. Most of the follow up studies in RNTCP are short term. Five year follow up studies have not been done previously in this region. The objective of the present study is to evaluate the outcome of Category I treatment in smear positive tuberculosis, five years after treatment in terms of relapse, sequelae and death and to know the associated factors.

***Material and Methods*:** Patients who had registered for Category 1 treatment during the period 2002 – 2004 were followed up at five years with clinical evaluation, Chest X-ray, ESR and sputum AFB smear.

***Results*:** Of the 224 patients who were studied, 81% patients were males. Addictions, including smoking and alcoholism, were prevalent in 136 patients (61%). Treatment success rate at six months was 94.2%. At the end of five years, 124 patients (57.9%) were symptomatic, 59% patients had radiological sequelae, relapse in 10 patients (4.5%), and mortality in 12 (5.4%) patients. Diabetes mellitus was the most common comorbid illness. Smoking and age ≥ 45 years were associated with radiological sequelae. Smoking was significantly associated with mortality. Smokers had worse outcomes in all parameters.

***Conclusions*:** Relapse rate was 4.5% and overall mortality was 5.4% at the end of five years. Significant proportion of patients has radiological sequelae. Smoking was the preventable risk factor associated with sequelae, relapse and mortality. **[*Indian J Tuberc 2012; 59:* 83-91]**

Key words: RNTCP, Relapse, Mortality, Sequelae

INTRODUCTION

Tuberculosis remains a worldwide public health problem and is one of the most challenging communicable diseases to control effectively. India is the highest tuberculosis burden country globally accounting for one-fifth of the global incidence. The tuberculosis control programme in India (Revised National Tuberculosis Control Programme) is now one of the largest public health programmes in the world. The programme has been remarkably successful although it still faces many challenges. Evaluation of disease outcome is central to the assessment of tuberculosis control programmes. Studies in this regard are necessary for possible improvements in the programme in order to achieve further success, especially in RNTCP, which is undergoing a phase change. Most of the follow up studies in RNTCP are at the end of two or three years after treatment as most cases of relapse are thought to occur in this time period. Five year follow up studies have not been done previously in this region. Therefore, the present study attempts to evaluate the patients treated under RNTCP for smear positive tuberculosis five years after the initiation of their treatment.

OBJECTIVES

1) To evaluate the outcome of Category I treatment in new smear positive pulmonary tuberculosis patients registered under RNTCP, five years after initiation of treatment and to know their status in terms of relapse, sequelae and death.

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2) To study the factors associated with these outcomes.

STUDY METHODOLOGY

Study population

All patients, who had taken Category 1 ATT under RNTCP for new smear positive pulmonary tuberculosis five years before, are included in the study.

Study period

November 2008 to October 2010

Inclusion criteria

- All new sputum smear positive cases registered at the RNTCP center at ICD during the period from 2002 to 2005 are included and these patients are followed up so as to know their status five years after treatment.
- Those patients who were on follow up at the department who had treatment records from RNTCP showing they had taken Category 1 treatment for smear positive pulmonary tuberculosis during the period 2002 to 2005 were also included in the study.

Exclusion criteria

- All smear negative pulmonary and extra pulmonary cases are excluded from the study.
- All cases transferred out of state are excluded from the study.
- All patients who cannot be traced are excluded from the study [e.g, patients in temporary dwellings like gypsies].

Study design

Cross sectional study

Sample size

Sample size was determined using the formula

$$\textbf{Sample size} = 4pq/d^2$$

p = Mortality rate of 5% was taken
q = 100 – p
d = standard deviation of 3% was taken
Sample size = $4 \times 5 \times (100-5)/ 3^2$
= 210 approximately

METHOD

An analysis of records maintained under RNTCP was made. All new sputum smear positive cases during the period 2002 to 2005 were identified. Cases were contacted by sending letters in envelopes to maintain confidentiality. Those people who respond to the letters were advised to attend the Out Patient department. Those patients who were already on follow up in the department, who had previous treatment records from RNTCP showing they had taken Category 1 treatment for smear positive pulmonary tuberculosis during the same period were also included in the study.

When these patients visited the OPD, they were evaluated by a detailed medical history including present symptoms, past history, family history – contact with smear positive case and personal history – addictions including smoking and alcoholism, occupation and annual income.

Physical examination was done and clinical diagnosis was made. Investigations including Hemoglobin, TC, DC, ESR, and RBS, Chest X – ray and Sputum AFB smear examination were done in all patients. FBS/PPBS, Renal Function Test, Liver Function Test and ECG were done in selected cases. Sputum AFB culture, spirometry, bronchoscopy and CT Thorax were done if required.

Classification of radiological lesions

The radiological lesions were classified in to three groups.

- Class 1 – Minimal lesions

Lesions of limited extend and slight or moderate density, either unilateral or bilateral that involve an area not greater than that contained by the space between the apex of lung to second costal cartilage or the body of the fifth thoracic vertebra. Lesions should not include demonstrable cavitation.

- Class 2 - Moderately advanced

This consists of lesions of slight to moderate density involving the area occupied by one lung. If the lesions are dense, then the area should be limited to one-third of a lung. The lesions may be unilateral or bilateral. Total diameter of cavitation should be less than 4 cms.

- Class 3 – Far advanced

- This signifies involvement in excess of the above.

Statistical analysis

Statistical analysis was done using SPSS software version 12.0. The Chi-square test was used for testing the difference in the proportions. Univariate analysis was performed to find the distribution of factors among patients who had sequelae, relapse and those who had died. Odds ratio (OR) and 95% confidence intervals (95% CI) were calculated. In multivariate analysis, significant factors were included to find independent association of factors with outcomes adjusting for confounding factors. P value >0.05 was considered statistically significant. Continuous variables were tested using student T test.

RESULTS

A total of 742 patients were identified as new smear positive patients registered for Category 1 treatment at the Institute of Chest Diseases. All patients were contacted through letters. Of these, 202 patients attended the OPD. 22 patients who attended the OPD, who had the RNTCP treatment cards showing they had taken Category 1 treatment under RNTCP from various tuberculosis units during the period from 2002 to 2004 were also included in the study. Therefore, a total of 224 patients were available for study.

At the time of diagnosis of tuberculosis

The mean age of the population at the time of diagnosis of tuberculosis was 47 years $\pm$ 15. The age range was 15 to 80 years. Among these, 182(81%) patients were males and 42 (19%) were females. Addictions including smoking and alcoholism were prevalent in 136 patients (61%). Those with a smoking score of more than 100 were considered to be smokers. 74% of the smokers had a smoking score of 400 or above. When the smoking behaviour was analyzed, it was found that 57% of the smokers had not stopped smoking at all . Of the smokers, 75% said that they had not received any advice regarding the ill effects of smoking.

The mean duration of symptoms before diagnosis of tuberculosis was 9.5 weeks $\pm$ 12 weeks. The duration ranged from one week to 52 weeks. When the sputum status at the time of diagnosis was analyzed, it showed sputum AFB smear grading of scanty bacilli in 19 patients (8.5%), 1+ in 75 patients (33.5%), 2+ in 81 patients (36.2%) and 3+ in 49 patients (21.9%). That is 130 patients (58%) had an initial sputum smear grading of 2+ or more.

The co-morbidities at the time of diagnosis of tuberculosis were analyzed and showed that diabetes mellitus was present in 23 patients (10.2%). Hypertension was seen in 12 patients (5.3%). Cerebrovascular disease was present in two patients (0.9%). Coronary artery disease was present in 11 patients (4.9%). COPD was diagnosed in one patient (0.4%).

The treatment outcome at the end of six months was treatment success (cure + treatment completed) in 211 patients (94.2%), failure in five patients (2.2%) and death in four patients (1.8%), There were four (1.8%) defaulters. Category two

treatment was given to 18 patients which included 10 relapses, five failures and three defaulters. All of these patients were cured and are sputum negative by culture now. Drug sensitivity testing reports were available for seven of these patients. Two among these, one failure case and one relapse, were subsequently diagnosed to have MDR tuberculosis, which were also successfully treated with second line drugs and are culture negative now (Figure-1).

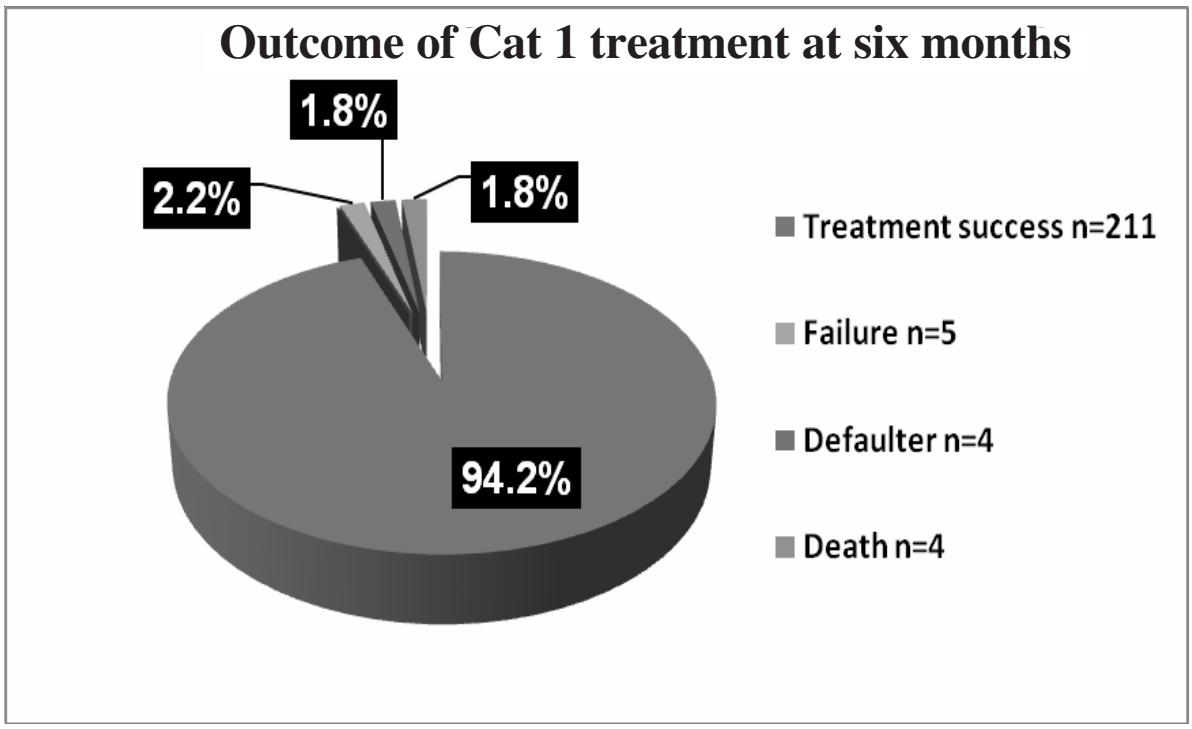

Figure 1: Treatment outcome at the end of six months

At the end of five years

124 patients (57.9%) were symptomatic. Fever was present in three patients (1.3%). Cough with expectoration was present in 91 patients (40.6%). Haemoptysis was present in 28 patients (12.5%). One patient had loss of weight (0.4%). Dyspnoea was present in 82 patients (36.6%).

71 patients had comorbid illnesses at present. 29 patients had diabetes mellitus (13.5%). Hypertension was present in eight patients. Four patients had both diabetes and hypertension. Five patients had CVA. Fifteen patients had coronary artery disease. COPD was present in 35 patients (16.3%). Carcinoma lung was diagnosed in two patients. One patient had Ca stomach.

The analysis of Chest X- ray revealed normal X-ray in 77 patients (36%), Class 1 shadows in 77 patients (36%), Class 2 shadows in 30 patients (14%), Class 3 shadows in 10 patients (4.7%), and hyperinflation in 14 patients (6.5%).

The final outcome at the end of five years

At the end of five years, 63 patients (28.1%) were normal , 68 (30.4%) patients were alive with sequelae, 26 (11.6%) patients were alive with

comorbidities and 45(20.1%) patients were alive with sequelae and comorbidities. Relapse was noted in 10 patients (4.5%), disease specific mortality in four patients (1.8%) and mortality due to other or unknown causes in eight patients (3.6%) (Figure-2).

Male sex (P value=0.001), low income (0.0001), initial weight loss (P value = 0.016), Category 2 treatment (P value = 0.0001), addictions (P value = 0.0001), smoking (P value = 0.0001), and age more than 45 years (P value=0.0001) were found to be significantly associated with **radiological sequelae** in univariate analysis. In multivariate analysis, smoking (P value=0.031) and age more than 45 years (P value = 0.032) were found to be significant (Table 1).

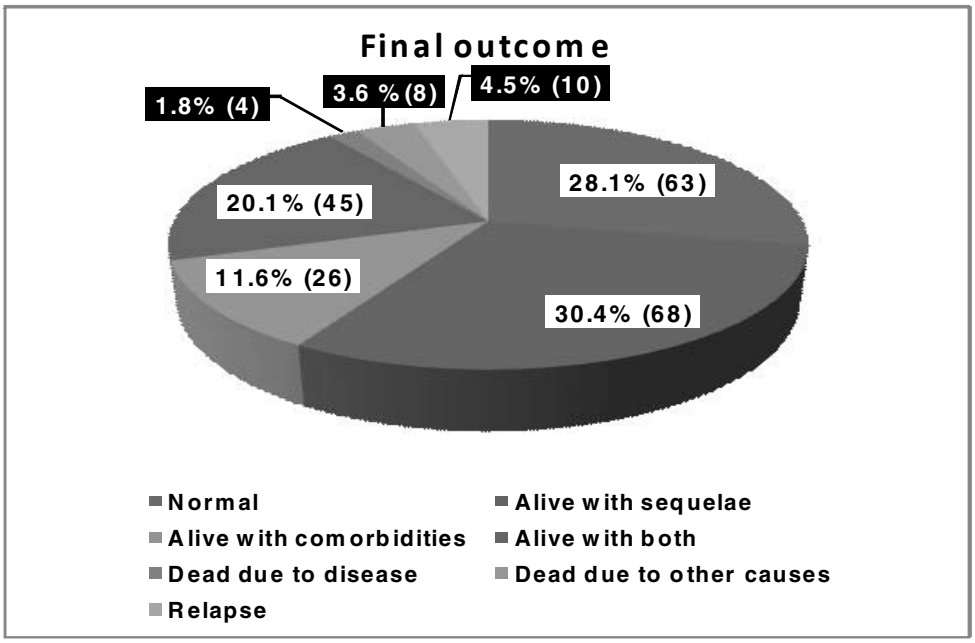

Figure 2: Treatment outcome at the end of five years' follow up

Table 1: Factors Associated with Radiological Sequelae

	P value univariate	OR (95% CI)	P value multivariate
Sex	0.001	1.884 (1.21 – 2.93)	0.432
Income	**0.000**	2.575 (1.34 – 4.96)	0.063
Initial Weight Loss	**0.016**	1.331(1.06 – 1.67)	0.126
Category 2 Treatment	**0.000**	1.876 (1.65 – 2.15)	0.696
Addictions	**0.000**		0.442
Smoking Behaviour	**0.000**		0.097
Smoking	**0.000**	**2.283 (1.632- 3.04)**	**0.031**
Age Group > 45 Years	**0.000**	**2.35 (1.7 – 3.04)**	**0.032**
Diabetes Mellitus	0.107		

The factors which showed significant association with **relapse** in univariate analysis were fever at initial presentation (P value = 0.007), addictions (P value=0.0001), drug resistance (P value = 0.002). Presence of diabetes mellitus was not significantly associated with relapse (P value = 0.992) (Table-2).

Twelve patients were dead at the end of five years (5.4%). Eleven patients were males and one patient was female. All the males who were dead were smokers and all were continuing to smoke till death. The disease specific **mortality** at the end of treatment period was four (1.8%). Of the remaining eight patients, two were in-hospital deaths. The cause of death was diabetic ketoacidosis in one patient and COPD with respiratory failure in another patient. Cause of death was not known in the remaining six patients. Smoking was found to be significantly associated with mortality in multivariate analysis (P value = 0.011) (Table-3).

Smokers had worse outcomes in all parameters studied. At the end of six months' treatment among the 13 patients who had worse treatment outcomes including death, failure and default, nine were smokers. At the end of five years, 63.5% (n=85) smokers had radiological sequelae compared to 31.2% (n=28) of non-smokers. Relapse rate was 5.2% (n=7) in smokers compared to 3.3% (n=3) in non-smokers. Overall mortality was 8.2% (n=11) in smokers compared to 1.1% (n=1) in non-smokers.

Table 2: Factors Associated with Relapse

	P value - Univariate	P value - Multivariate
Age $\geq$ 45 Years	0.937	
Male Sex	0.378	
Low Socio Economic Status	0.753	
Fever [at initial presentation]	**0.007**	0.790
Addictions	**0.000**	
Current Symptomatic	0.035	0.031
Diabetes Mellitus	0.992	
COPD	0.152	

Table 3: Factors Associated with Mortality

	P value Univariate	P value Multivariate
Smoking	**0.021**	**0.011**
Smoking Behaviour	**0.001**	
Initial Sputum Smear $\geq$ 2+	**0.015**	0.059
Weight Loss at Initial Presentation	**0.042**	0.096
Initial Dyspnoea	**0.046**	0.820
Diabetes Mellitus	0.859	
Age > 45 years	0.111	
Relapse	0.586	
Cat 2 Treatment	0.470	
Low Socio-economic Status	0.625	

DISCUSSION

Follow up studies of tuberculosis are few from India. In the present study, follow up rate is only 25% when compared to 42% reported in a similar study from Lucknow[1]. The mean age in our study population was higher in comparison to the available literature from this region[2]. The gender distribution showed a male predominance. The treatment success rate at the end of six months is better than that of the RNTCP status reports for the region in 2004.

At the end of five years, 57.9% were symptomatic. This is a significant number and is higher than that in similar studies[3,4]. This could probably reflect a selection bias as only symptomatic patients would attend the OPD of a tertiary medical centre.

59% of our study population was having radiological sequelae. But Class 3 shadows were seen in only in seven patients. This could reflect better case management in RNTCP including early diagnosis and effective treatment leading to decrease in numbers of patients with whole lung fibrosis or fibrothorax. The factors which were associated with radiological sequelae were age > 40 years, male sex, low income group, presence of relapse, and smoking. The studies dealing with post tubercular sequelae are few. One study conducted in Saudi Arabia by Al Hajjaj *et al* included 1080 patients with previous history of tuberculosis and found that old age, female gender, long duration of symptoms (delayed diagnosis), poor compliance with treatment and positive family history of tuberculosis were associated with poor radiological outcome[5]. A study conducted in Brazil analyzed data related to pulmonary function test in patients with post tubercular sequelae. The findings showed that the most prevalent was mixed ventilatory disturbance (17/50; 34%) and this was positively correlated with extend of radiological shadow[6].

The relapse rate was 4.5%. All relapses occurred within three years after treatment. An analysis of the factors associated with relapse showed a positive correlation for addictions including smoking and alcohol with the occurrence of relapse. The definition of 'bacteriological relapse' differs in various studies including clinical trials thus making comparison between studies difficult. Fully Intermittent regimens of six months' duration have shown a relapse rate of 7.7% (6%- 9.4%).

Among the major Indian studies, Vijay S *et al* studied the treatment outcome of 271 new smear positive patients treated under RNTCP in a metropolitan city 21/2 years later and found a relapse rate of 11.4%[3]. In a five-year follow-up study of RNTCP at Lucknow by S.K. Verma *et al*, outcome of 208 patients registered under RNTCP was studied. At the end of five years, 80 patients were available for follow up, of whom two had relapsed[1]. Thomas A *et al* in a study of predictors of relapse among pulmonary tuberculosis patients treated in a DOTS programme in South India followed up 503 patients for 18 months, there were 62 (12%) relapses during the 18-month period; 77% of the 62 relapses occurred during the first six months of follow-up. Irregular treatment (OR 2.5; 95% CI-1.4–4.6), initial drug resistance to isoniazid and/or rifampicin (OR 4.8; 95%CI- 2.0–11.6) and smoking were found to be predictors of relapse[7]. High initial smear grading has been found to adversely affect treatment outcome of tuberculosis in many studies[8,9].

In the present study, there were 19 diabetics. All had cure with six months of Cat I ATT. At the end of five years, none of them had relapsed or had significant sequelae. Several studies have reported a higher rate of adverse treatment outcomes including relapse in diabetics treated with short course chemotherapy[10]. Another important factor in determining the outcome is the presence of HIV infection. In the present study, there were no retro positive patients among the 224 subjects studied.

The next important outcome was the mortality in these patients. The overall mortality rate was 5.4%. Smoking behaviour and high initial sputum status has been found to be significantly associated with mortality in multivariate analysis. Tuberculosis ranks among the ten principal causes of death and disability worldwide, largely on the basis of mortality estimates. Only 59 of 213 countries in 2005 (including three in the World Health Organization Africa Region and one in the South-East Asia Region) had VR systems that reported tuberculosis deaths, corresponding to just 10% of

all estimated deaths attributable to tuberculosis[11]. There are few studies worldwide which have analyzed mortality in tuberculosis. In a study from Shanghai, China 7999 culture positive patients were studied and mortality analyzed during the time of ATT. The overall case fatality rate was 5.5% (440 cases), and half (50.5%) of the deaths were attributed to causes other than tuberculosis. The significant independent risk factors for mortality during anti-tuberculosis treatment were advancing age, male sex, sputum smear positivity, and the presence of a comorbidity[12].

Among the Indian studies, C Kolappan *et al* studied the mortality of tuberculosis patients in Chennai. The mortality rate among this cohort of tuberculosis patients was 60/1000 person-years. The excess general mortality expressed as standardized mortality ratio (SMR) was 6.1 (95% confidence interval (CI) = 5.4–6.9). Younger patients, men, patients with Category II disease, patients who defaulted on, or failed courses of treatment, and male smokers who were alcoholics, all had higher mortality ratios when compared to the rest of the cohort[13]. A study by Sadacharam *et al* in 2008 which followed up a cohort of smear positive patients treated under RNTCP found an overall mortality rate of 15.0%. In multivariate analysis, a higher mortality rate was independently associated with age, sex, occupation, treatment outcome and initial body weight of patients[14]. Another study conducted by Dhingra *et al* in Delhi which studied the mortality trends after the implementation of RNTCP showed that mortality due to tuberculosis has been considerably reduced in Delhi over the years with the Revised National tuberculosis Control Programme implementation since 1997[15].

Smoking was the major factor associated with worse outcome at the end of five years in the present study. Radiological sequelae, relapse, mortality and comorbid illnesses were higher in smokers. Smoking and tuberculosis are major public health problems and widely co-prevalent in the developing world. A recent review by Lin *et al* has shown that compared with people who do not smoke, smokers have an increased risk of having a positive tuberculin skin test, of having active tuberculosis, and of dying from tuberculosis[16]. A meta analysis by Bates and colleagues showed positive association between smoking and tuberculosis infection and disease[17]. In a narrative qualitative review, Chiang and colleagues summarized the evidence for the association between smoking and several tuberculosis outcomes including infection, active disease, and delay in diagnosis, relapse, drug resistance and mortality[18].

In India, only few studies have specifically looked into this aspect of smoking and tuberculosis[19,20]. Tuberculosis control programmes could consider tobacco control as a potential preventive intervention. Since smoking is a preventable and modifiable risk factor, there is considerable potential to design and implement tobacco cessation and prevention programmes as part of tuberculosis control programmes.

CONCLUSIONS

- **Category 1 treatment is effective in the treatment of new smear positive pulmonary tuberculosis patients.**
- **Relapse was present in 4.5% cases.**
- **Overall mortality at the end of five years was 5.4%.**
- **Significant proportion of patients has radiological sequelae at the end of five years after treatment.**
- **Smoking was the preventable risk factor associated with sequelae, relapse and mortality.**

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Original Article

UTILITY OF MPT 64 ANTIGEN DETECTION ASSAY FOR RAPID CHARACTERIZATION OF MYCOBACTERIA IN A RESOURCE CONSTRAINED SETTING

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Summary

Introduction: Important reasons for the negligible numbers of laboratories performing characterization of Mycobacteria in resource constrained settings are requirement of biosafety measures, longer turnaround time and laborious nature of tests. A rapid, accurate and simple test for characterization is required. "SD BIOLINE TB Ag MPT 64 Rapid®" is a rapid immunochromatographic test for differentiation of Mycobacteria into *M. tuberculosis* Complex (MTBC) and non-tuberculous mycobacteria (NTM).

Aim: To evaluate a commercial assay, SD TB Ag MPT64 Rapid® for characterization of Mycobacteria isolated on Lowenstein Jensen (LJ) medium.

Material and methods: 150 non duplicate isolates which were previously characterized as MTBC or NTM based on standard phenotypic characteristics were tested by the commercial assay after blinding. The result of the conventional phenotypic test and the commercial assay was compared. Any discordant result was referred for confirmation by genotypic Mycobacterium CM assay (Hain's life sciences, Germany). Sensitivity and specificity of the commercial assay was calculated using the results of conventional phenotypic and genotypic tests as gold standard.

Results: Phenotypically, 124 isolates were characterized as MTBC and 26 as NTM. The commercial assay gave concordant results for 149 isolates. One MTBC isolate did not demonstrate a band. The sensitivity, specificity, PPV and NPV was 99.19%, 100 %, 100% and 97.3% respectively. The total turnaround time for the rapid assay was 30 minutes compared to a few hours to days for phenotypic and genotypic method.

***Conclusion*:** "SD BIOLINE TB Ag MPT 64 Rapid®" is a simple, rapid and reliable test to differentiate MTBC from NTM. **[*Indian J Tuberc 2012; 59:* 92-96]**

***Key Words*:** *Mycobacterium tuberculosis*, MPT 64, Mycobacterial characterization.

INTRODUCTION

India has the highest burden of tuberculosis accounting for one fifth of the global incidence.[1] Tuberculosis is caused by members of *Mycobacterium tuberculosis* complex [MTBC], which includes *M. tuberculosis, M. bovis, M. africanum, M. canetti and M. microti*. In recent years, disease caused by mycobacteria other than tuberculosis [MOTT], also called as nontuberculous mycobacteria [NTM] are on the rise. This has been attributed to a parallel increase in HIV infection and other immunocompromised states.[2]

The clinical presentation of pulmonary disease due to NTM is similar to that caused by *M. tuberculosis* [MTB]. As a result, NTM as a cause of pulmonary tuberculosis are often under-diagnosed in resource constrained settings lacking culture and identification facilities. It is important to accurately characterize mycobacteria since NTM are inherently resistant to conventional anti-tuberculosis drugs, require modified treatment regimens and are often misdiagnosed as multidrug-resistant tuberculosis [MDRTB].[2]

Characterization of mycobacteria can be done phenotypically or genotypically. Conventional

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phenotypic methods for identification of mycobacterial species are based on the results of rate of growth, pigmentation of colonies and various biochemical reactions. These methods are time-consuming (growth on PNBA medium), involve use of hazardous chemicals, some of which are carcinogenic (niacin test) and are prone to subjective error in interpretation of results (nitrate reduction).[3] On the other hand, molecular methods that identify specific nucleic acid sequences are rapid, sensitive and specific, but are expensive and require trained personnel and special laboratory setup.[4] Hence there is need for a rapid, accurate and simple test for characterization of mycobacteria.

A variety of antigens have recently emerged for the immunodiagnosis of TB. The *Mycobacterium tuberculosis* protein 64 (MPT-64) antigen is an *M. tuberculosis* complex (MTC) specific antigen secreted during bacterial growth. It is also termed as protein Rv1980c which is a 24 kDa secretary protein secreted by MTBC, except some strains of M. *bovis* BCG.[5,6] This antigen is encoded by the RD2 region which is specific for MTBC and can be detected in culture isolates and biopsy samples.[7] MPT 64 induces a strong delayed type hypersensitivity reaction similar to that induced by purified protein derivatives in sensitized guinea pigs.[8]

Recently, Standard Diagnostics (SD, Korea) developed a simple and rapid assay, "SD BIOLINE TB Ag MPT 64 Rapid®" [commercial assay] to discriminate between MTBC and NTM by immunochromatography (ICT). The present study was undertaken to evaluate the commercial assay, SD TB Ag MPT64 Rapid® for characterization of Mycobacteria already isolated on Lowenstein Jensen (LJ) medium from cases of pulmonary and extrapulmonary tuberculosis.

MATERIAL AND METHODS

The study was carried out after obtaining Institutional Ethical Committee's permission. One hundred fifty non-duplicate strains included for analysis in this study were those isolated from clinical specimens routinely received for mycobacterial culture prior to the commencement of the study. It included isolates recovered from both pulmonary as well as extrapulmonary specimens. These strains were previously characterized as MTBC or NTM based on standard phenotypic characteristics which included rate of growth, pigment production, niacin test, growth on LJ medium containing PNBA, nitrate reduction and catalase test.[3] Any slow growing acid fast isolate, forming buff coloured colony on LJ medium, niacin and nitrate positive, unable to grow on PNBA and unable to produce a heat stable catalase was identified as MTBC. Of the 150 isolates tested, 124 were MTBC and 26 were NTM. Five reference strains (*M. tuberculosis* H37Rv, *M. fortuitum*, *M. gordonae*, *M. scrofulaceum* and *M. smegmatis*) obtained from mycobacterial repository at the Central JALMA Research Institute, Agra were used as controls. Ten nonmycobacterial strains (Gram positive as well as Gram negative bacteria) were included to detect any false positives. Twenty isolates (10 MTBC and 10 NTM) were tested in duplicate by the commercial assay to assess the reproducibility.

The work was carried out in a Class II Biosafety Cabinet and level two biosafety practices were followed. Strains to be tested by the commercial assay were first sub-cultured on LJ medium. When sufficient growth was obtained, the specimen numbers were blinded. The commercial assay, "SD BIOLINE TB Ag MPT 64 Rapid®", based on the principle of ICT, was performed as per the manufacturer's instructions.[9] The kit contains cassettes with nitrocellulose strip on which mouse monoclonal anti- MPT64 antibodies (test line) and goat anti-mouse antibody (control line) are immobilized. Another mouse monoclonal antibody recognizing a different epitope of MPT 64 antigen and conjugated with colloidal gold particles is present in the sample well. If MPT 64 antigen is added to the strip, it gets captured by both types of mouse monoclonal antibodies and gives a visible test band. The mouse monoclonal antibody conjugated with colloidal gold particles combines with goat anti-mouse antibody to give the control band.

Briefly, a suspension of the mycobacterial isolate to be characterized was prepared in 200µl extraction buffer provided with the kit. Where condensation fluid was present in medium, it was

directly used. 100 µl of the suspension or condensation fluid was added in the sample well. The inoculated cassettes were kept undisturbed at room temperature and were examined at the end of 15 minutes for presence of pink band in "Control" and "Test" region.

Interpretation

The appearance of control band confirmed the validity of the test. If the control band was not visible in 15 minutes, the result was considered invalid and the sample was retested. The presence of only control band in the absence of test band was considered a negative test and interpreted as absence for MPT64 antigen. Presence of both control and test band indicated a positive result and interpreted as presence of MPT64 antigen. Faint color band was recorded as weak positive and the sample was retested.

Analysis

The identification of the isolate was unblinded. The result of the conventional phenotypic test and the commercial assay was compared. Any discordant result was referred for confirmation by genotypic Mycobacterium CM assay (Hain Life Sciences, Germany). Sensitivity and specificity of the commercial assay were calculated using the results of conventional phenotypic and the genotypic test as the gold standard.

RESULTS

The control band was detected in all the 165 strains tested indicating validity of the test. H37 Rv strain showed the appearance of pink band in the test region indicating the presence of MPT 64 antigen. Reference NTM strains and the ten non-mycobacterial strains gave negative result indicating absence of MPT 64 antigen. The commercial assay was therefore considered satisfactory. Ten MTBC and 10 NTM strains which were tested in duplicate gave concordant results.

Of the 124 MTBC strains tested, 123 showed presence of MPT 64 antigen. The discordant strain was retested by both phenotypic methods as well as by the commercial assay. On obtaining the same result, the strain was referred for Genotype Mycobacterium CM assay (Hain Life Sciences Germany) and was identified as MTBC. Fourteen strains which gave a faint band by the commercial assay were retested and found to give weak positive result both the times, hence considered as MTBC for analysis. All 26 NTM strains gave a negative result. The specificity, sensitivity, PPV and NPV of the commercial assay were 100%, 99.19%, 100% and 97.3% respectively.

DISCUSSION

Tuberculosis is a major public health problem in India. In a scenario of NTM disease prevalence and increasing drug resistance in MTB, speciation and drug susceptibility testing have become a necessity today for appropriate patient management. There is a need for a rapid, simple yet accurate test for differentiation of mycobacteria. It was therefore decided to assess the utility of "SD BIOLINE TB Ag MPT 64®" assay marketed as a rapid ICT test in a resource constrained setting. The major attraction for using this test was its claim to characterize mycobacterial isolates accurately in 15 minutes. This assay was evaluated for rapid characterization of 165 culture isolates and whether it could replace the conventional phenotypic methods. An important observation of this study was 100% specificity and 99.19% sensitivity for the assay. Other studies have also demonstrated specificity of 100% and sensitivity ranging from 96.5% to 100%.[10-14] The test is based on the detection of MPT64, an antigen considered specific for MTBC. The MPT 64 antigen is a highly specific protein of MTBC which has been confirmed by cloning and sequencing of MPT 64 gene of H37Rv culture filtrate.[15] It has also been proved that MPT 64 antigen is found only in viable and actively dividing cells of MTB.[5] It is secreted in significant amounts during early period of culture and decreases with longer cultivation. This antigen is found in the culture fluid of MTBC isolation media. The MPT 64 antigen is absent in BCG strain of *M. bovis, M. leprae* and nontuberculous mycobacteria.[15]

In the present study, the commercial assay missed identifying one isolate as MTBC. As the test was performed from a recent sub-culture on LJ medium, nonviability or older culture could not be the reasons for the false negativity. A previous study has established that, MPT 64 once secreted into the culture medium, is stable for at least one year.[4] Therefore, instability of the antigen could also not be the reason for the false negativity. The genotypic test used, can characterize mycobacteria only into the broad MTBC / NTM group. They do not identify the members within the complex. It is possible that some members within the complex lack MPT 64. In a study by Li. H. *et al*, it has been observed that some substrains of *M. bovis* BCG lack MPT 64 production. The discordant strain could be such a variant.[16] It is suggested that Lowenstein Jensen medium with sodium pyruvate be routinely used to differentiate *M. bovis* from other MTBC strains. Though the turnaround time is longer in comparison to molecular assay, it is a simple and inexpensive test to incorporate. This was not part of the differentiation panel used in the present study which would have easily helped in characterizing *M. bovis* strain. Another possible explanation for the negative test result is that the strain had mutations within the *mpt64* gene, leading to the production of an incomplete protein. By sequencing, Hirano *et al* identified several such mutations, including deletion of nucleotides, point mutations, and an IS*6110* insertion mutation at nucleotide position 501.[17] False negativity may also be related to the low-level expression of the antigen.[12]

The only limitation of this assay is the requirement of biosafety procedures and equipment while manipulating the culture. This probably is not a true limitation since biosafety is a mandatory requirement for Mycobacteriology laboratories performing cultures.

The strengths of this commercial assay are its rapidity, simplicity, ease of use, economy and non-requirement of technical skill, equipment, hazardous chemicals and low temperature storage. The assay is based on the principle of ICT, and is therefore rapid. With only a suspension of an already isolated mycobacterium to be prepared for testing, it is simple. The kit is currently available at a cost of Rs. 4250/- and comes in a pack size of 25. With this, the cost per test for the ICT assay used in this study was Rs. 170/-. The single test cartridge is individually packed, so as manty tests can be used without the fear of wastage of remaining kit. The kit should be used only after validating with known positive and negative controls. This serves as the batch/lot validation and negates the need for running controls with each isolate/ test. In comparison, the cost of consumables for differentiation by the battery of biochemical tests recommended is approximately the same or slightly lesser. However, with the phenotypic tests, the storage of reagents at low temperature and the need for running separate positive and negative controls with each test would be the hidden limitation. The molecular assays on the other hand cost a minimum of Rs. 1000/- per isolate identification and do not differentiate within members of MTBC. The total time required to complete the assay is approximately 30 minutes, compared to many hours for the molecular characterization methods and few days for the phenotypic tests. The detection limit of this assay is 10^5 CFU/ ml which can be prepared by emulsifying three-four tiny colonies from a solid medium, obtained from early stages of subculture. This therefore shortens the overall time of identification of the clinical isolate by three-four weeks. Both culture suspension and culture filtrate gave equally satisfactory results. The assay also demonstrated good repeatability. With 99.19% sensitivity and 100% specificity, the test appears to be accurate and has the potential to replace the phenotypic methods of characterization.

CONCLUSION

"SD BIOLINE TB Ag MPT 64®" assay is a simple, rapid, economical and reliable test for characterization of clinical mycobacterial isolates that can be easily incorporated by myco-bacteriology laboratories. Rapid characterization of mycobacterial isolates is expected to facilitate treatment decisions in tuberculosis control programme. This assay should be further evaluated for identification of MPT 64 antigen directly in smear positive clinical samples.

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MULTIPLE VISCERAL ABSCESSES DUE TO TUBERCULOSIS

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***Summary*:** Tuberculosis is a global epidemic, especially in India. In immuno-competent host, abdominal tuberculosis most commonly presents as ileo-caecal tuberculosis and ascitis. Presented is a rare case of immuno-competent host with abdominal tuberculosis in the form of multiple visceral abscess. **[*Indian J Tuberc 2012; 59:* 97-99]**

***Key words*:** Tuberculosis, Abscess, Immuno-competent.

INTRODUCTION

Tuberculosis is a major health problem in developing countries. The gastrointestinal tract (GI) tuberculosis is reported to be the sixth most common extra-pulmonary site TB. Evidence suggests that 15 to 50% of GI tuberculosis patients may have co-existing pulmonary tuberculosis. Abdominal TB can involve the intestine, liver, spleen, lymph nodes, and peritoneum. The diagnosis of GI tuberculosis is often delayed, resultantly increasing the morbidity associated with this potentially treatable condition.[1]

CASE

A 35-year-old patient reported to us with a history of moderate grade fever, anorexia and weight loss of about 15 kg since three months. Past medical and surgical history was insignificant. His general physical examination was unremarkable. Systemic examination revealed mild diffuse abdominal tenderness. Rest all systems were normal. On investigation, all the routine blood investigations were normal. ESR was 90 mm in first hour. ELISA for HIV was non-reactive. On imaging studies, his CXR was normal but USG of the abdomen revealed multiple abscesses in the liver, spleen and psoas muscle. CT scan abdomen (Fig. 1) revealed liver abscess in segment six (arrow 1), right psoas abscess (arrow 2), splenic abscess (arrow 3) and necrotic enlarged lymph node at the porta (arrow 4). In addition, there were multiple enlarged lymph nodes at the porta and peripancreatic region.

Considering the history and imaging findings, a pigtail catheter was inserted to drain the psoas abscess. The drain was positive for AFB.TB-PCR of the collection was strongly positive and culture confirmed and substantiated our diagnosis by showing the growth of tubercle bacilli. Mantoux test was strongly positive with 30mm of induration in 48 hours (Fig. 2).

The patient was started on anti tuberculous therapy i.e DOTS thrice a week regimen according to WHO 2010 guidelines two months of isoniazid(H), rifampicin(R), pyrazinamide(Z) and ethambutol(E) followed by four months of isoniazid and rifampicin, and his fever subsided in one week period. His appetite improved and he gained 1.5 kg weight over a period of 15 days. The amount of drain gradually decreased. After 20 days, the drain decreased to less than 20 ml in 24 hours. The drain was removed and the patient was discharged. He was advised follow-up after one month for repeat ultrasound examination of abdomen for the status of abscesses.

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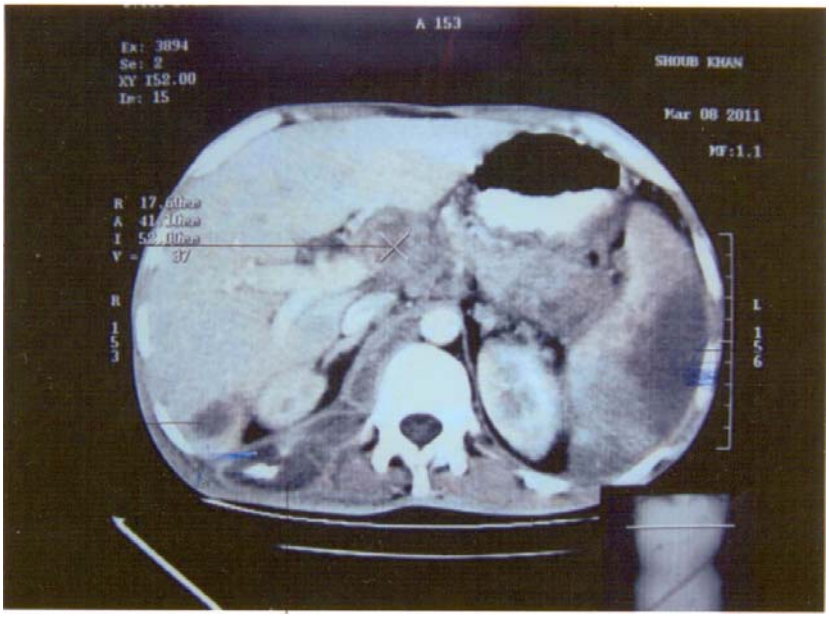

Figure 1: CT abdomen showing liver abscess in segment six, right psoas abscess , splenic abscess and necrotic enlarged lymph node at the porta

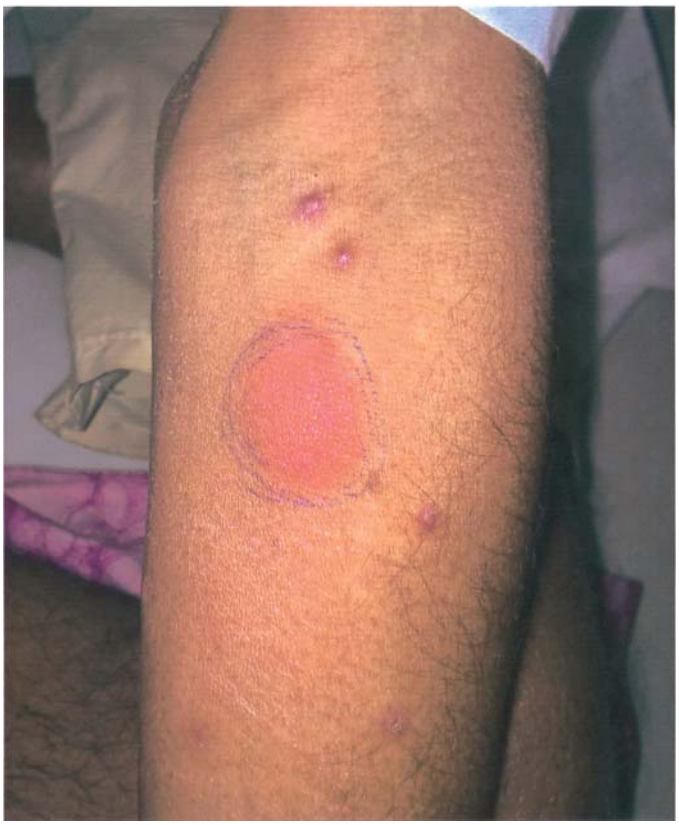

Figure:2: Strongly positive Mantoux test (30mm of induration)

DISCUSSION

The pathogenesis of GI tuberculosis postulates several mechanisms by which tubercle bacilli invade the GI tract. The possible mechanisms are hematogenous spread from primary lung focus, ingestion of bacilli in sputum from active pulmonary TB disease, direct spread from adjacent organs and through lymphatics from diseased nodes.[2] Tuberculosis of genito-urinary tract can involve kidney, perinephric tissue prostate and very rarely bladder. The possible explanations are ascending infections or hematogenous dissemination.

There are case reports suggesting tubercular abscesses involving isolated organs like liver, spleen, prostate and peri-nephric tissue.[3-6] The present case is one of the rarest presentations of disseminated abdominal TB involving abdominal organs and psoas muscle in an immuno-competent individual without lung involvement or miliary disease. The possible explanation of disseminated abdominal tuberculosis involving psoas muscle, liver, spleen and abdominal lymph nodes in the present case is by way of either local spread or hematogenous spread.

The basic approach to management of GI tuberculosis is anti-tuberculous chemotherapy. The recent WHO 2010 guidelines for tuberculosis management recommend two months of HRZE in intensive phase and four months of HR in continuation phase for a total duration of six months (H=5mg/kg for daily regimen,10mg/kg for alternate day regimen, R=10 mg/kg, Z=25 mg/kg for daily regimen and 35mg/kg for alternate day regimen and E=15mg/kg daily regimen and 30 mg/kg alternate day regimen). **This regimen is to be given daily or can be given alternate day/three times a week, if it is directly observed therapy.**[7]

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Case Report

SEIZURES WITH SINGLE THERAPEUTIC DOSE OF ISONIAZID

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Summary: Isoniazid (INH) is an integral component of treatment of tuberculosis. An acute overdose is potentially fatal and is characterised by the clinical triad of repetitive seizures unresponsive to the usual anticonvulsants, metabolic acidosis with a high anion gap and coma. A case of isoniazid induced seizures after therapeutic dose of 600 mg. as a part of CAT I thrice weekly intermittent anti-tuberculosis regimen for pulmonary tuberculosis is reported. The frequency of the usage of Isoniazid as antituberculosis therapy requires that physicians be aware of such toxicity. ***[Indian J Tuberc 2012;59: 100-102]***

Key words: INH, Seizures, Isoniazid, INH Toxicity

INTRODUCTION

Isoniazid is an effective and widely used drug in the treatment of tuberculosis. The administration of toxic amounts of isoniazid (INH) causes recurrent seizures, profound metabolic acidosis, coma and even death but therapeutic dose of isoniazid rarely causes seizures. Presented is a case of 65-year-old male who developed isoniazid induced seizures after first therapeutic dose as a part of CAT I thrice weekly intermittent anti tuberculosis regimen with isoniazid 600 mg, rifampicin 450 mg, ethambutol 1200 mg and pyrazinamide 1500 mg.

CASE REPORT

Mr S K, a 65-year-old non-smoker male, was admitted in emergency ward with history of seizure after administration of first dose of anti-tuberculosis chemotherapy for pulmonary tuberculosis. He was apparently well six months' ago when he developed cough and expectoration. He was diagnosed as a case of sputum smear positive pulmonary tuberculosis. He was advised CAT I intermittent anti-tuberculosis regimen with isoniazid 600 mg, rifampicin 450mg, ethambutol 1200 mg and pyrazinamide 1500 mg thrice weekly. On the very first day of anti-tuberculosis therapy, he developed convulsions after one hour. He was admitted in the nearest health facility and was administered oxygen therapy and injection phenytoin intravenously and was referred to our institute after six hours. Patient's wife told that he developed seizure one hour after taking two tablets of Isoniazid 300 mg and one capsule Rifampicin 450 mg. On admission, he was conscious, pulse rate was 82 per minute, blood pressure 120/80 mm of Hg and oxygen saturation of 95% at room air. Thorough nervous system examination revealed no abnormality. Routine blood examination revealed: haemoglobin 11.2 gm %, total leucocyte count 12400 cells / cu mm with polymorphs 94%, and lymphocytes 6% , blood urea 25mg% (normal 10-50mg%), serum creatinine 0.58 mg/dl (normal 0.20-1.20 mg%), serum sodium 140 m mmol/1(normal 133-145 mmol /L), serum potassium 5.6mmol /L (normal 3.8-5.56mmol /L), fasting blood sugar 100mg% (normal 70-110 mg%). Platelet count was 150,000/ cu mm and routine urine analysis was normal. There was no personal or family history of epilepsy or history of head injury; CT scan head was normal. There was no history of alcohol abuse. After admission, there was no seizure for two days, anti-tuberculosis chemotherapy with rifampicin 450 mg, ethambutol 1200 mg and pyrazinamide 1500 mg was administered orally. Isoniazid was withheld. He tolerated these drugs without any

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adverse event. Isoniazid is an integral component of treatment of tuberculosis, so it was decided to add isoniazid 600 mg. in the next dose along with rifampicin 450 mg, ethambutol 1200 mg and pyrazinamide 1500 mg under supervision. After forty five minutes of this treatment, he complained of dizziness and his body became stiff and head turned to one side followed by rhythmic tonic and clonic convulsions of both upper limbs. There was frothing at mouth. He was given injection 200 mg phenytoin intravenously stat and infusion of 600 mg phenytoin without any control over seizures. After 15 minutes, his attack was controlled with intravenous administration of 5 mg of diazepam. Later on, tablet pyridoxine 100mg twice a day was given. Isoniazid was thus proved as the offending drug. Subsequently, his sputum culture sensitivity test revealed *Mycobacterium tuberculosis* resistant to Isoniazid and sensitive to rifampicin, pyrazinamide and ethambutol. His follow-up period was uneventful with daily regimen consisting of rifampicin 450mg, ethambutol 800 mg and pyrazinamide 1500mg, with which he is improving well.

DISCUSSION

Isoniazid is an antimicrobial that has been used as a first-line agent for treatment of tuberculosis since 1952. Patients with active disease are put on a regimen of INH combined with other antituberculous medications. It is a very safe antitubercular drug, yet is known to cause varied adverse effects. A minority of patients receiving INH experience neurological side effects, including peripheral neuritis, dizziness, and insomnia. INH may precipitate convulsions in patients with seizure disorder, and rarely, in patients with no history of seizure.[1] Convulsions are reported in patients being treated with isoniazid, with no prior history of seizure, however few patients have developed seizures with a single conventional doses of isoniazid.[2] In our case, there was no previous personal or family history of epilepsy. All other possible causes of seizures were ruled out by thorough clinical examination and relevant investigations. Our patient developed severe acute isoniazid neurotoxicity with single therapeutic dose in the absence of overdose or any underlying conditions that would predispose him to such a severe adverse reaction. Remission and recurrence of seizures when isoniazid was stopped and reintroduced proved it to be an offending drug to induced seizures.

The adverse effects due to Isoniazid are divided into toxic, idiosyncratic and hypersensitivity reactions.[3] In patients receiving conventional low dose INH therapy, symptoms usually do not appear until six months. With high doses of INH, symptoms often appear within three to five weeks. [3] The earliest known and most widely recognized untoward effects of INH are the peripheral neuropathies. Pyridoxine, 50 mg daily, can prevent the occurrence of peripheral neuropathy in the high susceptibility groups. [3] Neurologic syndrome is dose-related and seizures are attributed to overdosage. [3] The susceptibility of adverse effects is the highest in individuals with liver disease, impaired renal function, epilepsy, psychosis, alcoholism, malnutrition and pyridoxine deficiency. [3] None of these factors was present in our cases. Acute ingestion of as little as 1.5 g of INH can cause mild toxicity in adults. Doses larger than 30 mg per kg often produce seizures that are usually refractory to anticonvulsant therapy. Ingestion of the drug in amounts greater than 80 to 150 mg per kg can rapidly lead to death.[4]

The presumed etiology of isoniazid-induced seizure involves a decrease in the availability of gamma-aminobutyric acid (GABA). Isoniazid metabolites, such as isoniazid hydrazones, inhibit pyridoxine phosphokinase. This enzyme converts pyridoxine (vitamin B-6) to its active form, pyridoxal-5-phosphate. Pyridoxal 5 phosphate, a co-factor for glutamic acid decarboxylase enzyme required for the synthesis of GABA, which is the major inhibitory neurotransmitter in the central nervous system. The consequent reduction in GABA increases the susceptibility to seizures. Thus, neurologic effects of isoniazid are specifically countered by administration of pyridoxine.[5] Five grams of IV pyridoxine given over 5-10 minutes is sufficient to abolish the neurologic effects of isoniazid in

most cases. In case of INH toxicity, pyridoxine should be administered in a dose equivalent to the suspected amount of isoniazid ingested (i.e., gram-per-gram replacement.[6] If amount of INH ingested is unknown, 5 gm of pyridoxine should be given intravenously. Repeat dosing may be required for persistent seizure activity. Prolonged use of isoniazid might lead to pyridoxine deficiency and seizures, which respond to pyridoxine administration. Pyridoxine administration did not help in the seizures with single conventional dose of INH. [2] In our case, as seizures occurred with single dose of isoniazid, pyridoxine deficiency could not be the cause of seizures. **In conclusion, physicians should be aware of possible isoniazid induced seizure even with therapeutic doses.**

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Case Report

ENCEPHALOPATHY DUE TO TUBERCULAR OTITIS MEDIA

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Summary: Middle ear infection due to *Mycobacterium tuberculosis* has been reduced from 3-5% to 0.05-0.9% in the last century due to advent of effective anti-tuberculosis therapy. On the other side, this decrease in frequency of tuberculous otitis media along with indistinguishable signs and symptoms of frequently occurring non-tuberculous otitis media makes clinicians vulnerable to delayed or misdiagnosis of the disease. A case of tubercular otitis media with atypical clinical manifestations in the form of encephalopathy is presented. ***[Indian J Tuberc 2012;59: 103-106]***

Key words: Encephalopathy, Tubercular otitis media

INTRODUCTION

Extra-pulmonary tuberculosis involving head and neck region, both in developing as well as developed nations, affects mainly cervical lymph nodes, and to a lesser degree, middle and external ear, tonsils, pharynx, mouth and the salivary glands.[1,2] In the beginning of 20th century, tuberculosis was the cause in 3% to 5% of all the causes for chronic suppurative otitis media cases but this rate has been decreased to 0.05 to 0.9% with the advent of effective anti-tuberculosis therapy.[3,4] In India, in the last 15 years, there are less than 10 reported cases of tubercular otitis media (TOM).[1,2,5-8] On the other side, this decrease in frequency of TOM along with indistinguishable signs and symptoms of frequently occurring non-tuberculous otitis media makes clinicians vulnerable to delayed or misdiagnosis of the disease. A case of TOM with atypical clinical manifestations in the form of encephalopathy is presented.

CLINICAL RECORD

A 40-year-old male, who had no co-morbid illness, was admitted at a private hospital with complaints of fever, nausea, vomiting and loose motions. Due to suspected acute gastroenteritis, empirical antibiotics were given. After one week of illness, patient developed altered sensorium for which CT scan of the brain was done which was normal. CSF study revealed 25 cells- 65% lymphocytes, protein 51 mg/dl, glucose 74 mg/dl; Gram's stain was negative for organisms, ADA was eight (not suggestive of TB). Other investigations revealed TLC -13100/mm^3, platelet count- 3.3 lakhs; while Dengue ELISA, Widal test, microscopy for Malaria and antigen were negative. So, provisional diagnosis of sepsis with septic encephalopathy was made. In view of persisting altered sensorium, nausea, vomiting and sepsis with septic shock along with oliguria, the patient was referred to our hospital's ICU.

On admission, he was febrile (38.9^{0}C) and was in altered sensorium (GCS-12), pupils were bilaterally equal and reacting to light; patient also had tachycardia, hypotension and tachypnoea. In view of worsening of altered sensorium and tachypnoea, he was intubated and kept on mechanical ventilation. His initial PaO_2/FiO_2 ratios were 220. He continued to have high grade fever, altered sensorium, repeated episodes of nausea and vomiting for the last six weeks, we thought of intracranial pathology. So, we did repeat CSF examination and got MRI brain done but both the reports were normal. As he continued to remain in altered sensorium, tracheostomy was done for the need of prolonged mechanical ventilation. During sixth week of illness (first week in our ICU), patient started having left ear discharge which was sent

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for Gram staining and culture sensitivity. Report showed growth of *Acinetobacter* species for which antibiotics according to culture sensitivity were started. Patient continued to remain febrile and in altered sensorium along with ear discharge for which ENT and neurology consultation was sought. HRCT of the head and paranasal sinuses revealed bilateral mastoiditis (Lt>Rt) along with sphenoidal sinusitis (Figure 1). For the source control, left cortical mastoidectomy was done by ENT surgeon in the sixth week of illness. Intra-operative findings revealed pale granulations filling antrum, aditus and attic. The tissue was sent for histopathological examination along with microscopy and cultures for bacterial, fungal and *Mycobacterium spp*. The histopathology of the lesion showed tissue bits lined by flattened lining epithelium containing granulation tissue composed of proliferating capillaries, fibroblasts and mixed inflammatory cells. Foci of calcification and hemorrhage were also present (Figure 2). Granulation tissue smear was positive for Acid fast bacilli (AFB) on Ziehl Neelsen (ZN)

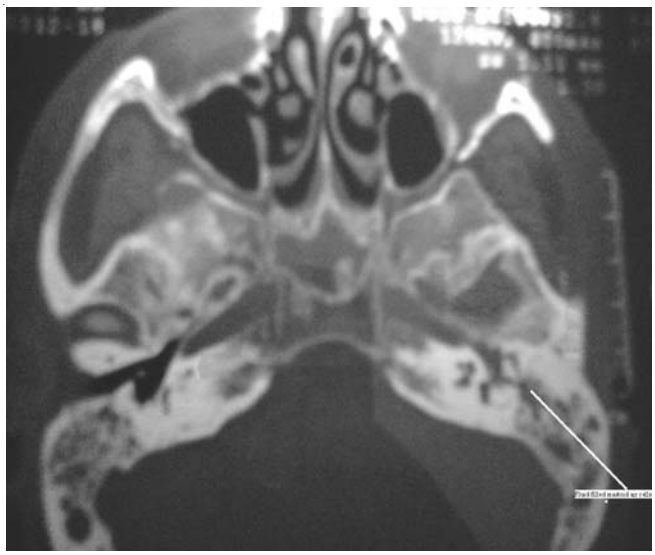

Figure 1: Axial cut of HRCT temporal bone showing left mastoiditis and sphenoid sinusitis

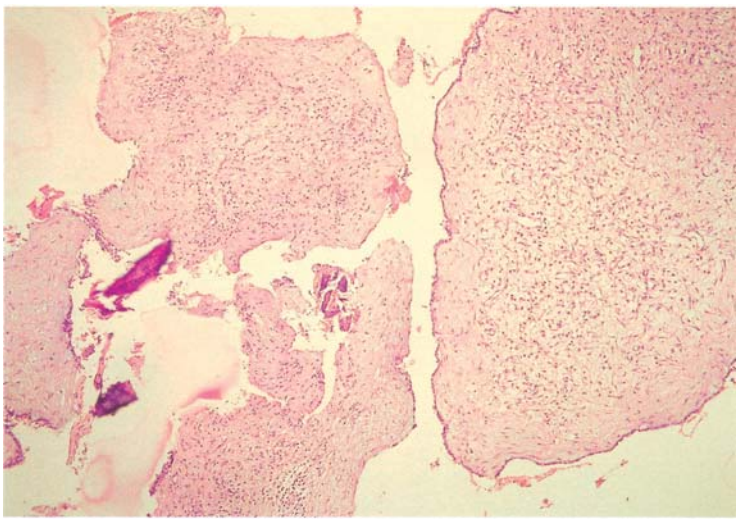

Figure 2: Granulation tissue composed of proliferating capillaries, fibroblasts and a few mixed inflammatory cells along with foci of calcification.

staining using 20% sulphuric acid for decolourisation. Also culture was positive for *Mycobacterium spp*. on LJ media after seven weeks of incubation aerobically at 37^{0}C. After starting anti-tubercular therapy (ATT), his sensorium gradually improved over next few days. Weaning from the mechanical ventilation was planned, but unfortunately, patient developed refractory septic shock due to ICU acquired multi-drug resistant bacterial infection and succumbed to death.

DISCUSSION

In one of the largest series of TOM, highest incidence was found during third decade of life.[9] The clinical features of TOM have been changing over the years. It used to present previously as the triad of pain-free otorrhea, multiple tympanic membrane perforations and peripheral facial palsy. In recent literature, some non-specific clinical presentations have been described like significant otalgia probably due to pressure caused by granulation tissue within the mastoid, serous otorrhea which may become purulent due to secondary bacterial contamination, severe and early hearing loss (sensorineural, mixed or conductive) in 90% of cases which may persist after the infection has been completely treated, especially if therapy was initiated late.[3,9,10] Single or multiple tympanic perforations, denuded hammer of the ear, erosion of ossicles and even of the cortical bone of the mastoid, which may involve the bone capsule of the facial nerve, pale granulations of the middle ear and mastoid cells are other non-specific findings.[3,9,10] Complications occur mostly when the diagnosis is late and may include: peripheral facial nerve paralysis, retroauricular fistulae, labyrinthitis, meningitis, tuberculous osteomyelitis of the petrous pyramid, subperiosteal, cerebral or cerebellar abscesses, acute mastoiditis and cellulitis [3,9,11]

The pathogenesis of TOM involves three major mechanisms: 1) aspiration of mucus through the eustachian tube; 2) blood-borne dissemination from other tuberculous foci and; 3) direct implantation through the external auditory canal and tympanic membrane perforation.[9] The diagnosis starts with thorough clinical history to rule out contact with a tuberculosis patient or previous treatment of this condition or to rule out concomitant lesions, which may be found in up to 50% of cases.[4,9] Patient with otorrhea having evidence of active tuberculosis in any other part of the body must be having tuberculosis of the ear until proven.

Laboratory examination should include direct sputum bacilloscopy, while bacteriology of ear secretions is not very reliable in the presence of other microorganisms that may interfere with the growth of Koch's bacillus and delay the diagnosis.[10,12] Chronic use of topical drops, such as neomycin, may alter the sensitivity of bacterial cultures. The test is positive for bacilli in 20 to 30% of cases.[3,4] Histopathology of granulation tissue is the most reliable diagnostic method. However, biopsies frequently need to be repeated for confirmation. Caseous necrosis and specific granulations with epithelioid cells and giant Langerhan's cells may be seen.

Other chronically suppurative diseases that do not improve with conventional therapy should be considered in the differential diagnosis such as cholesteatoma, syphilis, wegener's granulomatosis, fungal infection, eosinophillic granulomatosis and sarcoidosis.[3,10] **In India, as tuberculosis is quite rampant still and can present in any form, we should always rule out tuberculosis as early and meticulously as possible to improve the outcome.**

Thus, as a routine, whenever a patient with altered sensorium with otorrhea is managed, we should always keep in mind of ear and nose as the potential source of infection including tuberculosis, despite low incidence as these cavities are closely linked with intracranial cavity as well as external environment.

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Indian J Tuberc 2012; 59: 107-111

STATUS REPORT ON RNTCP*

RNTCP has sustained its achievements against the twin objectives of RNTCP during the fourth quarter, 2011 and has moved towards universal access to TB care. With this, it is evident that the programme, while sustaining its past achievements, is progressing satisfactorily towards achieving the TB related Millennium Development Goals, in terms of achieving the programme objectives.

RNTCP performance in fourth quarter 2011

During the quarter, over 1.9 million suspects were examined 2,15,171 sputum positive cases were diagnosed, and 3,49,454 TB cases were registered for treatment. The annualized total case detection rate is 116 cases per 100,000 population. With a total of 148,090 new smear positive cases being registered for treatment, the new smear positive TB case notification rate (annualized) for the fourth quarter 2011 is 49 per lakh population. In addition to this, 79,070 new smear negative cases, 50,293 new extra pulmonary cases, 46,999 smear positive re-treatment cases and 24,565 re-treatment Others' were also registered for treatment in this quarter. The treatment success rate amongst the new smear positive Pulmonary TB cases registered in the fourth quarter 2010 is 88.1% and the sputum conversion rate of patients registered during third quarter, 2011 is 90.4%. The default rates among NSP (5.4%), NSN (6.6%) and re-treatment cases (14.4%) continue to show the declining trend over the past several quarters.

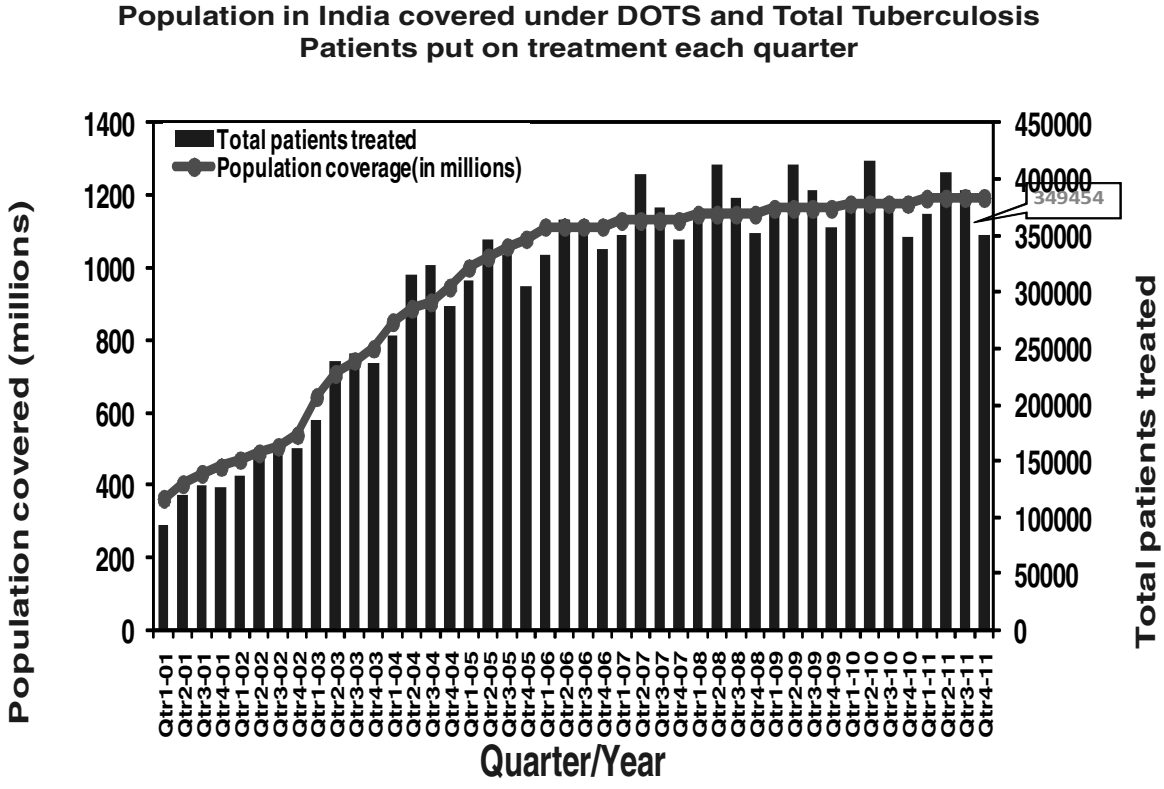

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Table: Performance of RNTCP Case Detection (2011, fourth quarter), Smear Conversion (2011, third quarter), and Treatment Outcomes (2010, fourth quarter)

State	Population (in lakh) covered by RNTCP[1]	No. of suspects examined	Suspects examined per lakh population	Rate of change in suspects examined per lakh population (compared to same quarter in previous year)	No of Smear positive patients diagnosed[2]	Suspects examined per smear positive case diagnosed	Rate of change in suspects examined per s+ case diagnosed (compared to same quarter in previous year)	Annualized smear positive case notification rate (reported by RNTCP DMCs)	Annualized smear positive case notification rate [from CFR: sm + cases (NSP + Rel + TAD) * 4 / Pop]	Total patients registered for treatment[3]	Annualized total case notification rate	Annualized new smear positive case notification rate	Annualized new smear negative case notification rate	Annualized new extra pulmonary case notification rate	Annualized previously treated case notification rate
Andaman & Nicobar	4	1154	304	55%	101	11	30%	106	102	240	253	79	69	67	37
Andhra Pradesh	847	140315	166	-2%	18665	8	-3%	88	75	27423	130	59	29	16	26
Arunachal Pradesh	14	2441	177	-12%	314	8	-27%	91	82	531	154	60	32	25	35
Assam	312	31740	102	-7%	4898	6	-4%	63	56	8535	110	46	29	14	21
Bihar	1038	96765	93	2%	10555	9	3%	41	36	17375	67	29	19	4	14
Chandigarh	11	4146	393	53%	536	8	4%	203	107	539	204	78	25	61	41
Chhattisgarh	255	27361	107	0%	3050	9	3%	48	44	6439	101	38	38	13	12
D & N Haveli	3	697	203	30%	70	10	34%	82	58	103	120	47	29	22	22
Daman & Diu	2	733	302	2%	48	15	17%	79	38	77	127	28	56	15	28
Delhi	168	39264	234	14%	5091	8	2%	122	101	10239	244	67	43	70	64
Goa	15	3607	247	6%	323	11	-10%	89	62	449	123	47	15	36	25
Gujarat	604	109673	182	-6%	13816	8	0%	92	81	18461	122	58	14	14	36
Haryana	254	40738	161	8%	5134	8	1%	81	70	8223	130	45	23	22	39
Himachal Pradesh	69	14494	211	-4%	1480	10	3%	86	75	2696	157	52	27	38	40
Jammu & Kashmir	125	21612	172	-5%	1944	11	10%	62	58	2834	90	46	11	17	16
Jharkhand	330	37403	113	1%	5080	7	4%	62	59	8820	107	51	31	7	18
Karnataka	611	131532	215	-1%	11185	12	-2%	73	59	17067	112	46	24	20	22
Kerala	334	86838	260	-2%	3540	25	-4%	42	37	6623	79	32	20	18	10
Lakshadweep	1	286	444	175%	4	72	-41%	25	43	8	50	25	0	6	19
Madhya Pradesh	726	99799	137	12%	12493	8	10%	69	60	22151	122	48	39	13	22
Maharashtra	1124	182169	162	-10%	18007	10	-4%	64	58	32635	116	45	25	20	26
Manipur	27	2908	107	-24%	259	11	6%	38	36	651	96	29	27	23	17
Meghalaya	30	5177	175	-1%	581	9	12%	78	67	1136	153	54	32	36	32
Mizoram	11	1896	174	-14%	164	12	-20%	60	55	512	188	41	49	63	36
Nagaland	20	3201	162	10%	396	8	11%	80	73	822	166	56	35	39	36
Orissa	419	53084	127	4%	6637	8	6%	63	56	11595	111	48	27	20	16
Puducherry	12	5822	468	31%	647	9	7%	208	73	395	127	57	23	27	20
Punjab	277	38896	140	-4%	4552	9	12%	66	63	7944	115	44	21	23	27
Rajasthan	686	106555	155	7%	15938	7	6%	93	79	25270	147	55	39	20	34
Sikkim	6	1403	231	-6%	162	9	-2%	107	101	366	241	78	50	60	53
Tamil Nadu	721	153750	213	-11%	10716	14	-5%	59	55	18788	104	42	25	19	18
Tripura	37	5027	137	4%	409	12	22%	45	42	604	66	37	9	12	8
Uttar Pradesh	1996	303603	152	11%	42593	7	4%	85	79	64456	129	63	28	13	25
Uttarakhand	101	15634	155	-7%	2031	8	5%	80	63	3078	122	43	25	19	34
West Bengal	913	142311	156	3%	13752	10	6%	60	56	22369	98	45	17	16	19
Grand Total	**12102**	**1912034**	**158**	**0%**	**215171**	**9**	**1%**	**71**	**63**	**349454**	**116**	**49**	**26**	**17**	**24**

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, data from DMCs

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

State	Annualized previously treated smear positive case notification rate	No (%) of pediatric cases out of all New cases		3 month conversion rate of new smear positive patients	Success rate of new smear positive patients	3 month conversion rate of retreatment patients	No (%) of Smear Positive cases started RNTCP DOTS within seven days of diagnosis		No (%) of all Smear Positive cases registered within one month of starting RNTCP DOTS treatment		No (%) of all cured Smear Positive cases having end of treatment follow- up sputum done within seven days of last dose		No (%) of cases (all forms of TB) registered receiving DOT through a community volunteer		Proportion of all registered TB cases with known status	Proportion of TB patients known to be HIV infected among tested	Proportion of TB patients known to be HIV infected among registered	Proportion of HIV infected TB patients put on CPT(RT report)	Proportion of HIV infected TB patients put on ART(RT report)
Andaman & Nicobar	27	21	10%	87%	80.3%	81%	101	100%	94	93%	72	84%	55	23%	28%	1%	0%		
Andhra Pradesh	18	1042	5%	91%	89.1%	74%	14675	90%	15830	97%	11193	84%	22718	83%	89%	10%	9%	88%	47%
Arunachal Pradesh	23	50	12%	90%	88.6%	88%	271	93%	284	98%	154	89%	152	29%	69%	0%	0%		
Assam	12	313	5%	87%	83.3%	67%	3836	86%	4179	94%	2485	73%	2830	33%	31%	1%	0%	43%	86%
Bihar	8	1041	8%	89%	89.3%	74%	8243	87%	9311	98%	5025	71%	12043	69%	13%	4%	0%	14%	48%
Chandigarh	33	39	9%	91%	90.9%	73%	276	95%	290	99%	261	98%	104	19%	93%	1%	1%	67%	17%
Chhattisgarh	6	295	5%	90%	87.3%	71%	2482	88%	2764	98%	1551	71%	3365	52%	16%	2%	0%	0%	50%
D & N Haveli	12	4	5%	89%	81.6%	85%	47	94%	49	98%	37	95%	22	21%	60%	2%	1%		
Daman & Diu	10	2	3%	88%	87.5%	80%	21	91%	23	100%	26	100%	26	34%	74%	4%	3%	100%	0%
Delhi	37	912	12%	89%	86.6%	71%	3910	89%	4291	98%	3164	92%	949	9%	72%	2%	1%	71%	77%
Goa	17	25	7%	84%	85.2%	75%	207	88%	234	100%	178	95%	74	16%	98%	3%	3%	98%	70%
Gujarat	24	768	6%	92%	87.5%	69%	11370	92%	12199	98%	9335	90%	10655	58%	93%	5%	4%	96%	73%
Haryana	28	325	6%	91%	86.4%	75%	4127	89%	4407	95%	2835	86%	2374	29%	60%	1%	1%	67%	36%
Himachal Pradesh	27	140	7%	93%	87.4%	82%	1291	96%	1306	97%	1044	87%	480	18%	43%	1%	1%	50%	33%
Jammu & Kashmir	13	152	7%	92%	89.8%	78%	1823	98%	1860	100%	1646	97%	297	10%	16%	0%	0%	0%	80%
Jharkhand	8	410	6%	93%	90.8%	83%	4194	86%	4827	99%	2770	70%	5846	66%	24%	2%	0%	0%	78%
Karnataka	15	1131	8%	88%	82.8%	63%	7996	86%	8891	96%	5382	80%	8673	51%	94%	13%	12%	99%	75%
Kerala	7	969	17%	83%	84.9%	69%	2869	87%	3008	91%	1966	76%	4057	61%	68%	2%	2%	76%	83%
Lakshadweep	19	0	0%	100%	100.0%	0%	7	100%	7	100%	1	0%	0	0%	0%		0%		
Madhya Pradesh	14	2310	13%	91%	89.8%	73%	9885	89%	10863	97%	6769	78%	13045	59%	27%	2%	1%	11%	53%
Maharashtra	14	1718	7%	90%	86.0%	68%	14597	88%	16200	98%	10784	81%	10970	34%	83%	10%	8%	95%	63%
Manipur	9	35	7%	89%	88.3%	74%	249	97%	249	97%	218	86%	347	53%	53%	9%	5%	46%	35%
Meghalaya	17	137	15%	85%	84.1%	63%	472	90%	502	96%	328	90%	664	58%	11%	0%	0%		
Mizoram	17	71	17%	91%	228.2%	78%	158	100%	156	99%	89	79%	99	19%	65%	14%	9%	98%	44%
Nagaland	20	83	13%	92%	91.7%	77%	293	78%	341	91%	321	86%	335	41%	64%	7%	5%	81%	59%
Orissa	10	533	5%	89%	87.5%	71%	5026	84%	5837	97%	3318	70%	8489	73%	30%	3%	1%	0%	0%
Puducherry	19	31	9%	90%	83.6%	76%	181	77%	181	77%	144	95%	0	0%	85%	3%	2%	100%	67%
Punjab	20	325	5%	91%	88.8%	77%	4143	93%	4373	98%	3646	92%	2304	29%	67%	2%	1%	74%	65%
Rajasthan	25	1025	5%	92%	93.7%	78%	11461	83%	13119	95%	9015	81%	3593	14%	30%	1%	0%	53%	37%
Sikkim	36	19	6%	88%	80.7%	61%	168	98%	172	100%	115	94%	126	34%	0%	0%	0%		
Tamil Nadu	13	1137	7%	90%	86.0%	70%	8260	82%	9683	96%	6149	81%	5215	28%	89%	7%	6%	89%	62%
Tripura	6	11	2%	89%	85.4%	79%	317	80%	380	96%	271	77%	270	45%	40%	3%	1%		
Uttar Pradesh	17	3041	6%	92%	90.0%	79%	35843	90%	39656	99%	25346	85%	46959	73%	23%	1%	0%	12%	49%
Uttarakhand	22	143	6%	89%	85.8%	73%	1455	89%	1598	98%	1072	83%	1870	61%	44%	1%	0%	75%	25%
West Bengal	13	912	5%	88%	85.6%	66%	10586	80%	12580	95%	8581	82%	6002	27%	48%	2%	1%	87%	78%
Grand Total	**16**	**19170**	**7%**	**90%**	**88.1%**	**73%**	**170840**	**88%**	**189744**	**97%**	**125291**	**82%**	**175008**	**50%**	**52%**	**6%**	**3%**	**91%**	**62%**

Major activities during the quarter

Programme review Supervision, Monitoring and Training

The third National Co-ordination Committee meeting for reviewing Global Fund Round 9 projects in Tuberculosis in India was held in October 2011 in Bhopal.

National Stakeholders Meeting for Tuberculosis and Diabetes Mellitus Collaborative activities was in October 2011 at Delhi which was attended by Programme Officials from RNTCP and the Non-Communicable Disease Control Programme and State TB Officers.

The Biannual National State TB Officers' and RNTCP Consultants' Review Meeting was held in November 2011 at New Delhi. The theme for the meeting was 'Quality services for universal access under RNTCP' with objectives to review the performance and quality of RNTCP services (DOTS, DOTS-Plus, TB-HIV, PPM, ACSM) and to prepare focused action plan for underperforming areas and to update the STOs and Consultants on newer initiatives, policy changes, etc.

Progress in accreditation of Intermediate Reference Laboratories (IRL)

RNTCP has accredited 35 Culture and DST laboratories in the country which include four National reference laboratories (NRL), 17 Intermediate Reference laboratories, eight medical colleges, two ICMR laboratories, three NGO laboratories and one private laboratory. The Line Probe Assay (LPA) has been introduced in the programme and three NRLs, nine IRLs and six Medical College Laboratories have been accredited. Laboratories of four NRLs and one private sector are accredited for Liquid culture DST. The programme has planned to conduct operational feasibility study of cartridge based nucleic acid amplification test (CB-NAAT) in different field settings.

Progress in the Programmatic Management of Drug Resistant TB (PMDT) services

RNTCP achieved its PMDT Vision for 2011 by introducing services in all 35 states in some districts with variable access and scaling up. 508 million (43%) population have access to services through 260/662 (40%) districts that varies from states to state. 11/35 (31%) States-UTs have achieved 100% complete geographical coverage and are progressing towards achieving universal access. Since the inception of PMDT services in India, a cumulative total of 38,155 MDR TB Suspects have been examined for diagnosis; 10,263 MDR TB cases have been confirmed and 6,994 MDR TB cases have been initiated on regimen for MDR TB through 50 DOTS Plus Sites across the country.

In fourth quarter 2011, central level PMDT appraisals were conducted in Gujarat (six districts), Maharashtra (eight districts), Lakshadweep Islands, West Bengal (two districts), Uttarakhand (five districts), Uttar Pradesh (six districts) and Andhra Pradesh (seven districts). Five batches of National Training in PMDT were conducted at Gujarat (two batches), New Delhi (two batches) and Kerala (one batch) in this quarter.

Regional PMDT Review meetings with key state officials of 20 preparatory and implementing states were conducted at Shimla and Pune in October and November 2011 respectively, to closely monitor the progress made by every state against their respective state PMDT scale up plans, further accelerate and organize timely intervention from central and state level to support the states to complete pending preparatory activities.

Progress in PPM & ACSM activities

National ACSM Workshop: The National ACSM (Advocacy, Communication and Social Mobilization) Workshop was organized by Central TB Division in November 2011 in NCR, Delhi. The State TB Officers, State IEC Officers of RNTCP, RNTCP WHO ACSM Consultants and ACSM Consultants of the states and Communication Facilitators of 35 states and UTs participated in this residential

workshop. The workshop focused chiefly on strengthening the programmatic aspects of ACSM in the perspective of achieving the targets of Universal Access that has been planned in the next five years' strategy (2012 – 2017) of RNTCP.

Tenth National Task Force (NTF) workshop for enhancing the involvement of Medical Colleges under RNTCP was held on 21st and 22nd December, 2011 at the Lala Ram Sarup (LRS) Institute of Tuberculosis and Respiratory Diseases, New Delhi.

Progress in TB HIV Collaborative Activities

Scale-up of Joint TB/HIV collaborative activities continues to progress impressively. CTD and NACO completed TOT in Jharkhand, Bihar and five Union territories. Thus, apart from Jammu and Kashmir, intensified TB/HIV package is now implemented across the country and nationwide coverage is expected to be achieved in second quarter, 2012.

In fourth quarter, 2011, nationally 51.6% TB patients were tested for HIV and 6% of them were detected HIV infected. More than 91% HIV infected TB cases were initiated on CPT, but only 62% on ART in the fourth quarter, 2010 cohort. Performance in high performance states improved further in fourth quarter, 2011 with Andhra Pradesh, Karnataka, Tamil Nadu, Maharashtra, Gujarat and Goa achieving HIV testing of more than 80% and CPT initiation of more than 90%. Karnataka, Gujarat and Goa also achieved ART initiation in more than 70% patients. But performance remains sub-optimal in low HIV prevalence states like Uttar Pradesh, Madhya Pradesh, Rajasthan, Assam, Jharkhand, Orissa, Chhattisgarh, etc., which managed to test less than 30% TB patients. Although challenges like mismatch in service delivery points between RNTCP and NACP exist in these states, efforts to improve performance need strengthening. Facilitation for establishment of HIV testing facilities at all RNTCP DMC with respective SACS will help boost these performance levels.

As per decision of NTWG, RNTCP facilitated conduct of operational research study for HIV testing among TB suspects in Karnataka and Andhra Pradesh, and preparations are completed for 10 selected districts in low prevalent states.

The programme has achieved many milestones over last one decade and is moving towards universal access to TB Care.

Short Communication

FATE OF SPUTUM SAMPLES TRANSPORTED IN BOTTLES CONTAINING CETYLPYRIDINIUM CHLORIDE AND SODIUM CHLORIDE: A NATIONAL REFERENCE LABORATORY STUDY

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***Summary*:** The transportation of sputum samples may sometimes take more than one week which results in an increased contamination rate and loss of positive cultures. The current study was planned to analyze the recovery rate of mycobacteria from transported samples with and without Cetylpyridinium chloride (CPC). Addition of CPC is useful for isolation of *M. tuberculosis* from sputum subjected to long-term storage. **[*Indian J Tuberc 2012; 59:* 112-115]**

***Key words*:** Cetylpyridinium chloride, Tuberculosis, Transport

INTRODUCTION

Tuberculosis (TB) remains a public health problem throughout the world in spite of the potent Anti Tubercular Treatment (ATT) and effective TB control programmes. Condition is more serious in developing countries where laboratory facilities are under developed and TB is rampant.

The situation has been further compounded by the emergence of MDR and XDR strains of *Mycobacterium tuberculosis (M. tuberculosis)* and HIV pandemic. These drug resistant strains are the major hurdle of TB control programmes. Due to the lack of accreditated laboratories in our country, the samples are transported to the National Reference Laboratories for culture and Drug susceptibility testing (DST). The transportation may sometimes take more than one week which results in an increased contamination rate and loss of positive cultures[1-3]. This long time gap between sample collection and culture may occur in places where transportation from the collection site is subjected to a logistic constraint and samples are gathered to limit the cost of shipment.

Cetylpyridinium chloride (CPC) is a quaternary ammonium compound, when added to sputum specimen at final concentration of 0.5% (1% CPC and 2% Sodium Chloride solution is added to equal volume of sputum specimen), not only decreases the number of cultures lost by contamination as a result of prolonged transit time but also decreases the laboratory time required for processing the specimens. CPC is inexpensive and tubercle bacilli remained viable after eight days of exposure to this solution[1,4].

LRS Institute is a tertiary care hospital and serves eight states of India as National Reference Laboratory (NRL) (Haryana, Delhi, Manipur, Arunachal Pradesh, Mizoram, Nagaland, Meghalaya and Tripura). As per the National Tuberculosis Control Programme guidelines, the samples received by our laboratory belong to the patients who have already failed the first line ATT which makes every sample more precious for us. So, keeping the importance of the recovery of these transported specimens in mind, the current study was planned to analyze the recovery rate of mycobacteria from a large number of transported samples with and without CPC.

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MATERIAL AND METHODS

A total of 1210 sputum samples from treatment failure patients were received from different states during a period of 2.5 years (Jan 2008- Sep 2010). 77% (931/1210) of the samples were received within seven days and 23% (279/1210) were received after seven days from the day of collection. Out of these, 541 samples were received in CPC and the rest 660 were without CPC. Fifty one (4.2%) samples had leaked and could not be processed further. The remaining 1159 samples were processed for culture.

The stock solution of CPC was prepared by dissolving 1gm CPC and 2 gm Sodium chloride in 100ml of distilled water and autoclaved at 121°C for five minutes. The solution was distributed in 5ml quantities in sterile Mc Cartney bottles with date of manufacture and date of expiry. The bottles were distributed to respective District Tuberculosis Centres for sample collection. The bottles not used within one month were returned back to the IRL (Intermediate Reference Laboratory).

Direct smears were made from samples received with CPC. The Mc Cartney bottles were filled to near the top with sterile distilled water, capped and then centrifuged at 3000xg. The liquid was decanted and the sediment in each tube was resuspended in 1-2 ml of sterile distilled water. The resuspended sediment was inoculated on slants of Lowenstein Jensen (LJ) medium. For the samples without CPC, direct smear was made and stained with Ziehl Neelsen (ZN) method. Culture was performed as per standard modified Petroff's method[5]. All the samples after decontamination were inoculated on to the LJ slants and incubated for eight weeks at 37°C. The inoculated LJ slants were examined weekly for the appearance of growth.

RESULTS

Six hundred fifteen sputum samples without CPC were processed, of which 292 (47%) grew *M. tuberculosis* and one was identified as Non Tubercular Mycobacteria (NTM). Sixty two (10.1%) got contaminated including 12 smear positive samples (Table 1).

Five hundred thirty five sputum samples with CPC were processed for culture, of which 289 (56%) grew *M. tuberculosis*. Seventeen (3.2%) cultures were contaminated, of which 13 were smear positive (Table- 2).

Table 1: Correlation of Smear positivity with culture among samples without CPC

	Smear +ve	**Smear –ve**	**Total**
Culture + ve	242 (74%)	51 (22.6%)	292 (*M. tuberculosis)* +1(NTM) (53%)
Culture –ve	86 (26.3%)	174 (77.3%)	260 (47%)
Total	328	225	553

Cultures Contaminated - 62/615-10.1%
12 samples were smear positive and 50 smear negative

Table 2: Correlation of Smear positivity with culture among samples in CPC

	Smear +ve	**Smear –ve**	**Total**
Culture + ve	183 (71%)	106 (41%)	289 (56%)
Culture –ve	76 (29%)	153 (59%)	229 (44%)
Total	259	259	518

Cultures Contaminated: 17/535- 3.1%
13 smear positives four smear negatives

DISCUSSION

WHO recommends the use of 1% CPC if the samples are likely to be exposed to room temperature for more than 48 hours to properly homogenize and be decontaminated[6]. Our observations also prefer the use of transport media to lower the contamination rate.

The possible reason for the smear positive and culture negative samples (26.3% in samples without CPC and 29% in samples with CPC) may be due to the presence of non-viable bacilli as the patients were on ATT or could be due to the loss of viability of the organism during transportation. No significant difference was observed in the culture positivity among the smear positive samples received with and without CPC. This supports the previous findings that tubercle bacilli remain viable even after storage of sputum samples in CPC for a long time.

A major difference was found in the recovery of tubercle bacilli from smear negative samples with CPC (22.6% in samples without CPC *versus* 41% in samples with CPC) (Tables 1 and 2). Earlier studies have also shown that culture positivity of smear negative sputa collected with CPC was statistically higher than that of sputa collected without CPC[3,7].

10.1% samples were lost due to contamination when processed by NaOH method as compared to only 3.2% by CPC method. The difference was found to be statistically significant ($P < 0.05$). Other studies have also shown that CPC increases the culture positivity and reduces contamination in sputum cultured on solid media between 1-2 weeks of collection[8,9].

A recent study has shown that CPC might interfere with the fluorescence if the samples are processed for liquid culture system like BACTEC MGIT 960. This may limit the use of CPC for the liquid culture system[10].

For transport of sputum specimens requiring storage up to seven-eight days, the CPC-NaCl method has advantages over the standard NaOH method in reducing the contamination and yielding more positive cultures. Other advantages of the CPC method are that the reagent is stable at room temperature, easy to prepare, inexpensive and self-sterilizing.

If transportation is expected to take a long time, the use of 1% CPC may be a good approach to avoid the loss of smear positive samples and better isolation from smear negative cases also. Since 4.2% of the precious samples were lost due to improper packaging, the safe and proper transport of samples is to be ensured. Correct collection and transportation of sputum samples is crucial to ensure the recovery of *M. tuberculosis.*

Transport of samples with CPC may be considered as an inexpensive and alternate decontamination method for isolation of tubercle bacilli from sputum specimens that remain in transport for more than 24 hours.

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NATCON - 2012

The 67th National Conference on Tuberculosis and Chest Diseases (NATCON 2012), under the joint auspices of the Tuberculosis Association of India and the Bihar Tuberculosis Association, will be held at Patna. The dates and other details will be announced in due course.

Commentary *Indian J Tuberc 2012; 59:* **116-118**

CHANGING TRENDS OF CUTANEOUS TUBERCULOSIS IN THE ERA OF DOTS STRATEGY

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Cutaneous tuberculosis has a worldwide distribution. In the past, it was more prevalent in temperate countries with cold and humid climate with a few hours of daily sunlight but now it is being encountered in tropical countries like India. Malnutrition and low socio-economic conditions are predisposing factors for Cutaneous tuberculosis.[1] Two decades back, a decline was observed in the incidence of Cutaneous tuberculosis, but recently there is resurgence of Cutaneous tuberculosis due to multidrug resistant strains of *Mycobacterium tuberculosis*. As a result, unusual manifestations of the disease have appeared. Oral Ulcers are now seen commonly in Orofacial Tuberculosis. Moreover, it has been reported at sites of Laproscopic surgery, sites of previously healed Scrofuloderma, following corticosteroids injection or sites of nose piercing and tattoing. Various other factors implicated in the rising incidence of extra-pulmonary TB could be ease of migration of people across the globe, the rise in immunosuppressive therapy, the decline in TB-control efforts and the emergence of resistant strains of *M. tuberculosis*.[2]

In developing countries like India, the incidence of Cutaneous tuberculosis has fallen from 2% to 0.15%.[3] This decline in incidence may be attributed to the availability of effective anti-tubercular drugs, elimination of milk-herds and general improvement in the living standard and effective implementation of DOTS strategy. The global impact of the converging dual epidemics of TB and HIV is one of the major health challenges of our times. In the present scenario, the disease is fast reappearing due to the HIV pandemic and due to emerging resistance to the conventional treatment. In a survey carried out among new tuberculosis patients under the Revised National TB Control Programme in 2007, HIV seroprevalence varied widely and ranged from 1% to 13.8% across 15 districts.[3,4]

Just as with systemic tuberculosis (TB), the Cutaneous variants have a variable clinical appearance, significance and prognosis .Since extra pulmonary, disseminated and sputum smear-negative manifestations are more common in patients with advanced immunosuppression, newer diagnostic tests, which are not only sensitive and specific but also easy to use in remote settings and are cost effective, are required for dealing with this emerging epidemic.

DOTS is an effective way to treat Cutaneous TB although there was a paucity of literature on it.

The diagnosis of Cutaneous TB is based on clinical features, skin biopsy, culture, and in recent years, PCR (Polymerase Chain Reaction)

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revolutionises the diagnosis. However, the yield from culture and PCR is often low and diagnosis may need to depend on clinical features, histological findings, and retrospective review of response to treatment. According to other reports, the yield from PCR has been low in pauci-bacillary cases. The low yield from PCR and culture may be due to the low number of viable bacilli within the specimen and/or to degradation of DNA material.[5, 6] Compared with PTB, the number of bacilli encountered in Cutaneous TB is low. Moreover, culture of *M. tuberculosis* is time-consuming and the yield is low. PCR is a rapid method of diagnosis but requires expertise, as it is prone to contamination and false positives. However, tissue culture remains the gold standard for diagnosis and for monitoring the emergence of drug-resistant strains.[5,6]

Finally, Cutaneous TB remains one of the least studied and reported variants of TB. The diagnosis in a country like India still relies on tests like the Mantoux, the chest x-ray, histopathology, and sputum smear examination, none of which is absolute in terms of diagnosis. PCR is a rapid method of diagnosis but requires expertise, as it is prone to contamination and false positives. Tissue culture remains the gold standard for diagnosis and for monitoring the emergence of drug-resistant strains, but it is time-consuming. Cutaneous TB can therefore be difficult to confirm, and on occasions, the diagnosis is only established retrospectively, after response to a therapeutic trial. Cutaneous TB is less common than PTB and therefore non-dermatologists may be less familiar with the entity, possibly resulting in underreporting.

DNA amplification by PCR is a rapid and sensitive method for detection of *M. tuberculosis* organisms in samples. Fewer than micro-organisms could be detected. Though precise estimation of the sensitivity is difficult, the primer directed amplification system used is both highly sensitive (98%) and specific (100%) in the detection of *M. tuberculosis* complex DNA from clinical specimens. Thus in one study, Tan *et al* concluded that PCR based detection of *M. tuberculosis* DNA in skin samples may extend and improve the diagnostic panel for cutaneous tuberculosis and may be also used to differentiate atypical mycobacterial infections in an immuno-compromised patient with negative culture, if the technique is prudently and properly used. Moreover, PCR has not been found to be a useful complement to the clinical and histologic diagnosis of "paucibacillary" forms of cutaneous tuberculosis in their experience.[9, 10] PCR results can be obtained within days, and a PCR based technique may facilitate the diagnosis of cutaneous tuberculosis.

The utility of PCR in the diagnosis of orificial tuberculosis with miliary spread, erythema induratum, papulonecrotic tuberculid, and other forms of cutaneous tuberculosis has been reported from different parts of the world.[11,] There are other limitations to the use of PCR as well. It is a technically complex method that requires trained hands to perform the test. The inherent sensitivity of PCR amplification can lead to amplification of non-specific sequences and contaminants. Greater care and meticulous technique are essential for a reliable PCR result. An important limiting factor is the inability of PCR to differentiate between the live and dead organisms which are likely to persist in inactive or treated cases. In view of this, PCR positivity does not necessarily indicate active disease.

Finally, we conclude that in all patients with Cutaneous TB, HIV testing should be necessary and in proven HIV other diagnosis should be entertained. DOTS therapy is the standard of care for treatment of Cutaneous TB. The quality control, reproducibility of results and variations in results from different laboratiories are still important issues to be sorted out. The cost of the PCR technique is another

important limiting factor in employing this method in routine diagnosis.

Moreover, higher levels of Collagen, Elastin, Fibronectin, Transforming growth factor-beta were noted in active lesions of Cutaneous TB as compared to healed one, indicating that effective therapy with DOTS may decrease the fibrosis which occurs as a sequel of active infection which again elucidates the effectiveness of DOT Strategy.

Thus in the era of resistant Tuberculosis, with resurgence due to HIV, changing clinical manifestations, availability of effective DOTS strategy and newer modalities like LAP, it is expected that the incidence of Cutaneous tuberculosis will further decrease.

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ABSTRACTS

Mycobacterial Interspersed Repetitive Unit typing in *Mycobacterium tuberculosis* isolates from Sichuan Province in China

Jian-hua Guo, Wen-liang Xiang, Geng Zhang, Tao Luo, Ning Xie, Zhi-rong Yang and Qun Sun. *Indian J Med Res* 2011; **134**: 362-8.

Emergence and spread of drug resistant *Mycobacterium tuberculosis* is a serious threat to tuberculosis (TB) control programme. Therefore, the objective of this study was to genotype drug-resistant *M. tuberculosis* strains isolated from patients in Sichuan, China, using Mycobacterial Interspersed Repetitive Units (MIRV) for epidemiological analysis. Drug-resistance testing of *M. tuberculosis* isolates from pulmonary TB patients was confirmed by proportion method. Twelve MIRV loci were analyzed on 80 drug-resistant and 9 susceptible isolates by polymerase chain reaction and agarose gel electrophoresis. Hunter-Gaston discriminatory index (HGI) values were determined for each 12 MIRV loci for the evaluation of their discrimination power. Among 12 MIRV loci examined, polymorphic bands could be generated on 11 loci. Sixty five isolates had distinct MIRV patterns, while other 24 belonged to eight clusters and resistant to at least one anti- TB drug tested. The association between the MIRV patterns and the mutation patterns of drug- resistance relevant target genes was not significant among the drug-resistant isolates. The results showed that with a satisfactory discrimination power exhibited, the 12 loci based MIRV typing could be a valuable tool for epidemiological studies in *M. tuberculosis* isolates from Sichuan.

Rifampicin-monoresistant *Mycobacterium tuberculosis* disease among children in Cape Town, South Africa

Dramowski A., Morsheimer M.M., Jordaan A.M., Victor T.C., Donald P.R. and Schaaf H.S. *The International Journal of Tuberculosis and Lung Disease* 2012; **16(1)**: 76-82.

The study was carried out at the Tygerberg Children's Hospital (TCH) and Brooklyn Chest Hospital (BCH), South Africa. The objective was to describe paediatric cases of rifampicin (RMP) monoresistant tuberculosis (RMR-TB) disease. Records of children with culture-confirmed RMR-TB between 1 March 2003 and 28 February 2009 were identified from a prospectively recorded database of drug-resistant TB at TCH and BCH. Mutation analysis was performed on available specimens. Eighteen children with a median age of 6.9 years (range 2 months-12.8 years) were identified. Nine (50%) were human immunodeficiency virus (HIV) infected and four (22%) were HIV-exposed but noni nfected. Eleven (61%) had had previous TB treatment or prophylaxis. Nine children (50%) had cavitary disease and five children (22%) had extra-pulmonary disease. Twelve (67%) had adult TB source cases, including five (42%) adults with known RMR-TB. Primary transmission occurred among 11 children (61%) and acquisition of RMR-TB was possible in seven (39%) with prior RMP exposure. Median delay to specific RMR-TB treatment was 70 days (range 23-188). One child died from RMR-TB meningitis. Gene mutations consistent with RMR-TB were confirmed in five available samples. RMR-TB disease is increasingly encountered, particularly among HIV-infected and HIV-exposed non-infected children. Delay in commencing appropriate treatment for RMR-TB and high rates of cavitary disease could be a source of RMR-TB transmission.

A national infection control evaluation of drug-resistant tuberculosis hospitals in South Africa

Farley J.E., Tudor C., Mphahlele M., Franz K., Perrin N.A., Dorman S. and Van der Walt M. *The International Journal of Tuberculosis and Lung Disease* 2012; **16(1)**: 82-90.

The importance of infection control (IC) in health care settings with tuberculosis (TB) patients

has been highlighted by recent health care-associated outbreaks in South Africa. To conduct operational evaluations of IC in drug-resistant TB settings at a national level. A cross-sectional descriptive study was conducted from June to September 2009 in all multidrugresistant (MDR-TB) and extensively drug-resistant TB (XDR-TB) facilities in South Africa. Structured interviews with key informants were completed, along with observation of IC practices. Health care workers (HCWs) were asked to complete an anonymous knowledge, attitudes and practices (KAP) questionnaire. Multilevel modeling was used to take into consideration the relationship between center and HCW level variables. Twenty-four M(X)DR-TB facilities (100%) were enrolled. Facility infrastructure and staff adherence to IC recommendations were highly varied between facilities. Key informant interviews were incongruent with direct observation of practices in all settings. A total of 499 HCWs were enrolled in the KAP evaluation. Higher level of clinical training was associated with greater IC knowledge ($P < 0.001$), more appropriate attitudes ($P < 0.001$) and less time spent with coughing patients ($P < 0.001$). IC practices were poor across all disciplines. These findings demonstrate a clear need to improve and standardize IC infrastructure in drug-resistant TB settings in South Africa.

Patterns of *pnc* A mutations in drug-resistant *Mycobacterium tuberculosis* isolated from patients in South Korea

Kim H.J., Kwak H.K., Lee J., Yun Y.J., Lee J.S., Lee M.S., Min S.Y., Park S.K., Kang H.S., Maeng Y.H., Kim S.Y., Kook Y.H., Kim Y.R. and Lee K.H. *The International Journal of Tuberculosis and Lung Disease* 2012; **16(1)**: 98-104.

Pyrazinamide (PZA), one of the most effective anti-tuberculosis drugs, becomes toxic to *Mycobacterium tuberculosis* when converted to pyrazinoic acid by pyrazinamidase (PZase). PZA resistance is caused mainly by the loss of enzyme activity by mutation. The aim was to investigate the patterns of *pnc*A mutations in PZA-resistant mycobacteria isolated from South Korean patients. Mycobacterial isolates with clinically proven drug resistance were cultured to determine susceptibility to anti-tuberculosis agents. *pnc*A mutations were recognised by sequencing and compared with the relevant wild-type DNA sequence. Among 108 isolates, 102 were successfully cultured and underwent drug susceptibility testing; all were multidrug-resistant (MDR). *pnc*A mutations were found in 86 cultured isolates (85.1%): 55 (84.6%) in MDR and 31 (86.1%) in extensively drug-resistant isolates. Substitution of a single nucleotide was most common. The most frequent mutations were a deletion that caused a frameshift at nucleotide (nt) 71, a substitution at nt 403 and a substitution at nt 11. Combined, these accounted for ~ 40% of all mutations. However, 15 samples (14.9%) with defective PZase activity showed no mutation. *pnc* A mutation in *M. tuberculosis* is a major mechanism of PZA resistance in MDR isolates from patients in South Korea. The patterns of mutation might be more scattered and diverse. DNA-based diagnosis of PZA resistance has potential for the rapid detection of drug resistance.

Tuberculosis contact investigation in a high-burden setting: house or household?

S.S.VanWyk, A.M. Mandalakas, D.A. Enarson, R.P.Gie, N.. Beyers, A.C. Hesseling,.*The International Journal of Tuberculosis and Lung Disease* 2012; **16(2)**: 157-62

The study was done in a high tuberculosis (TB) burden setting, South Africa. Two frequently used definitions for household' are 1) 'all dwellings on the same plot of land that share the same residential address'; and 2) 'a group of persons who live together in the same dwelling unit and who have the same eating arrangements'. The objective was to characterise a household and the outcome of investigations in household child contacts using definition 1 compared to definition 2 during a TB contact investigation. Access to a household (definition 1) was gained via an adult TB case. Children were assessed for TB infection and disease. Household enumeration indicated 25 members of three families living in a main house and a fourth family living in an adjacent structure. Three children were diagnosed with TB and two referred for isoniazid preventive therapy. Families living in the main house shared the main kitchen, while the yard house family used its own kitchen. This household would

have been classified as two separate households if definition 2 had been used, and children with TB disease and infection would have been missed. The definition of household in TB contact investigation should provide a framework that is broad enough to capture the majority of children at risk.

DOT associated with reduced all-cause mortality among tuberculosis patients in Taipei, Taiwan, 2006-2008

Y. F. Yen, T. C. Rodwell, M.Y. Yen, H.C. Shih, B.S. Hu, L.H. Li, Y.H. Shie, P. Chuang, R.S. Garfein.*The International Journal of Tuberculosis and Lung Disease* 2012; **16(2)**: 178-84(7).

The objectives of the study were to determine whether patients receiving directly observed treatment (DOT) had lower all-cause mortality than those treated with self-administered treatment (SAT) and to identify factors associated with mortality among tuberculosis (TB) patients. Patients in Taipei, Taiwan, diagnosed between 2006 and 2008 were included in a retrospective cohort study. Among 3624 TB patients, 45.5% received DOT, which was disproportionately offered to older patients and those with more underlying illness and severe TB disease. After controlling for patients' sociodemographic factors, clinical findings and underlying comorbidities, the odds of death were 40% lower (AOR 0.60, 95%CI 0.5-0.8) among patients treated with DOT than those on SAT. After adjusting for DOT, independent predictors of death included non-Taiwan birth, increasing age, male, unemployment, end-stage renal disease requiring dialysis, malignancy, acid-fast bacilli smear positivity and pleural effusion. DOT was associated with lower all-cause mortality after controlling for confounding factors. DOT should be expanded in Taiwan to improve critical treatment outcomes among TB patients.

High prevalence of drug resistance amongst HIV-exposed and infected children in a tuberculosis prevention trial

A.C. Hesseling, S. Kim; S. Madhi; S. Nachman, H.S. Schaaf, A, Violari, T.C. Victor, O. McSherry, C. Mitchell, M.F. Cotton. *The International Journal of Tuberculosis and Lung Disease* 2012; **16(2)**: 192-5(4).

An emergence of drug-resistant tuberculosis (DR-TB) in settings affected by human immunodeficiency virus (HIV) and tuberculosis (TB) has been observed. We investigated the prevalence of DR-TB in P1041, a multicentred, randomised, double-blind trial which compared the administration of isoniazid (INH) to placebo, in HIV-exposed, non-infected and -infected African infants in the absence of any documented TB exposure. The prevalence of multidrug-resistant TB (MDR-TB) was 22.2% (95%CI 8.5-45.8) and INH monoresistance 5.6% (95%CI 0.1-27.6) among culture-confirmed cases, with all MDR-TB occurring in a single site. There was no association between INH treatment or placebo group, or between HIV infection status, and DR-TB prevalence. There was a high prevalence of DR-TB among HIV-exposed and -infected children. Surveillance of DR-TB among children in high-burden TB-HIV settings should be routine.

Global isoniazid resistance patterns in rifampin-resistant and rifampin-susceptible tuberculosis

S.E.Smith,E.V.Kurbatova, J. S. Cavanaugh, J.P. Cegielski. *The International Journal of Tuberculosis and Lung Disease* 2012; **16(2)**: 203-5(3).

Following the World Health Organization's endorsement of the Xpert® MTB/RIF assay, which rapidly and simultaneously diagnoses tuberculosis (TB) and detects resistance to rifampin (RMP), the question arises to what extent RMP resistance is an adequate marker for multidrug-resistant TB (MDR-TB). A retrospective analysis of data from >81 countries and subnational settings demonstrated that >40% of RMP-resistant isolates from new TB cases did not display resistance to isoniazid (INH) in settings with relatively low MDR-TB prevalence (one third of all countries and subnational settings). Results indicated the need for INH susceptibility testing in addition to RMP susceptibility testing.

Fluoroquinolone and pyrazinamide resistance in multidrug-resistant tuberculosis

C. Pierre-Audigier; C. Surcouf, V. Cadet-Daniel, A. Namouchi S. Heng, A. Murray, B. Guillard, B Gicquel. *The International Journal of Tuberculosis and Lung Disease* 2012;. **16(2)**: 221-3(3).

In a study performed in Cambodia, a higher number of tuberculosis (TB) strains with mutations in the *pnc*A gene associated with pyrazinamide resistance (PZA-R) was found in fluoroquinolone-resistant (FQ-R) multidrug-resistant (MDR) strains (93%), compared with 47% in MDR and 3% in non-MDR strains. This emphasises the need for easy and rapid tests for identification of PZA-R for efficient treatment of MDR-TB.

Recovery of *Mycobacterium tuberculosis* from Lowenstein-Jensen media contaminated with other organisms

Nagarajan, S. Anbarasu, V. Kumar, N. Selvakumar. *The International Journal of Tuberculosis and Lung Disease* 2012; **16(2)**: 230-31(2).

Growth of contaminating organisms along with *Mycobacterium tuberculosis* on Lowenstein-Jensen (LJ) medium is common. However, there is no documented evidence on the decontamination procedure adopted in mycobacteriology laboratories to recover *M. tuberculosis* from the contaminants grown on LJ medium. At the National Institute for Research in Tuberculosis, of 1048 LJ slopes with *M. tuberculosis* received from intermediate reference laboratories, 98 (9%) were contaminated. Of these, 87 (89%) *M. tuberculosis* cultures were retrieved after decontamination with 1% cetrimide. The use of cetrimide as a decontaminating agent to retrieve *M. tuberculosis* cultures grown with contaminants is documented.

Using likelihood ratios to estimate diagnostic accuracy of a novel multiplex nested PCR in extra-pulmonary tuberculosis

V. Vadwai, A. Shetty, C. Rodrigues. *The International Journal of Tuberculosis and Lung Disease* 2012; **16(2)**: 240-47(8)

The study was done in a tertiary care centre in Mumbai with a referral bias towards treatment failures. The objectives were to standardise and evaluate a novel single tube multiplex nested polymerase chain reaction (PCR) targeting insertion sequence (IS) *6110*, *mpb*64, *rrs* and *rpo*B genes for rapid diagnosis of extra-pulmonary tuberculosis (EPTB). The PCR assay was evaluated among 489 consecutive consenting patients, and results were compared against a composite reference standard comprising smear microscopy, culture, clinical symptoms, radiological scan and histology. PCR assay reported a pooled sensitivity of 94.5% (242/256, 95%CI 91-97): 91.9% (125/136, 95%CI 86-96) for smear-negative composite reference standard (CRS) positive cases and 97.5% (117/120, 95%CI 93-99) for smear-positive CRS-positive cases. The PCR positivity rate increased from 91.7% (235/256, 95%CI 88-95) when presence of IS*6110* was considered alone for reporting a test as positive to 94.5% (242/256, 95%CI 91-97) when used in combination with other three gene targets, with a specificity of 96.4% (212/220, 95%CI 93-98). A positive likelihood ratio of 26 (95%CI 13-51) and a negative likelihood ratio of 0.06 (95%CI 0.03-0.09) makes the test useful for ruling out and ruling in the disease. Culture should not be replaced by PCR as a gold standard; however, PCR can be used as a rapid, accurate tool in the diagnosis of EPTB.

Prevalence of HIV among blood donors in a tertiary care centre of north India

R.N. Makroo, Mohit Chowdhry, Aakanksha Bhatia, Bhavna Arora and N.L. Rosamma. *Indian J Med Res* 2011; **134(6)**: 950-3.

India has the second highest HIV population in the world with about 2.5-3.0 million cases. HIV-2 cases among general and blood donor population have also been reported mostly from west and south India. This single centre study was carried out to observe the HIV-1 and HIV-2 prevalence among blood donors from north India. A total of 2,04,677 people were screened for the presence of HIV infection over the 11 year period (1999 to 2009). Till 2004, a third generation ELISA kit was used. From 2005 till January 2009, all tests were done using the fourth generation ELISA kit which detected the presence of HIV-1 P24 antigen and anti-HIV antibodies. From February 2009 onwards, the kits used were Genscreen ULTRA HIV Ag-Ab Assay. A total of 506 (0.247%) donors were found to be repeat reactive for HIV. Of these, 486 (96%) donors tested using the Western blot were found positive for HIV-

1 infection. Twenty (4%) donors showed a negative Western blot result, none of the donors were found reactive for HIV-2 infection. The prevalence of HIV was 0.249 per cent among blood donors of north India. No HIV-2 case was found among the studied blood donor population indicating that it is not a threat currently.

Barriers to ART adherence & follow ups among patients attending ART centres in Maharashtra, India

N. Joglekar, R. Paranjape, R. Jain, G. Rahane, R. Potdar, K.S. Reddy and S. Sahay. *Indian J Med Res* 2011; **134(6)**: 954-9.

Adherence to ART is a patient specific issue influenced by a variety of situations that a patient may encounter, especially in resource-limited settings. A study was conducted to understand factors and influencers of adherence to ART and their follow ups among patients attending ART centres in Maharashtra. Between January and March 2009, barriers to ART adherence among 32 patients at three selected ART centres functioning under national ART roll-out programme in Maharashtra, were studied using qualitative methods. Consented patients were interviewed to assess barriers to ART adherence. Constant comparison method was used to identify grounded codes. Patients reported multiple barriers to ART adherence and follow up as *(i)* Financial barriers where the contributing factors were unemployment, economic dependency, and debt, *(ii)* social norm of attending family rituals, and fulfilling social obligations emerged as socio-cultural barriers, *(iii)* patients' belief, attitude and behaviour towards medication and self-perceived stigma were the reasons for sub-optimal adherence, and *(iv)* long waiting period, doctor-patient relationship and less time devoted in counselling at the centre contributed to missed visits. Mainstreaming ART can facilitate access and address 'missed doses' due to travel and migration. A 'morning' and 'evening' ART centre/s hours may reduce work absenteeism and help in time management. Proactive 'adherence probing' and probing on internalized stigma might optimize adherence. Adherence probing to prevent transitioning to suboptimal adherence among patients stable on ART is recommended.

Efficacy and Safety of Linezolid in the Treatment of Extensively Drug-Resistant Tuberculosis

Shen-Jie Tang, Qing Zhang, Lin-Hai Zeng, Hua Sun, Jin GU, Xiao-Hui Hao, Yi-Dian Liu, Lan Yao and He-Ping Xiao. *Jpn J Infect Dis* 2011; **64**: 509-12.

Linezolid is a new antibiotic with activity against *Mycobacterium tuberculosis in vitro* and *in vivo*. This study aims to evaluate the efficacy and safety of linezolid in the treatment of extensively drug-resistant tuberculosis (XDR- TB). We used a linezolid-containing regimen in the treatment of 14 XDR- TB patients. Two years of individualized chemotherapy regimens were adopted on the basis of the patients' medication history and the results of drug susceptibility testing. The patients received 600 mg of linezolid twice a day for the first 1-2 months, followed by once a day thereafter. Eleven patients (78.6%) showed significant improvement in clinical symptoms. Chest computed tomography revealed that 10 patients (71.4%) showed cavity closure. Smear conversion and culture conversion were achieved in all patients (100%) with an average of 64 and 63 days, respectively. The exact proportions of serious and minor adverse events determined by linezolid were 21.4% (3/14) and 64.3% (9/14), respectively. These data show that linezolid-containing chemotherapy for the treatment of XDR- TB may significantly improve clinical symptoms, promote lesion absorption and cavity closure, and accelerate sputum conversion. Further, adverse reactions can be tolerated and resolved with suitable intervention.

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I, Shri Tejinder Ahluwalia, 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.

Tejinder Ahluwalia
Secretary General
On behalf of the Tuberculosis Association of India