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Editorial

NEW GENERATION METHODS FOR DRUG SUSCEPTIBILITY TESTING FOR TUBERCULOSIS

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There is a well accepted strategy to manage tuberculosis at the global level [1]. For the treatment of fresh untreated cases of tuberculosis drug susceptibility testing is not required. This is based on the fact that the drug resistance (especially multi-drug resistance – MDR) is low in fresh untreated cases with figures ranging from 1% to 5% from different parts of India [2, 3]. However, during the last one decade there has been increase in reported incidence of drug resistance in Category II cases especially those treated with improper regimens, and also in an irregular fashion. The figures for MDR rates in such cases vary from area to area and overall 10% to 15% MDR rates have been estimated in well conducted studies. This makes the drug susceptibility testing important for this category particularly in those showing poor response to Cat II DOTS regimen. The experience from studies conducted by our Institute in Agra, Kanpur and Banda also showed the same trends for MDR rates as are reported from most other parts of the country. In addition the problem of extensive drug resistance (XDR), though may be small in terms of absolute numbers, is a therapeutic challenge with very high chances of failure. India has already been listed as one of the 41 countries from which XDR has been reported [1]. However, despite sporadic initial reports exact magnitude of XDR is not known in India.

As a part of Revised National Control Programme (RNTCP) of Government of India different State Health Authorities have followed a well-planned strategy to face the challenge of drug resistance in tuberculosis [3] . These measures are essentially preventive and include increasing coverage with DOTS, operational research to monitor the trends in drug resistance, research in methods for rapid detection of drug resistance and improving methods/regimens for prevention and management of drug resistant cases. DOTS plus is being introduced in a phase by manner for which drug susceptibility testing (DST) is very crucial. To get an accurate estimate of drug resistance at public health level and also provide reliable tools of DST to the clinicians, there is need to debate the methods which are available or are under different stages of development. As this testing differs significantly from other bacteria, the clinicians are often perplexed because of the wide choice being offered by different laboratories particularly when adequate validation is not documented and quality assurance is not guaranteed [4]. For these reasons there should be debate periodically about the status of different methods for DST of *M.tuberculosis*.

The conventional microbiological methods for DST have been well-tested and their strengths as well as limitations are known. Among the conventional methods, the three widely known approaches are proportional, resistance ratio and absolute concentration methods. Proportion method has been considered the most reliable and is taken as a reference for comparing any method. These conventional microbiological procedures, though quite robust, take several months (can be reduced by direct testing in specimens with sufficient number of bacteria) and as a result the search for alternatives has been accelerated in recent years. Various alternative methods for drug susceptibility testing are based on various genomic and phenotypic characteristics [5-10]. Among these alternatives **BACTEC 460** with turn around time of 7-14 days is the most acceptable method [6] . Experience from different parts of the world shows that it is a rapid and highly sensitive method but has the limitations of being hazardous due to radioactivity. Equipment

is expensive and running costs are high. Mycobacterial growth Indicator tube (**MGIT)** which is being considered as a replacement to BACTEC 460 is a fluorometric, rapid and sensitive method for early detection of the growth of mycobacteria and also for DST. **BacT/Alert** is another emerging rapid colorimetric, fully automated system with online data access system. The high running cost and expensive equipment are the limitations in its becoming a popular method. This method also requires about an average of seven days for drug susceptibility testing of various drugs including pyrazinamide [7].

E test is another easy and simple method. This method is based on use of E test strips containing gradients of impregnated antibiotics applied on the surface of agar medium inoculated with test strain. This approach has shown good result in various microbes including pneumococcus, neisseriae and also mycobacteria. While the results are good with rifampicin, modifications in the cut-off are required for INH [8].

A number of other techniques based on **oxidation reduction** of different indicator dyes/ systems need special mention [9]. These methods utilize dyes such as, *alamar blue*, *Resazurin* and *nitroblue tetrazolium* (NBT). This approach is very simple and user friendly. Easy detection of growth or its inhibition by different drugs is possible and drug susceptibility levels can be determined by making a titration of drug concentration in the micro titrate plate. Further the results of drug susceptibility testing can be easily interpreted. From the published literature and also our own experience these methods have been found to be quite promising for rifampicin, INH and also drugs like ethambutol [9]. For DST for pyrazinamide, assay(s) based on *pyrazinamidase* have also been reported to be promising [7]. While it is too early to recommend any of these methods for the time being as more rigorous evaluation is required, all these approaches are attractive, easy to perform and would be suited to low cost settings. Combining these methods with identification by simple protein detection techniques will be worth considering for a composite approach of early detection , identification and DST. As such, these need to be encouraged and evaluated on priority.

Use of **mycobacteriophages** as indicators of viability has been well established for drug susceptibility testing against *Mycobacterium tuberculosis*. These techniques utilize mycobacteria as indicator strains and or reporter systems such as luciferase/ beta galactosidase. Some of mycobacteriophage based systems are also available commercially and are expected to be used in a big way in future.

Molecular methods are also being extensively used for understanding the mechanisms of drug resistance not only in tuberculosis [5] , but also for viruses, other bacteria, fungi, parasites and even cancer cells. Specificity and speed are major advantages of molecular assays [5,6]. In case of tuberculosis, mutations in the target sites, are considered most important mechanism of isolate becoming resistant. While DNA sequencing is the gold standard for the detection of mutations in the genes responsible for drug resistance, other simple approaches like line probe hybridization assay , PCR – SSCP, PCR hetero duplex formation, PCR-RFLP etc have been described for detection of mutations. Techniques such as line probe hybridization are simple to use and more suited for laboratories with small infrastructure. While a line probe assay for rifampicin is commercially available (Inno-LIPA), improved versions have been developed in India. PCR-SSCP as well other approaches have shown varied sensitivity and specificity and there is a clear need to evaluate these methods rigorously. Usefulness of molecular detection of drug resistance also varies for different drugs. For example the detection of mutations appears to be quite reliable for rifampicin but it is not so efficient and reliable for other drugs because of different known (multiple targets sites, presence of sites other than the known targets and efflux pumps) and unknown mechanisms. In case of INH, mutations in katG, inhA, ahpC, oxyR and many other loci have been shown to be related to resistance. Similarly, in case of streptomycin, ethambutol, pyrazinamide and quinolones , mutations in known targets

explain only part and have shown an overall co-relation with DST in 50 -60% isolates only. Further, the mutations may be different in isolates from different geographical settings as seen in different Indian studies. It would be thus important to keep on enlarging the data-base about the sequences/ molecular mechanisms from different geographical areas. Further, it is also necessary to focus on mutations which are clinically relevant as low degree of resistance is not clinically meaningful. *Real Time PCR* protocols for detection of mutations in target genes such as rpoB for rifampicin and katG for INH have been published for tuberculosis [10]. These include melting curve analysis bi-probe systems, and taqmen probes. Most of these real time PCR methods have been described to be promising but certainly need more rigorous evaluation.

As the detection of mutations in the target sites is not proving successful for drugs other than rifampicin, there is an increasing focus on investigating alternate mechanisms such as permeability barriers, inactivation of drugs by enzymatic machinery of the bacteria and extrusion of drugs by efflux pumps. All these mechanisms play varying role in different isolates for various drugs and a comprehensive picture is yet to emerge. Unknown mechanisms can be better understood by microarray and also proteomic approaches. With microarray upregulation of several genes in INH resistance, involvement of new pumps in ofloxacin as well as multi-drug resistance has been identified in *M.tuberculosis* (JALMA : Indian Patents filed). These investigations have provided several leads which have undoubtedly potential applications in the development of new compounds and also new molecular methods for detection of drug resistance. However, this is all in the realm of the future and no clear recommendations can be made at the moment.

Overall, we can conclude that several conventional and new generation methods for DST against tuberculosis are available. References cited are mainly either review articles or personal experiences with some of these alternate methods and thus are by no means comprehensive. There is wealth of literature on the subject which needs to be referred to. It would be worthwhile to continue the debate and develop a data base(s) on the emerging experience of application of these methods especially from endemic countries like India. Finally it would be essential to adequately test methods for second line drugs and establish standards for them.

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Original Article

SURVIVAL OF TUBERCULOSIS PATIENTS TREATED UNDER DOTS IN A RURAL TUBERCULOSIS UNIT (TU), SOUTH INDIA

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Summary

***Objective*:** To estimate survival probabilities and identify risk factors for death of tuberculosis (TB) patients during treatment period.

Methods: TB patients registered during May 1999 to December 2004 from a rural TB unit (TU) with a population of 580 000 in Tiruvallur district, South India, formed study population. Life table and Cox's regression methods were used.

***Results*:** Of the 3818 TB patients who were initiated on treatment, 96, 94 and 97% of category – I, II and III respectively, were surviving after completion of treatment. Higher death rates were independently associated with patient's age (45 years), previous history of treatment, alcoholism and initial body weight (<35 kgs).

Conclusion: The survival probability was found to be similar in all patients irrespective of categorization. Necessary actions need to be initiated in the programme to improve body weight and abstain from alcoholism.

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***Key words*:** Tuberculosis, Death, Cox proportional hazard model, Life table, Risk factors.

INTRODUCTION

Tuberculosis (TB) is an infectious disease. It continues to be a leading cause of death[1]. Globally, there are almost nine million new cases of TB each year, two million of which results in death. More than one-third of these cases and deaths are in India and China[2]. TB incidence in India accounts for one fifth of the global incidence[3]. In India, every year, almost 1.8 million new cases occur, of which almost half are infectious and 370, 000 die[3]. Patients with infectious pulmonary TB disease can infect 10-15 persons in a year[3]. Directly Observed Treatment, Short-course (DOTS), strategy in TB control has considerably improved the quality of diagnosis and treatment outcome globally. In India, DOTS was implemented since 1993 for effective management of TB treatment. The goal of TB control programme is to decrease mortality and morbidity due to TB and cut transmission of TB in the community. For the individual patients and the DOT providers, the treatment outcome is of more interest in particular the probability of surviving the disease. The duration of the treatment is also important in deciding the survival of the patients. In this study, we attempted to estimate survival probabilities and identify risk factors for death of TB patients during treatment period.

METHODS

It is a retrospective study from a rural TB unit (TU) with a population of 580000 in Tiruvallur district, South India. The study area includes 209 villages and nine urban clusters scattered across approximately 200 sq. kms. The DOTS strategy was implemented in this area since May 1999[4]. There are 17 governmental health facilities (HFs) participating in the programme and of these, seven offer diagnostic facilities for sputum examination.

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All the patients diagnosed with TB at one of these Health Facilities (HFs) are given DOT in accordance with RNTCP policies[5]. Every dose of treatment is to be directly observed during intensive phase (IP) and at least first of the three doses is to be directly observed during continuation phase.

During May 1999 – December 2004, 5366 TB patients were registered for treatment under DOTS at the HFs in this area. The data on socio- economic demographic profile was collected within a week of starting the treatment and the treatment profile at the end of the IP. Trained field staff interviewed 3818 (71%) patients at their residence and collected information on socio-economic demographic profile, body weight at initiation of treatment, history of previous treatment, smoking and drinking habits, whether they took treatment under supervision and type of DOT provider using a semi-structured questionnaire. Category and treatment outcome were collected from TB register maintained by TU. Category I is defined as all new cases that are sputum smear positive or seriously ill patients with smear negative or extra-pulmonary disease. Category II patients are cases who have had previous anti-tuberculosis treatment. Category III regimen is prescribed to new patients who are smear negative or extra-pulmonary TB and are not seriously ill. The anti-tuberculosis regimens used for Category I, II and III patients were $2H_3R_3Z_3E_3/4H_3R_3$, $2H_3R_3Z_3E_3S_3/1H_3R_3Z_3E_3/5H_3R_3E_3$, and $2H_3R_3Z_3/4H_3R_3$ respectively (H = isoniazid; R = rifampicin; Z = pyrazinamide; E = ethambutol; S = streptomycin. Numbers before the letters indicate the duration of the treatment phase in months and numbers in subscript indicate the number of times the drug is given each week). Treatment for category I and II patients was extended by another month if the sputum smear remained positive at the end of IP. Standard international definitions were followed to classify TB patients according to outcome[5].

Data were scrutinized and entered twice in order to ensure accuracy, corrected for discrepancy and missing information. Life table method was used to estimate survival and hazard functions of survival times of TB patients for three categories separately. Using this analysis survival curves were constructed for the three categories of patients. Wilcoxon (Gehan) test was used to compare survival times of TB patients between categories. The Life

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

Factors	**Total (%)** n = 3818
Sex	
Female	1048 (27)
Male	2770 (73)
Age (Years)	
<45	2013 (53)
≥45	1805 (47)
Education	
Illiterate	1626 (43)
Literate	2192 (57)
Occupation	
Unemployed	935 (24)
Employed	2883 (76)
Weight (Kg)*	
<35	495 (13)
>35	3054 (80)
Smoking*	
No	2230 (58)
Yes	1586 (42)
Alcoholism*	
No	2619 (69)
Yes	1197 (31)
Category	
I	1944 (51)
II	449 (12)
III	1425 (37)

*The number of patients is less than 3,818 due to the non-availability at the time of interview within a week after treatment began.

Table 2: Estimates of life survival and hazard functions for Categories I, II and III

	Category I					Category II					Category III				
Interval	n_j	c_j	d_j	S(t)	h(t)	n_j	c_j	d_j	S(t)	h(t)	n_j	c_j	d_j	S(t)	h(t)
0	1944	20	4	.9979	.0021	449	18	0	1.0000	.0000	1425	22	1	.9993	.0007
1	1920	48	8	.9937	.0042	431	26	4	0.9904	.0096	1402	43	3	.9971	.0022
2	1864	38	2	.9926	.0011	401	26	6	0.9751	.0156	1356	29	4	.9941	.0030
3	1824	35	14	.9850	.0078	369	24	1	0.9724	.0028	1323	22	5	.9904	.0038
4	1775	50	13	.9776	.0075	344	22	0	0.9724	.0000	1296	25	10	.9826	.0078
5	1712	157	10	.9716	.0061	322	19	0	0.9724	.0000	1261	153	6	.9777	.0051
6	1545	809	8	.9648	.0070	303	20	5	0.9558	.0172	1102	757	3	.9736	.0042
7	728	339	1	.9631	.0018	278	72	3	0.9439	.0125	342	196	2	.9656	.0082
8+	388	378	10	.9147	***	203	198	5	0.8986	***	144	142	2	.9392	***

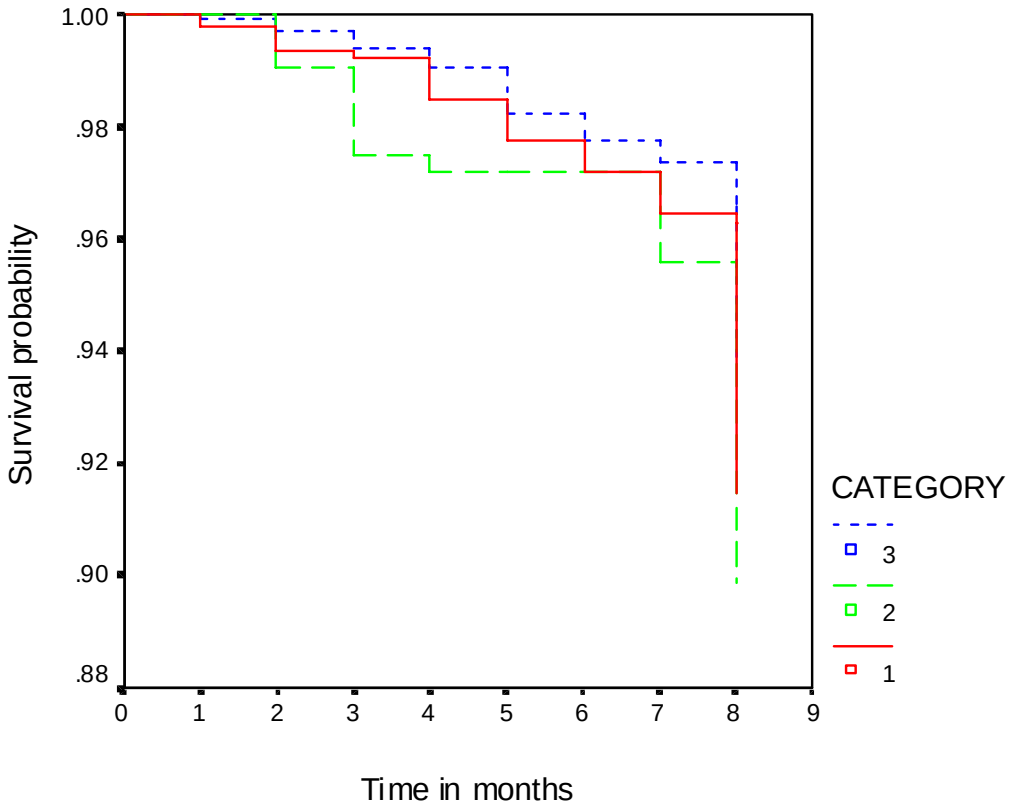

Figure: Survival curves of TB patients under three categories

table estimate of survival function S(t) and hazard function h(t) are given by

$$h(t) = d_j \Big/ (n_j^{/} - d_j / 2)(\tau_j)$$

Where n_j = Number of individual who are alive and therefore at risk of death, at the start of j'th interval
d_j = Number of deaths in the j'th time interval, j =1,2,…,m.
c_j = Number of censored survival time in this interval
$n_j^{/}$ = average number of individuals who are at risk during this interval = $n_j - c_j / 2$
$\hat{o}_j$ = the length of j'th time interval.
Cox's proportional hazards model was performed to identify risk factors for death during treatment period using backward stepwise procedure by SPSS version 10.0. Adjusted hazard ratio and 95% confidence interval (CI) were estimated for the risk factors. The level of statistical significance was defined as $p<0.05$.

RESULTS

Among 3818 TB patients registered under DOTS programme, 2770 (73%) were males, 1805 (47%) were 45 years and above, 3054 (80%) had weight more than 34 kgs, 1626 (43%) were illiterate and 935 (24%) unemployed. The life style indicators for the patients were: 1586 (42%) smokers and 1197 (31%) alcoholics. Of the 3818 patients treated, 1944 (51%) were under category I, 449 (12%) under category II and 1425 (37%) under category III (Table 1). For these patients, the treatment outcomes were as follows: 1538 (40.3%) (Category I- 70.2% and Category II- 38.8%) were cured, 1468 (38.4%) (Category I- 10.0%, Category II-9.8% and Category III- 86.2%) had completed treatment, 535 (14%) (Category I- 11.5%, Category II- 38.5% and Category III- 9.8%) defaulted, 130 (3.4%) (Category I- 3.6%, Category II- 5.3% and Category III- 2.5%) expired, 144 (3.8%) (Category I- 4.7%, Category II- 7.6% and Category III- 1.3%) failed treatment and 3 (0.1%) (Category I- 0.1%, and Category III- 0.1%) were transferred out.

Using Life Table estimate method, survival probabilities of the patients were 99.4%, 99.0% and 99.7% for category I, II and III respectively after the first month of treatment. The corresponding survival probabilities were 98.5%, 97.2% and 99.0% respectively after the three months and 96.3%, 94.4% and 96.6% respectively after seven months of treatment (Table 2). Using Wilcoxon (Gehan) test, there was no significant difference between the survival times of TB patients among the three categories ($p = 0.1$). The survival probability against time in months for category I, II and III is separately shown (Fig). The gap between the three curves distinguishes the difference between survival distributions, where the curve for category II decreases compared to that of category III and I. From this curve, it is understood that the survival probabilities of category III patients is more than category I patients during treatment period but survival probabilities of category II is less than category III and I.

Of the 3818 patients, 305 cases with missing values were excluded for multivariate analysis (Cox proportional hazard model). Of 3513 patients, 109 (3.1%) deaths occurred during treatment period. Duration in months from diagnosis of TB to end of the treatment period and treatment outcomes was measured for 3404 (96.9%) those who were alive at the end of treatment. The variables considered for the model were sex, age, category, education, occupation, body weight at initiation of treatment (<35 kg), history of previous anti TB treatment, smoking and drinking habits, type of DOT providers, whether patient took treatment under supervision in intensive phase and continuation phase. Age (≥45 years), previous history of treatment, alcoholism and body weight at initiation of treatment (<35 kg) were found to be risk factors for death during treatment period. The corresponding adjusted hazard ratios were 2.345 (95% C.I.: 1.557, 3.533), 1.615 (95% C.I.: 1.102, 2.368), 2.015 (95% C.I.: 1.357, 2.992) and 3.709 (95% C.I.: 2.434, 5.652) respectively (Table 3). The other factors namely; sex, category, education,

Table 3: Results of Cox's proportional hazard model of risk factors for death during treatment period.

Factors		No. Registered	No. of Death	p	Adjusted hazard ratio (95% CI)
Sex	Female	965	26		1
	Male	2548	83	0.364	1.317 (0.726, 2.391)
Age	<45Yr	1846	34		1
	≥45	1667	75	< 0.001	2.345 (1.557, 3.533)
Category	I	1784	61		1.189 (0.760, 1.859)
	II	397	17		0.766 (0.390, 1.507)
	III	1332	31		1
Education	Literate	2001	48		1
	Illiterate	1512	61	0.215	1.278 (0.867, 1.883)
Occupation	Employed	2665	72		1
	Unemployed	848	37	0.124	1.375 (0.917, 2.064)
Previous history of treatment	No	2492	64		1
	Yes	1021	45	< 0.05	1.615 (1.102, 2.368)
Smoking	No	2060	57		1
	Yes	1453	52	0.239	0.733 (0.437, 1.229)
Alcoholism	No	2434	59		1
	Yes	1079	50	< 0.005	2.015 (1.357, 2.992)
Type of DOT providers	Government	1388	50	0.379	1.309 (0.718, 2.384)
	Community	1582	45	0.973	0.990 (0.542, 1.807)
Friends, relatives, self and others		543	14		1
Supervision under IP	Always	2619	78		1
	Never	894	31	0.468	1.168 (0.768, 1.778)
Body weight at initial stage of treatment	< 35 kg	488	34	< 0.001	3.709 (2.434,5.652)
	≥ 35	3025	75		1

occupation, smoking, type of DOT providers and supervision under IP were not found to be significant for risk of death during treatment period.

DISCUSSION

Our study findings show that 94-97% of patients were surviving at the end of seven months of anti-tuberculosis treatment, irrespective of categories. A multivariate analysis of the study found that age (≥45), body weight at initiation of treatment (<35 kgs), previous history of treatment and alcoholism were risk factors associated with death during anti-tuberculosis treatment period. A study[6] conducted by our centre in Chennai city showed that 91% patients treated for TB survived the entire follow-up period of 600 days from the date of start of treatment and 9% patients died during the follow-up period. The risk factors identified for TB mortality were young age, male sex, smear postivity, treatment default, treatment failure and the combination of smoking and alcoholism. In the study, survivors were followed up after completion of treatment for a minimum period of 600 days. But our study was confined to the treatment duration and this could be the reason for age (≥45) identified as risk factor. In a study from Netherlands[7], survival probabilities for tuberculosis patients were estimated as 95% after six months of treatment and 6% died within one year while on treatment. The study identified the following risk factors for mortality of tuberculosis patients: male sex, age (>65 years), presence of malignancy, human immune-deficiency virus (HIV) infection, addiction to alcohol or drugs, localization of tuberculosis (pulmonary and extra pulmonary tuberculosis) and type of medical

officer having made the diagnosis (specialist internal medicine). An earlier report from South India[4] showed that 39 (6%) of 676 TB patients died during treatment period and higher death rates were independently associated with weight <35 kgs and history of previous treatment. The study recommended that possible role of nutritional interventions should be explored among underweight patients to reduce mortality. A Russian study[8] reported 183 (9.6%) deaths occurred among 1916 TB patients and older age, previous treatment for TB, multi-drug resistance TB and alcoholism were risk factors for death during treatment period.

In the present study, the survival probability was found to be similar in all patients irrespective of categorization. Age, initial body weight, previous history of treatment and alcoholism were risk factors for higher death rate. Necessary actions need to be initiated in the programme to improve body weight and abstain from alcoholism. The mortality rate was slightly lower compared to that found in other studies. The study population consists of patients registered during 1999-2004, so the average morality would have come down due to patients treated under DOTs over the period of time. The analysis was restricted to study population during the treatment period, so this could be reason for finding similar survival probabilities irrespective of categorization. The deaths reported in this study are those who expired during the treatment period and all the deaths might not be due to TB. In this study other risk factors for deaths, not due to TB, could not be identified. This warrants for a larger study involving patients died due to TB only by evaluation of proper document like death certificate.

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Original Article

A COMPARISON OF CONVENTIONAL AND RADIOMETRIC METHODS FOR THE ASSESSMENT OF ANTI-TUBERCULAR ACTIVITY OF DRUGS AGAINST *MYCOBACTERIUM TUBERCULOSIS* IN MICE AND MACROPHAGE MODELS

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Summary

***Background*:** Presently, *in vitro* and *in vivo* screening of anti-tubercular drugs is a time-consuming exercise. Therefore, it is important to develop faster methods.

Material and Methods: Towards this end, conventional plating and radiometric BACTEC methods of anti-tubercular screening were compared to determine the efficacy of anti-tubercular drugs (isoniazid and rifampicin) and morphine in *Mycobacterium tuberculosis* H37Rv-infected mice and macrophages.

Results: A linear correlation ($R^2 = 0.95$) was observed between number of colony forming units (CFUs) and growth index (GI) values. BACTEC method was found to be faster and sensitive as compared to plating method. Further, BACTEC method, being a closed system, appeared to be less susceptible to microbial contamination and poses less biohazard.

Conclusion: We conclude that BACTEC method can be employed for easy, precise, and rapid screening of anti-tubercular compounds and morphine in mice and macrophage models ***[Indian J Tuberc 2008; 55: 70-76]***

Key Words: *Mycobacterium Tuberculosis*, BACTEC, CFU, GI, Macrophage, Mice, Morphine.

INTRODUCTION

Tuberculosis (TB) is a major health problem worldwide. An estimated one-third of world population is exposed to *M. tuberculosis* leading to two to eight million deaths anually. Further, eight million new cases appear every year[1]. Chemotherapy of drug susceptible TB consists of three or four drugs regimen, administered for six months. The long duration of therapy results in poor compliance leading to emergence of multi-drug resistant strains of *M. tuberculosis*[2,3]. There has been resurgence in TB cases due to HIV epidemic because HIV/AIDS patients show an increased susceptibility to infections and TB appears early in the course of HIV infection. Whereas vaccine is definitely a long-term solution of TB control[4], but prospectus of having an improved vaccine appears remote. Thus, chemotherapy has to be the mainstay of TB control. Development and evaluation of new chemical entities (NCEs) against TB is a challenging task. The NCEs are first tested *in vitro* for direct anti-tubercular activity by employing broth dilution, agar dilution, alamar blue dye or BACTEC method followed by evaluation in macrophage and *in vivo* models. The *in vivo* screening requires four weeks of NCE/drug administration to *Mycobacterium*-infected mice followed by CFU enumeration in the target organs (lungs, spleen and/or liver) which requires an additional three to six weeks of incubation for colonies to develop on solid medium. Therefore, it takes about eight to ten weeks for evaluating *in vivo* activity of a NCE/drug. The macrophage model requires six to seven days of exposure to NCE/drug followed by three to six weeks of incubation for CFU enumeration. Further, the highly nutritive solid media (Middlebrook 7H10, Middlebrook 7H11, Lowenstein Jenson medium, etc.) employed for CFU enumeration are susceptible to microbial contamination during long durations of incubation (3-6 weeks).

The previous reports of correlation between CFU and GI were limited to the estimation of

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mycobacterial load and the effect of direct-acting anti-tubercular drugs[5,6]. Apparently, no study has demonstrated the correlation between CFU and GI for drugs lacking direct anti-tubercular activity like morphine. In the present study, *M. tuberculosis*-infected mice were treated with various drugs and organ homogenates (lungs and spleen) were inoculated on Middlebrook 7H11 agar medium plates and in BACTEC 12B medium vials. Similarly, *M. tuberculosis*-infected macrophages were incubated with different drugs and macrophage lysates were inoculated on Middlebrook 7H11 agar medium plates and in BACTEC 12B medium vials. The CFU counts obtained on Middlebrook 7H11 agar medium were compared with GI values**.** Here, we report the observed correlation between CFU and GI for direct-acting drugs and morphine in mouse and macrophage models of TB.

MATERIAL AND METHODS

Animals

Swiss albino mice (20 ± 2 g, both sexes) were obtained from the Central Animal Facility of the institute and maintained in 12-hour light/dark cycle. The animals were provided food and water *ad libitum*. All studies were carried out as per protocol approved by Institutional Animal Ethics Committee (IAEC) adopted from the guidelines of the Care and Use of Animals in Scientific Research, Indian National Science Academy, New Delhi.

Organism

M. tuberculosis H37Rv was obtained from Tuberculosis Research Centre, Chennai, India. The mycobacteria were grown in Middlebrook 7H9 medium (HiMedia, India) supplemented with 10% ADC (HiMedia, India). Log phase cultures were centrifuged, washed twice with sterile saline and adjusted to McFarland standard corresponding to $1x10^7$ CFU/ml. The size of inoculum was confirmed by plating serial dilutions on Middlebrook 7H11 media (HiMedia, India) plates supplemented with 10% OADC (HiMedia, India). The plates were incubated for four weeks prior to CFU enumeration.

Infection of mice

The mycobacterial suspension was sonicated (Bandelin, Germany; 20 KHz, 10 seconds, 10 cycles), and mice were infected intravenously with 0.1 ml of the suspension ($1x\ 10^7$ CFU/ml)

Drugs

Morphine was obtained from Government Opium and Alkaloid Factory, Ghazipur, India. Isoniazid and rifampicin were obtained from Sigma, USA. Morphine was dissolved in sterile saline and administered subcutaneously. Stock solution of isoniazid was prepared in sterile water and rifampicin was prepared in 0.1 % Tween-80 (Sigma, USA).

Drug treatment

Mice were divided in different experimental groups (n=10/group). One group was treated with saline, and is referred to as vehicle-treated control. One group received isoniazid (25 mg/kg, six days/week) and another group received rifampicin (20 mg/kg, six days/week) for four weeks by oral gavage. The other groups received morphine at various graded doses (0.5, 5, 10, 50 and 100 mg/kg), subcutaneously, on day 0 and day +15 of infection.

CFU enumeration

Mice were sacrificed on day +30 of infection by cervical dislocation. Lungs and spleen were aseptically removed and homogenized in saline. Viable mycobacterial counts were determined by plating 10-fold serial dilutions on Middlebrook 7H11 medium supplemented with 10% OADC. CFUs were enumerated after four weeks of incubation at 37°C in 5% CO_2 atmosphere.

Cultivation of mouse peritoneal macrophages

Thioglycollate-elicited peritoneal macrophages were isolated as described elsewhere[13]. The cells were suspended in Dulbecco's Modified Eagle's Medium (DMEM; PAA Lab., Austria)

supplemented with 10% fetal calf serum (FCS; PAA Lab., Austria), 2.0 mM L-glutamine, 0.01 M HEPES and 40µg/ml gentamicin (complete DMEM; CDMEM). Cells were seeded in 24-well tissue culture plates at 1 x 10^6 cells/well in a total volume of one ml per well. The cells were incubated overnight at 37°C / 5% CO_2 atmosphere for adherence.

Intramacrophage killing of *M. tuberculosis*

After overnight incubation in 24-well tissue culture plate, non-adherent cells were removed by washing with warm Hank's balanced salt solution (HBSS; PAA Lab, Austria) and replaced with CDMEM without gentamicin. Log phase *M. tuberculosis* culture was centrifuged, washed thrice with sterile saline and suspended in DMEM. Adherent macrophage monolayers were infected with mycobacterial suspension at a multiplicity-of-infection of 1:10 for 4h at 37°C in 5% CO_2 atmosphere. Unphagocytosed mycobacteria were removed by washing (5x) with HBSS. Fresh DMEM, with or without drugs, was added and incubated at 37°C in 5% CO_2 atmosphere. Macrophages were lysed on day 0, 4 and 7 by addition of 0.4 ml of 0.25% sodium dodecyl sulphate and 1.1 ml of Middlebrook 7H9 medium. After 10 minutes of incubation at 37°C, 0.5ml of 20% bovine serum albumin was added and again incubated for 10 minutes. Ten-fold serial dilutions of macrophage lysates were prepared in saline and plated on Middlebrook 7H11 medium in triplicate. The plates were incubated for three to four weeks at 37°C in 5% CO_2 atmosphere.

BACTEC 460 TB determination

The standard radiometric (BACTEC) method was employed[7]. In brief, different dilutions of organ homogenates and macrophage lysates were inoculated in BACTEC 12B vials (Becton Dickinson, USA) in duplicate and vials were incubated at 37°C in 5% CO_2 atmosphere. The GI of inoculated vials was taken at 24h intervals in BACTEC 460TB system (Becton Dickinson, USA). Appropriate positive and negative controls were also included.

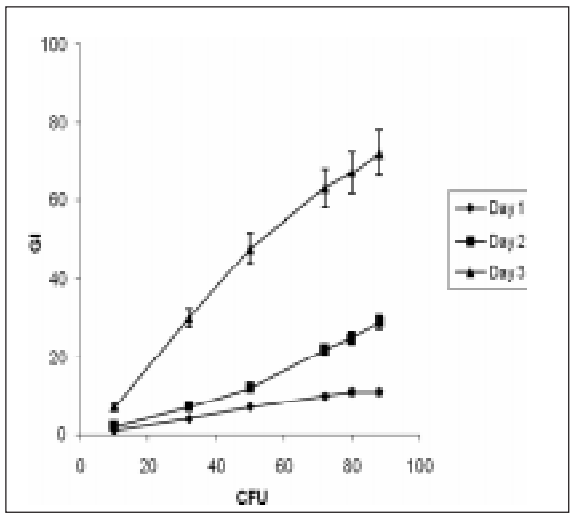

Fig. 1: Correlation between CFU and GI in log phase cultures of *M. tuberculosis* H37Rv

RESULTS

The correlation between CFU and GI was studied in log phase *M. tuberculosis* culture, organ homogenates and macrophage lysates infected with *M. tuberculosis*. We have observed that BACTEC method can be employed for easy and rapid screening of anti-tubercular drugs.

Correlation between CFU and GI in log phase cultures of *M. tuberculosis* H37Rv

Correlation between CFU and GI at different dilutions of log phase cultures of *M. tuberculosis* was evaluated by plotting CFUs against GI values at different intervals. There appeared to be a linear correlation (R^2 = 0.99) between CFU counts and GI on different days (Fig. 1).

Correlation between CFU and GI in lungs of *M. tuberculosis* H37Rv-infected mice treated with isoniazid, rifampicin and morphine

In lungs, again a linear correlation (R^2 = 0.99, Fig 2) was observed between CFU and GI in isoniazid- and rifampicin- treated animals. Similarly, in morphine-treated animals a linear correlation (R^2 = 0.99), was observed (Fig. 3)

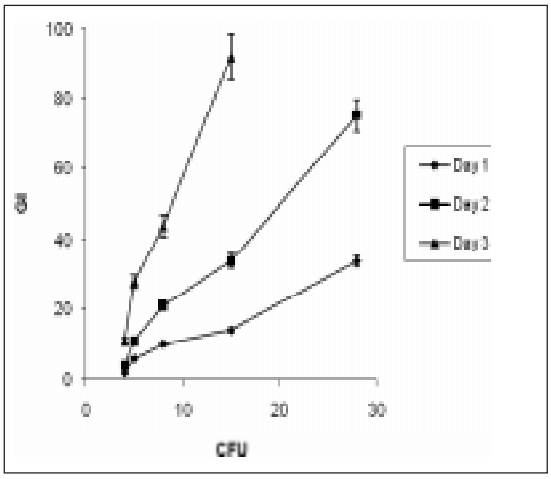

Fig. 2: Correlation between CFU and GI in lungs of *M. tuberculosis* H37Rv-infected mice treated with Izoniazid and Rifampicin

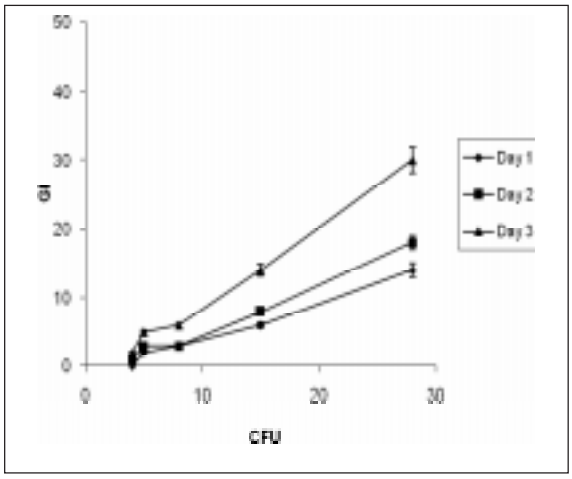

Fig. 4: Correlation between CFU and GI in spleen of *M. tuberculosis* H37Rv-infected mice treated with Isoniazid and Rifampicin

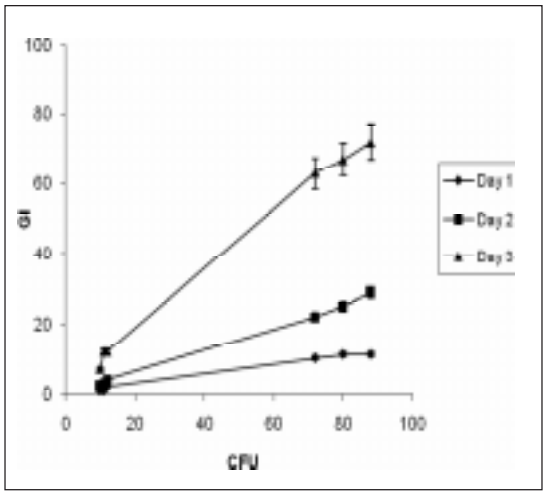

Fig. 3: Correlation between CFU and GI in lungs of *M. tuberculosis* H37Rv-infected mice treated with morphine

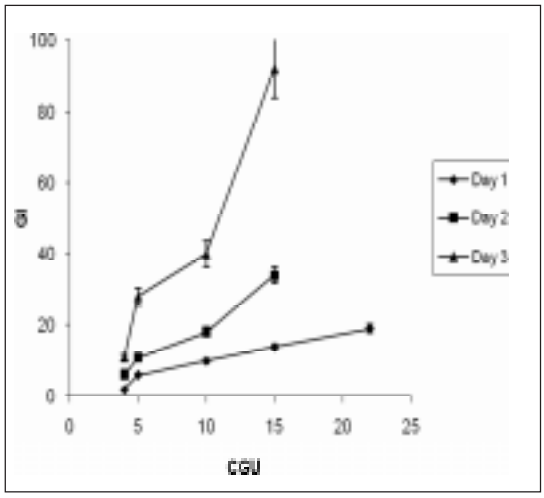

Fig. 5: Correlation between CFU and GI in spleen of *M. tuberculosis* H37Rv-infected mice treated with morphine

Correlation between CFU and GI in spleen of *M. tuberculosis* H37Rv-infected mice treated with Isoniazid, Rifampicin and morphine

In spleens also, a linear correlation was observed between CFU and GI in isoniazid- and rifampicin- ($R^2 = 0.99$, Fig. 4) and morphine-treated ($R^2 = 0.98$, Fig. 5) animals.

Correlation between CFU and GI in macrophage model treated with Isoniazid, Rifampicin and morphine

A linear correlation was obtained between CFU and GI of control macrophage, isoniazid- and rifampicin- treated and morphine-treated lysates with $R^2 = 0.99$ and 0.98 (Figs. 6 and 7).

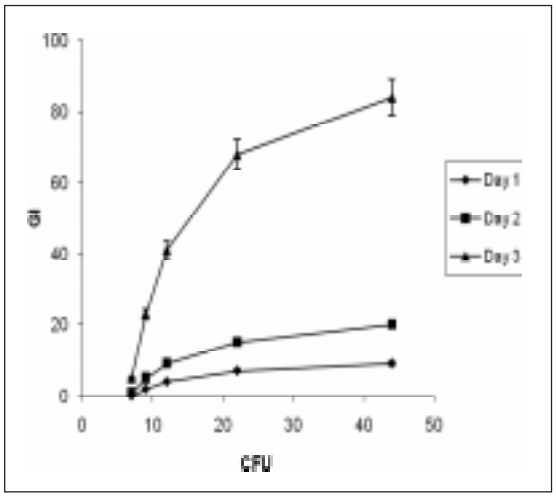

Fig. 6: Correlation between GI and CFU in macrophage model with Isoniazid, Rifampicin

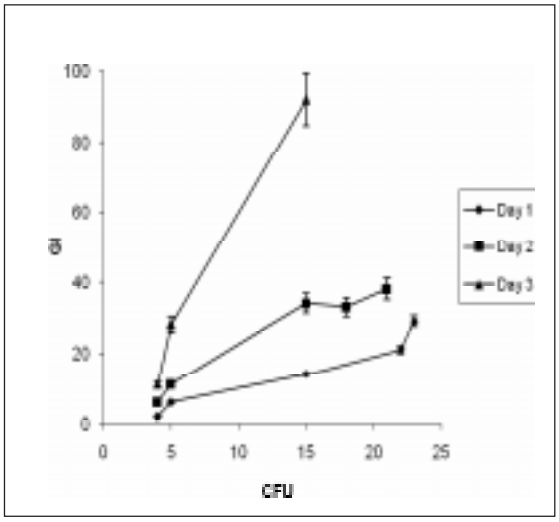

Fig. 7: Correlation between GI and CFU in macrophage model with morphine

Microbial contamination

Microbial contamination was found to be less frequent in BACTEC vials as compared to conventional method, the frequency of microbial contamination being 2-5% in BACTEC vials and 10-15% in media plates (detailed data not shown).

DISCUSSION

Currently, CFU enumeration and BACTEC methods are most widely employed for studying direct-acting anti-tubercular compounds. Here, we report that BACTEC method is superior to the conventional plating method in many aspects.

In this study, we have compared conventional plating and BACTEC methods for screening of anti-tubercular agents in *in vivo* and macrophage models. The *M. tuberculosis*-infected mice were treated with morphine and anti-tubercular agents and mycobacterial load was assessed in target organs (lungs and spleen) using plating and BACTEC method. The results of *in vivo* study showed a good correlation between the CFU counts and GI readings for all the drugs (R^2 = 0.95-0.99; Figs. 2-5). Further, these drugs showed a good correlation between the CFU and GI values in macrophage model of infection (R^2 = 0.95-0.99; Figs. 6, 7).

Previous studies have established a correlation between CFU counts and GI values in *in vivo*[6] and *in vitro*[5] models. Another approach employed for correlation involves determining the days to positivity (number of days required for GI to cross 30) and correlating it to CFU counts[8, 9]. However, our study differs from the previous studies in many ways. Firstly, the study by Reddy *et al*[6] was limited to determination of GI values 24h after inoculation of BACTEC vials whereas in the present study GI was determined over a time period of 10 days or even more. This offers many advantages over a single reading after 24h, which include increased sensitivity, determination of linearity of correlation after different durations of incubation and allows recovery of injured/slow-growing, but viable, mycobacteria. Secondly, in previous studies BACTEC method has been employed for determination of activity of direct-acting anti-tubercular drugs[5] while we have evaluated the activity of direct-acting drugs (isoniazid and rifampicin) as well as morphine. Morphine was chosen for this study because it shows a dose-dependent immunomodulatory effect, low doses being immunostimulant while higher doses being immunosuppressive. The immunomodulatory activity of morphine can be attributed to activation

of hypothalamus-pituitary-adrenal axis and by direct action on immune cells like macrophages and lymphocytes[10-12]. Thirdly, we have determined the GI values at different dilutions of the inoculum (organ homogenates and macrophage lysates; Fig II-VII). This is because mycobacteria tend to form clumps and could lead to erroneous results when inoculum with high bacterial counts is inoculated in BACTEC vials. Additionally, erroneous results in *in vivo* studies may arise due to improper homogenization of organs and can be overcome by dilution of the samples. When different dilutions of organ homogenates and macrophage lysates were inoculated in BACTEC vials, a better correlation was observed between CFU and GI (II-VII). In general, GI values were more sensitive and results were obtained in shorter periods. Moreover, microbial contamination was negligible in BACTEC method as compared to CFU enumeration (2-5% and 10-15%, respectively).

It has been observed that the number of mycobacteria differed in different samples depending on the drug, dose/concentration of drug and dilution of the inoculum. This resulted in different GI values in different BACTEC vials; the vials inoculated with higher number of mycobacteria showed higher GI values. The drugs, which showed higher anti-tubercular activity, produced little or no change in GI whereas those showing little or no anti-tubercular activity produced rapid increase in GI during the incubation period. Depending upon the number of mycobacteria, some BACTEC vials showed GI of 999 after 24h while others showed no increase in GI until four to six days. This problem has been addressed by inoculating different dilutions and subsequently analyzing the correlation. Hence, mycobacteria in different vials were detected after varying durations of incubation and reading can be obtained in one to ten days. We have observed that the inoculum which showed no CFU even after eight weeks of incubation in conventional method, showed a measurable change in GI after eight to ten days of incubation in BACTEC vials. This observation is in agreement with previous studies that indicate better recovery in broth cultures[13] and contribute to better sensitivity of BACTEC method in comparison to conventional plating method. The present study substantiates the claims of superiority of BACTEC method over CFU enumeration[6].

On the other hand, usefulness of conventional plating method in studying the colony morphology and purity of mycobacterial strain on Middlebrook 7H10 or 7H11 plates cannot be ignored. However, in our opinion, the disadvantage of BACTEC method is involvement of radioactive material and limitation of GI reading range (0-999)[6]. Although the risk of radioactivity in BACTEC method can be overcome by employing plating method or newer methods like fluorimetric Mycobacteria Growth Indicator Tube[14] and MB/BacT[15,16], but these methods may, sometimes, require longer durations of incubation and have higher microbial contamination rates[5, 14].

In conclusion, BACTEC method can be employed for screening of potential anti-tubercular compounds by inoculating organ homogenates and macrophage lysates. This method is useful in evaluation of compounds that possess direct anti-tubercular activity as well as morphine[10, 11]. Addition of antibiotic cocktail PANTA (Polymyxin-B, Amphotericin-B, Nalidixic acid, Trimethoprim and Azlocillin) to BACTEC vials could overcome the problem of contamination. Additionally, random plating of some dilutions may be done on Middlebrook 7H10 or 7H11 media plates along with GI determination for confirmation of results obtained by BACTEC method. The use of BACTEC method will significantly reduce the testing time and biohazard while giving better sensitivity and precision.

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Original Article

INCREASED YIELD OF SMEAR POSITIVE PULMONARY TB CASES BY SCREENING PATIENTS WITH ≥2 WEEKS COUGH, COMPARED TO ≥3 WEEKS AND ADEQUACY OF 2 SPUTUM SMEAR EXAMINATIONS FOR DIAGNOSIS.

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Summary

Background

RNTCP recommends examining three sputum smears for AFB from Chest Symptomatics (CSs) with cough of ≥3 weeks for diagnosis of Pulmonary TB (PTB). A previous multi-centric study from Tuberculosis Research centre (TRC) has shown that the yield of sputum positive cases can be increased if duration of cough for screening was reduced to ≥2 weeks. Other studies have shown that two smear examinations are adequate for diagnosis of smear positive PTB . To validate the above findings, a cross sectional multi-centric study was repeated in different settings in five geographical areas in India.

Methods

Three primary and secondary level health facilities with high out-patient attendance were selected from two Tuberculosis Units (TU) in each of the 15 selected districts to screen about 10,000 new adult outpatients from each state. For patients who did not volunteer history of cough, symptoms were elicited using a structured simple questionnaire. All the CSs were referred for sputum examination.

Results

A total of 96,787 out-patients were registered. Among them 69,209 (72%) were new adult out-patients. Using ≥2 weeks of cough instead of ≥ 3 weeks as the criterion for screening, there was an overall increase of 58% in CS and 23% increase in the detection of smear-positive cases. Among 211 patients, 210 were positive at least by one smear from the initial two specimens. Increase in the work-load if 2 smears were done for patients with cough of ≥2 weeks cough were 2 specimens (i.e.13 to 15) per day for an adult OPD of 150.

Conclusion

The yield of sputum positive PTB cases can be improved by screening patients with ≥2 weeks cough and two specimens are adequate for diagnosis. **[*Indian J Tuberc 2008; 55: 77-83*]**

***Key words*:** Chest symptomatic, RNTCP, Sputum smear examination-AFB

INTRODUCTION

Following a phased rapid expansion from 1997, the Government of India's DOTS-based Revised National Tuberculosis Control Programme (RNTCP) achieved nation-wide coverage in early 2006.[1] RNTCP recommends that the diagnosis of Pulmonary TB (PTB) is made by the examination of three sputum smears for AFB from chest symptomatics (CSs) with a history of cough of three weeks or more.[2]

A previous multi-centric study undertaken by Tuberculosis Research Centre (TRC), Chennai, compared the yield of sputum smear positive PTB cases among CSs with a cough of ≥ 2 weeks versus ≥ 3 weeks, and found an increase in the yield if the duration of cough for screening was reduced to ≥ 2 weeks.[3] Various studies have shown that PTB cases can be diagnosed by doing two smears examination rather than the present recommended three, saving time as well as cost.[4-6] A recent systematic review suggested that reducing the recommended number of specimens from 3 to 2 could also potentially increase case detection by improving the quality of examination of the first two specimens[7]. To validate these findings, we undertook the current study in different settings in five geographical areas in India.

*Tuberculosis Research Centre, Chennai, **RNTCP, Maharashtra, # RNTCP, Rajasthan, $ RNTCP, West Bengal, @ RNTCP, Andhra Pradesh, RNTCP, Orissaბ, § World Health Organisation.

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OBJECTIVES

1. To assess the yield of smear positive cases among chest symptomatics with cough of two weeks or more compared to three weeks or more.
2. To compare the efficacy of two smear examinations instead of three to diagnose smear positive PTB cases among chest symptomatics.

METHODS

Study design

In this cross sectional multi-centric study, adopting a multi-stage stratified sampling procedure, five States; Andhra Pradesh, Maharashtra, Orissa, Rajasthan and West Bengal where RNTCP coverage was >50%, were conveniently selected. From each of these States, three districts were selected; one each with a low, medium and high case detection rate as per the RNTCP performance report of the fourth quarter 2004. A convenient sample of two TB Units (TUs) [refer footnote [1]] in each district was selected, and three primary and three secondary level health facilities with high Out Patient Deparment (OPD) attendance were selected in those TUs, to screen about 10,000 new adult outpatients from each State. From the primary health facilities, one non-microscopy and two microscopy centres with a daily average of 50 new adults out-patients were selected. Similarly from the secondary level health facilities, two taluk level hospitals and one Government hospital with daily about 100 new adults patients were included. The duration of the data collection for the primary level centres was a minimum of 10 days or until the target of 500 was achieved. For secondary level centres, a minimum of one week or until the target of 700 was reached. Duration of intake was from December 2005 to March 2006.

Definitions

New adult out-patient – A patient making their first visit to a health facility for the current illness, aged ≥15 years, during the study period.

Chest Symptomatic (CS): A new adult out-patient with a history of a cough of two weeks or more, with or without expectoration, or with a history of haemoptysis. The chest symptomatics were analysed in two groups. First ,those who had cough of ≥ 2 weeks and the second group , those who had cough of ≥ 3 weeks.

Smear positive PTB: At least one sputum, positive for AFB by smear microscopy.

Medical officers and health workers in the study centres were trained about the study procedures and were asked to ensure that sputum examination was done on all patients with chest symptoms who fulfilled the criteria of a chest symptomatic. All out-patients were asked about their main complaints. If a patient did not spontaneously give a history of cough, then it sought after using a structured simple questionnaire.

All CSs were given a referral slip for sputum examination. In case of non-microscopy centres, arrangements were made for collection and transport of sputum specimens to the nearest microscopy centre. Patients with chest symptoms were asked to return on the following day to provide an early morning sample and a second spot specimen. Daily out-patient load at each facility according to age and sex, were collected using the OPD attendance register maintained at the facility.

To ensure accuracy of data collection, the respective State and District TB Officers, and TRC staff made frequent supervisory visits to the health facilities involved in the study.

Statistical analysis

The data were scrutinised and computerized, verified keying in twice, edited and corrected for discrepancy and missing information. Data was analysed using SPSS/PC version 13.0. The Chi-square test was used to test difference in proportions. The level of statistical significance was defined as $p \leq 0.05$.

[1]A TB Unit under RNTCP is a sub-district supervisory and monitoring unit covering approximately 500,000 population.

RESULTS

During the study period, a total of 96,787 out-patients were registered in the selected centres of the five states. Among them, 69,209 (72%) fitted the criteria for new adult out-patients; 13,394 from Andhra Pradesh, 16,002 from Maharashtra, 11,017 from Orissa, 12,916 from Rajasthan and 15,880 from West Bengal. (Table 1). Using ≥ 2 weeks instead of ≥ 3 weeks as the criterion for screening of chest symptomatics for sputum microscopy, there was an overall increase of 58% in chest symptomatics i.e. an increase from 1,625 to 2,560 (range 50-69%). The increase in the detection of smear-positive PTB cases was 23% (range 18 to 40%). Among the 17% (441/2,560) who did not complain of a cough spontaneously, the rate of smear positivity was 5.7% (25/435) compared to 9.1% (190/2088) among those who complained spontaneously, a non-significant difference.

The increase in the number of CSs using cough of ≥ 2 weeks for screening was maximum among 15-25 year age group (Table 2) and minimum among the 56-65 age group. There was 38% increase in positivity rate among the younger age group. There was, however, no difference in the increase in the CSs and positivity rate between males and females (62% vs 55%, and 29% vs 21%).

Table 1: Proportion of chest symptomatics and sputum positivity rate state-wise

State	New adult out patients	Cough ≥ 2 weeks*				Cough ≥ 3 weeks				Increased %	
		CS	S+ve	Per 100000 new adult OPD		CS	S+ve	Per 100000 new adult OPD		CS	S+ve
		n(%)	n(%)	CS	S+ve	n(%)	n(%)	CS	S+ve		
(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)	(k) (e-i)/e	(l) (f-j)/f
Andhra Pradesh	13394	465 (3.5)	64 (13.8)	3472	478	309 (2.3)	54 (17.5)	2307	403	50	19
Orissa	11017	423	43 (3.8)	3840 (10.2)	390	250 (2.3)	34 (13.6)	2269	309	69	26
Maharastra	16002	640 (4.0)	40 (6.2)	4000	250	398 (2.5)	34 (8.5)	2487	212	61	18
Rajasthan	12916	355 (2.7)	33 (9.3)	2749	255	233 (1.8)	28 (12.0)	1804	217	52	18
West Bengal	15880	677 (4.3)	35 (5.2)	4263	220	435 (2.7)	25 (5.7)	2739	157	56	40
Total	69209	2560 (3.7)	215 (8.4)	3699$^{\alpha}$	311$^{\alpha}$	1625 (2.3)	175 (10.8)	2348$^{\alpha}$	253$^{\alpha}$	58$^{\beta}$	23$^{\beta}$

* including all cases > 3 weeks

CS = chest symptomatic; S+ve = smear positive.

$^{\alpha}$ Estimated for one lakh New adult OPD based on the total

$^{\beta}$ Calculated based on the formula given

New adult out-patients screened in the primary and secondary health facilities were 25,650 and 43,559 respectively. The increase in the CSs was 57% and 58% and positivity rate were 19% and 25% in primary and secondary facilities respectively.

Among 935 CSs with ≥ 2 weeks of cough, 23 (2.5%) patients did not give sputum. Amongst the 1,625 CSs with ≥ 3 weeks of cough, 14 (0.9%) did not give sputum which was statistically significant (p<0.01).

Three samples were given by 2383 patients. As per the existing RNTCP definition of a smear positive PTB case, two or three specimens were positive for 199 (8.4%). If only the first 2 specimens were considered, both specimens were positive in 161 (6.8%). This was statistically significant (p<0.001) by McNemar's test. However, if one positive sputum was taken as the criterion for diagnosis of a smear positive PTB case, the positivity was similar whether two or three specimens were considered i.e. 211 (8.9%) cases detected if three specimens were considered and 210 (8.8%) if only the first two specimens were considered.

Table 2: Distribution of chest symptomatics in different age groups

Age	New adult out patients	Cough ≥ 2 weeks*				Cough ≥ 3 weeks				Increased %	
		CS	S+ve	Per 100000 new adult OPD CS	S+ve	CS	S+ve	Per 100000 new adult OPD CS	S+ve	CS	S+ve
		n(%)	n(%)			n(%)	n(%)				
(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)	(k) (e-i)/e	(l) (f-j)/f
15 - 25	21689	551 (2.5)	47 (8.5)	2540	217	317 (1.5)	34 (10.7)	1462	157	74	38
26 - 35	16375	526 (3.2)	51 (9.7)	3212	311	339 (2.1)	41 (12.1)	2070	250	55	24
36 - 45	11627	453 (3.9)	50 (11.0)	3896	430	287 (2.5)	44 (15.3)	2468	378	58	14
46 - 55	7933	396 (5.0)	38 (9.6)	4992	479	248 (3.1)	31 12.5)	3126	391	60	23
56 - 65	7789	422 (5.4)	20 (4.7)	5418	257	301 (3.9)	17 (5.6)	3864	218	40	18
> - 66	3796	212 (5.6)	9 (4.2)	5585	237	133 (3.5)	8 (6.0)	3504	211	59	13
Total	69209	2560 (3.7)	215 (8.4)	3699[α]	311[α]	1625 (2.3)	175 (10.8)	2348[α]	253[α]	58[β]	23[β]

* including all cases ≥ 3 weeks
CS = chest symptomatic; S+ve = smear positive.
[α] Estimated for one lakh New adult OPD based on the total
[β] Calculated based on the formula given

We estimated the increase in the work-load for the laboratory in the respective health facility by reducing the duration of cough to ≥ 2 weeks for screening. Considering an average daily OPD attendance of 150, the number of specimens to be examined for diagnosis will be around 13 per day for screening chest symptomatics if a criterion of ≥ three weeks cough is utilised. If ≥ 2 weeks' cough and two sputum examinations were utilised, the average work-load per day would be 15 specimens per day. Thus with a change to ≥ 2 weeks' cough and 2 smear examinations, the increase in work-load will be only two specimens per day (Table 3).

DISCUSSION

According to the current RNTCP recommendations, any patient with cough of ≥ 3 weeks should be screened for TB by doing three sputum examinations. This study confirms that the detection of smear positive PTB cases can be improved if the screening criterion is changed to a history of cough for ≥ 2 weeks. The findings of this study validate those shown by the earlier study done in 2002 in a different setting which showed 47% increase in sputum positive cases among CSs with ≥ 2 weeks cough.[3] Baily et al in 1967 reported an increased yield of 16% smear positive cases when the screening criterion used was cough of ≥ 2 weeks (44 of 622 with ≥ 2 weeks vs 37 of 275 with ≥ 3 weeks).[8] Our study also has similar findings.

In this study, the highest incremental yield of CSs and smear positive cases were found in the age group of 15-25 years. Vigilance should thus be high for history of cough when screening the

Table 3: Estimated work-load per microscopy centre per day in primary and secondary health facilities

	Primary health facility		Secondary health facility		Total		Cough 2 weeks or more with 2 specimens
	Cough ≥2 weeks	Cough ≥3 weeks	Cough ≥2 weeks	Cough ≥3 weeks	Cough ≥2 weeks	Cough ≥3 weeks	
New adult out patients per day (n)	46	46	104	104	150	150	150
Out patient with cough %	4.0	2.4	3.7	2.3	-	-	-
Patients with cough per year (n)	508	329	1157	719	1664	1048	-
Smear examined per year (n)							
For diagnosis (three smears per patients)	1523	988	3470	2157	4993	3145	3328
For follow-up	381	247	868	539	1248	786	1248
Total	1904	1235	4338	2696	6241	3931	4576
Average smears per day	6	4	14	9	21	13	15

younger age groups attending out-patient facilities in India.

Another important finding of our study was the similar smear positivity rate among OPD subjects who voluntarily complained of cough and those from whom the symptom was elicited. Similar finding was reported in an earlier study also.[3] This emphasises the need to actively detect symptomatics among the passively attending OPD attendees.

This study also investigated the diagnostic value of the third sputum specimen for the diagnosing of smear positive PTB cases. Three specimens were given by 2,383 patients, of whom 211 were positive on at least one specimen out of the three. Only one patient, however, was positive on the third specimen alone (0.5%). Another study from this centre also showed just 0.8% (8/962) of suspects positive by the third specimen alone.[4] A recent study in Moldova and Uganda also concluded that the third serial sputum smear examination was inefficient in diagnosing sputum smear positive PTB.[9]

As per the RNTCP definition for a new smear positive PTB case, two or three specimens were positive among 199 patients. If only the first two samples were considered, 161 patients were positive on both smears, with thus a loss of 38 (19%) patients. As, however, many, studies have reported that the incremental yield from the third specimen is negligible. If the first two specimens are considered with a case definition of at least one positive smear, then 210 out of 211 smear positive PTB cases would be detected i.e. two smears are an efficient method to diagnose smear positive PTB.[4-7,9,10] Even though the positive smear was not confirmed by culture in the study since it was done under programme conditions, it has been reported that among patients with only one of the two smears positive, 90% were positive by culture[11].

A concern raised was that if the screening criterion for a chest symptomatic is reduced from $\geq$ 3 weeks to $\geq$ 2 weeks, there would be an increase in the laboratory work-load. However, we estimated from the study findings that the increase would only be in the order of two to three specimens per day if the number of specimens is also reduced from the present recommended three to two. This small increase is manageable by virtually all microscopy centres under RNTCP.

CONCLUSION

The yield of sputum smear positive PTB cases can be improved if patients with $\geq$ 2 weeks history of cough are screened to diagnose such cases. In addition, two sputum smear examinations are as efficient as three for diagnosis. There is no significant increase in the work-load by screening CSs with $\geq$ 2 weeks cough if a methodology of only two sputum smear examinations is utilised.

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

Original Article

CLINICO-RADIOLOGICAL PROFILE OF NEW SMEAR POSITIVE PULMONARY TUBERCULOSIS CASES AMONG YOUNG ADULT AND ELDERLY PEOPLE IN A TERTIARY CARE HOSPITAL AT DEHERADUN (UTTARAKHAND)

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Summary

***Setting*:** Patients of pulmonary tuberculosis (TB) attending the out and in patient department of pulmonary medicine, Himalayan Institute of Medical Sciences (HIMS), a post graduate institute and a large tertiary care center in Dehradun.

***Objective*:** To compare the clinico-radiological pattern of pulmonary tuberculosis in the young adult (18-59 years) and elderly ($\geq$ 60 years) patients.

***Design*:** Prospective observational study of pulmonary and associated extra pulmonary tuberculosis cases, diagnosed between October 2005 to September 2006 in pulmonary medicine department of HIMS.

***Result*:** Mean age of young adult and elderly patients was 35.71±5.7 years and 68.57±3.03 years respectively. Elderly patients had a higher number of co-morbidities like diabetes mellitus, hypertension, and malignancy. Tuberculin positivity was less among elderly patients (36.0%) as compared to young adults (65.9%). Hemoptysis (29.5% vs. 6%), fever (95.4% vs. 76%) and night sweats (54.5% vs. 18.0%) were significantly higher in the young adult patients than the elderly. As for roentgenographic abnormalities, a higher involvement of lower zone (24.0% vs. 7.9%) and far advanced lesions (32.0% vs. 14.7%) were seen in the elderly patients as compared to young adults. The elderly showed a higher frequency of TB related mortality (8% vs. 1.1%) and associated extra pulmonary involvement (40% vs. 7%).

***Conclusion*:** Young adults are more likely to have hemoptysis, night sweats and positive PPD response while lower lung field involvement is more common in elderly. **[*Indian J Tuberc 2008; 55: 84-90*]**

***Key words*:** Pulmonary tuberculosis, Young adults, Elderly patients.

INTRODUCTION

Pulmonary tuberculosis represents an important worldwide public health problem. Due to increase in life expectancy, the population and absolute number of elderly have increased all over the world. India is the second largest country in the world with 72 million elderly person (Above 60 years of age) next only to China[1]. Demographic projections indicate that this figure will increase to 100 million by 2016. These figures emphasize the extent and importance of this group of population in India in near future. Tuberculosis has remained a public health problem in India since long. It is increasingly becoming more common in older age group.

Many studies have been published on the problem of pulmonary tuberculosis in elderly[2-9]. Some have suggested that pulmonary TB in the elderly presents with somewhat atypical symptoms[5,6] or radiological finding[7-9] or both. However, many studies have reported[11-13] that TB in the young adult and elderly patients shows similar clinical, bacteriological and radiological features. Most of these studies have been conducted outside India. In the Indian published literature, [14-15]there are very few comparative studies. This paucity of medical literature shows poor attention to geriatric pulmonary tuberculosis in our country. Sometime elderly tuberculosis may mimic pediatric tuberculosis and pose problem in diagnosis. Therefore this study was planned to compare the clinico-radiological presentation of pulmonary tuberculosis in the young adult and elderly age group.

MATERIAL AND METHODS

The study was carried out on patients attending the OPD/IPD of department of pulmonary

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medicine, Himalayan Institute of Medical Sciences, Dehradun, Uttrakhand, between October 2005 to September 2006. All patients having symptoms suggestive of pulmonary tuberculosis were subjected to sputum smear for AFB examination, Mantoux test, X ray chest PA view and hematological investigations. Bronchoscopy and washing for AFB detection were performed only in selected patient. We included only smear positive newly diagnosed pulmonary tuberculosis patients and pulmonary

Table 1: Population characteristics of the young adult and the elderly pulmonary tuberculosis patients.

	Young adult (n=88)		Elderly (n=50)	
	Number	Per cent	Number	Per cent
Age (mean ± SD)	35.71±5.7		68.57±3.03	
Gender				
Male	59	67.0	37	74.0
Female	29	33.0	13	26.0
Residence				
Rural	60	68.1	38	76.0
Urban	28	32.9	12	24.0
Religion				
Hindu	71	80.6	41	82.0
Other than Hindu	17	19.4	9	18.0

Table 2: Co-morbidities of the young adult and the elderly pulmonary tuberculosis patients.

	Young adult (n=88)		Elderly (n=50)	
	Number	Per cent	Number	Per cent
Diabetes mellitus	6	6.81	5	10.0
Hypertension	7	7.95	11	22.0*
COPD	4	4.54	13	26.0*
Chronic kidney disease	3	3.40	1	2.0
Malignancy	1	1.13	4	8.00*
Rheumatic heart disease	2	2.27	–	–

* $p < 0.05$

tuberculosis associated with extra pulmonary tuberculosis. All other forms of extra pulmonary tuberculosis, treatment failure, relapse, drug resistant tuberculosis and HIV seropositive patients were excluded from the study to allow better data comparison. A total of 138 patients were included in the study (Table 1). For further analysis patients were divided in two groups: young adult (18-59 years) and elderly (60 years and above). A total of 50 patients were in the elderly group while 88 patients made the young adult group. A prospective case series study was designed to see similarity/ dissimilarity between both groups of patients.

Two radiologists who were aware of the diagnosis but not clinical history reviewed the initial radiographs. The radiological evaluation was simple enough as to consider that potential biases, if any, were relatively small. The tubercular lesions were classified according to the site of lesion (unilateral, bilateral) and extent of lesion. Extent of lesion again divided in three, minimal lesion: disease with a combined area of less than that of the right upper lobe. Moderately advanced: Disease with a combined area of less than that of the right lung but more than that of the right upper lobe. Far advanced: Disease with a combined area of more than that of the right lung. The upper and lower lung fields defined as the lung above and below the hilar level respectively. Cavitation was considered to be present only when its diameter was more than 2 cms.

RESULTS

The study comprised 138 patients, 50 were in elderly and 88 were in young adult age group. The demographic profile of both groups is summarized in table 1. The younger patients ranged from 18 to 59 years of age and the elderly patients ranged from 60 to 82 years. Male predominance was seen in both the groups. Majority of patients in both groups belonged to rural area.

The prevalence of co-morbidities such as diabetes, COPD and malignancies was higher in elderly patients while chronic kidney disease was more frequently found in young adult patients (Table 2).

The proportions of patients with different symptoms are compared in Table 3. Lower prevalence of fever, night sweats and haemoptysis

Table 3: Symptomatology of pulmonary tuberculosis in young adult and elderly patients.

	Young adult (n=88)		Elderly (n=50)	
	Number	Percent	Number	Percent
Cought and /or sputum	80	90.9	42	84.0
Hemoptysis	26	29.5	3	6.0*
Fever	84	95.4	38	76.0
Night sweats	48	54.5	9	18.0*
Weight Loss	61	69.3	43	86.0
Anorexia	65	73.8	42	84.0
Chest pain	13	14.7	7	14.0
Dyspnea	6	6.81	15	30.0*

*P<0.05

as well as higher prevalence of dyspnoea were found in elderly patients as compared to young adults. Tuberculin positivity was significantly less among elderly patients as compared to young adults and this difference was found to be statistically significant ($p < 0.05$).

Table 4: Radiological and laboratory findings of the young and elderly pulmonary tuberculosis patients.

	Young adult (n=88)		Elderly (n=50)	
	Number	Percent	Number	Percent
Site of lesion				
Unilateral	58	65.9	21	42.0
Bilateral	30	34.0	29	58.0
Extent of lesion				
Minimal	35	39.7	11	22.0
Moderate	40	45.4	23	46.0
Far advance	13	14.7	16	32.0*
Involvement of zone				
Upper zone	60	68.1	22	44.0
Lower zone	7	7.9	12	24.0*
Multiple zone	21	23.8	16	32.0
Cavitatory lesion				
Yes	42	47.7*	11	22.0
NO	46	52.2	39	78.0
Laboratory finding				
Anaemia(<10 gm)	15	17	17	34.0*
? Albumin	9	10.0	11	22.0*
Diagnostic criteria of TB				
Sputum smear For AFB +ve	84	95.45	45	90.0
BAL fluid AFB +ve	4		3	
FNA/Biopsy	0		2	

*P<0.05

Table 5: Pulmonary tuberculosis associated with extra-pulmonary involvement.

	Young adult (n=88)		Elderly (n=50)	
	Number	Per cent	Number	Per cent
T.B Laryngitis	1	1.1	1	2.0
Pleural effusion	8	9.0	8	16.0
Disseminated/milliary	2	2.2	5	10.0*
Pott's Spine	1	1.1	3	6.0*
TBM	1	1.1	2	4.0
Hydropneumothorax	2	2.2	1	2.0
Total	15	17.0	20	40.0

*P<0.05

The radiological features of both groups are summarized in Table 4. Bilateral involvement, far advances disease and a lower zone involvement were more frequently found in elderly patients as compared to young adult patients. Radiographic image of cavitations were more frequently found in young patients. In laboratory finding, anemia and hypoalbuminemia were found more commonly among elderly patients (Table 4).

Among patients of pulmonary tuberculosis, associated extra pulmonary involvement was more common in elderly group as compared to young adult group (Table 5). TB related mortality occurred 1(1.1%) in young patients and 4(8%) in elderly.

DISCUSSION

Despite extensive tuberculosis control efforts in the past by WHO and local health departments, the tuberculosis epidemic continuous to ravage the developing world; affecting all susceptible individuals including aging adult. Several factors such as increase in the elderly population, immuno- compromised host, patients on steroids, anti-cancer drugs and immunosuppressive drugs and re-activation of dormant infection have contributed to this increasing proportion of TB in elderly.

In relation to the gender of the patients, we found that male predominance was found both in young adults and elderly patients. Other workers in India [14,15] and abroad[2-7] have also reported similar male predominance. One possible explanation for this male predominance may be that in most countries young men usually have more social and labour activities than women, thus favouring the transmission of the disease.

In our study, elderly tuberculosis patients had significantly higher occurrence of co-morbidities like hypertension, COPD, malignancy and diabetes. These results correspond to those of Alvarez et al[5], Umeki et al[7], Vanden Brande[16]. The exact relationship between these disorders and disease produced by M. tuberculosis is not fully understood because none of the studies include control non-tubercular group.

Our data suggest that symptoms like hemoptysis, febrile sense and night sweats occurred significantly more in young adults; whereas anorexia, weight loss and dyspnoea were more frequent in elderly patients. The above results are in agreement

with those of others[5,6,19] who found more classical symptoms in young adults. Umeki[7] and Vanden Brande et al[16] also reported similar results, though they found that hemoptysis occurred equally in both groups. However, differences in symptoms frequencies between these studies and the present study may be explained by earlier pulmonary TB detection by mass survey in former studies.

In this study, we found that tuberculin test was less frequently positive in elderly patients as compared to young adults (36% vs. 65%). Similar observations have been reported by other studies[5-7]. The possible explanation for these findings may be due to the fact that tuberculosis sensitivity is said to wane with age.

As for roentgenographic abnormality, in the present study, a higher involvement of lower lung field, more advanced lesion and less frequent cavitations were observed in the elderly patients than young adults. There had been much debate concerning the atypical radiographic findings of TB in the elderly. Some have reported no major differences[12,13] while other have reported a higher involvement of the middle and lower lung fields in the elderly[7,8,19]. Our findings are consistent with those of Perez[9] and chan[10] et al in this respect. It has been suggested that the higher frequency of lower lung disease and less cavity formation in the elderly is due to immunologic abnormalities, higher frequency of primary tuberculosis and higher VA/VQ ratio and PAO_2 in the lower lobe in elderly people. Therefore age induced changes should flavor multiplication of mycobacterium tuberculosis in lower lung zone.

On investigation the incidence of anemia and hypoalbumenia was significantly more in the elderly. These finding could be explained by the fact that older persons suffer from malnutrition much more frequently than younger people. Our findings are consistent with those of Alvarez[5] and Morris[17] et al.

Several studies have reported[5,6,17,19,20] higher TB related mortalities in the elderly patients. In the present study, all young pulmonary TB patients survived except one whereas four of the elderly patients died of TB.

There was no difference in the proportion of patients with bacteriologically proven disease between the two groups. Higher positivity rate may be due to the reason that we excluded all smear negative pulmonary tuberculosis patients for having minimal diagnosis bias.

Among patients of pulmonary tuberculosis, associated extra pulmonary involvement was more common in elderly group as compared to young adult (40% vs. 17%). Our results correspond to those of Maria K.K[6] and Zamarron et al[18].

In conclusion, this study shows that elderly pulmonary tuberculosis patients are more likely to present with non-specific symptoms and atypical radiographic findings. Moreover, we found a higher frequency of co-morbidities and higher TB related mortality in elderly TB patients. Thus, atypical clinico-radiological manifestation of tuberculosis in older persons can result in delay in diagnosis and initiation of treatment. This can lead to unfortunately higher rates of morbidity and mortality from this treatable infection in this population. So maintenance of a high index of suspicion for tuberculosis in this vulnerable population is undoubtedly justifiable.

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CHANCHAL SINGH MEMORIAL AWARD - 2008

The Tuberculosis Association of India awards every year a cash prize of Rs. 1000/- to a medical graduate (non-medical scientists working as bacteriologists, biochemists, etc, in the field of tuberculosis included) who is below 45 years of age and is working in the field of tuberculosis, for an original article not exceeding 30 double spaced foolscap size pages (approximately 6,000 words, excluding charts and diagrams) on tuberculosis. Articles already published or based on work of more than one author will not be considered. Papers may be sent, in quadruplicate, to reach the Secretary-General, Tuberculosis Association of India, 3, Red Cross Road, New Delhi-110001, before 30th June, 2008.

Indian J Tuberc 2008; 55: 91-93

STATUS REPORT ON RNTCP*

In the year 2007, RNTCP has achieved significant milestones- consolidating the achievements and launching of DOTS plus services for the management of MDR-TB. We hope to continue and maintain the same momentum in the year 2008.

RNTCP performance in fourth quarter 2007

During the quarter, over 1,645,983 TB suspects were examined and 200,223 sputum positive cases were diagnosed. More than 346,566 TB cases were registered for treatment, bringing the annualized total case detection rate to 123 cases per 100,000 population. The new smear positive (NSP) TB case detection rate (annualized) for the 4th quarter 2007 was 65%, with a total of 136,805 new smear positive cases being registered for treatment. In addition, 94,756 new smear negative cases (NSN), 47,061 new extra pulmonary cases, 47,656 smear positive re-treatment cases and 21,574 'others' were also initiated for treatment in this quarter. The success rate amongst the new smear positive PTB cases registered in the 4th quarter 2006 was 86%. The sputum conversion and cure rate among the new sputum positive cases was 90% and 84% respectively. The relatively high default rate among NSP (6.1%), NSN (7.7%) and re-treatment cases (16%) continues to be an area of deep concern which the programme managers at all levels must focus upon.

There is an encouraging progress in the DOTS plus services which have recently been initiated in the states of Gujarat and Maharastra. In this quarter, 85 MDR TB suspects have been tested, 42 MDR TB cases have been diagnosed and 33 have been put on treatment in the two states. From this quarter onwards, data on MDR TB cases on RNTCP Category IV treatment regimen will be routinely included in the quarterly performance reports.

Population in India covered under DOTS and total tuberculosis patients put on treatment each quarter

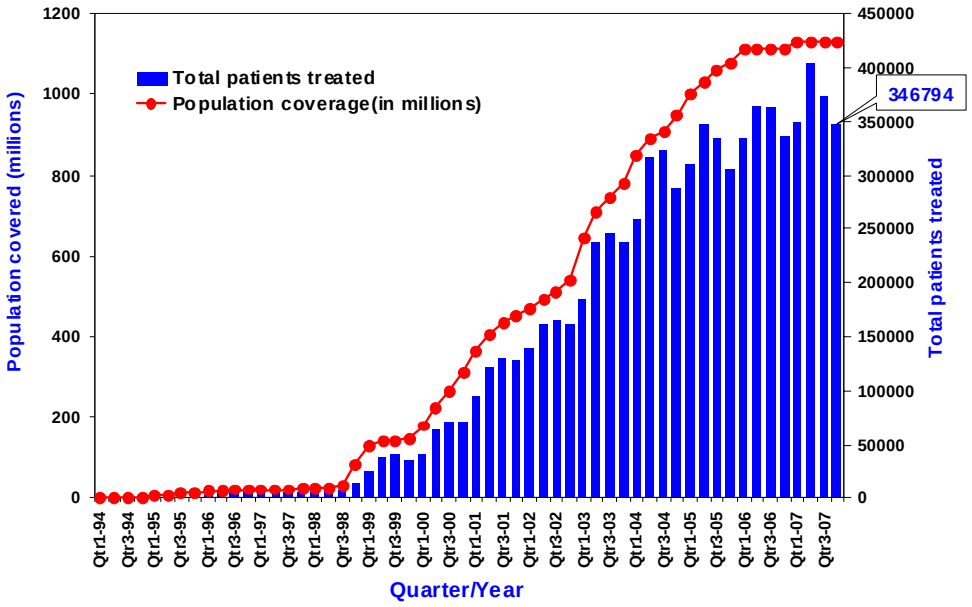

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Table: Performance of RNTCP Case Detection (2007 Fourth quarter), Smear Conversion (2007, Third quarter), and Treatment Outcome (2006, Fourth quarter)

State	Population (in lakh) covered by RNTCP[1]	Suspects examined per lakh population	No of Smear positive patients diagnosed[2]	Total patients registered for treatment[3]	Annualized total case detection rate	New smear positive patients registered for treatment	Annualized new smear positive case detection rate (%)		No of new smear negative cases registered for treatment	No of new EP cases registered for treament	No of smear positive retreatment cases registered for treatment	3 month conversion rate of new smear positive patients	Cure rate of new smear positive patients	Success rate of new smear positive patients
Andaman & Nicobar	4	233	75	185	184	52	52	69%	65	51	12	91%	85%	85%
Andhra Pradesh	813	166	18283	28350	139	12264	60	80%	8515	2573	3813	92%	86%	88%
Arunachal Pradesh	12	216	289	593	200	189	64	85%	160	89	84	88%	87%	89%
Assam	295	109	4922	8779	119	3847	52	70%	2440	956	886	91%	86%	87%
Bihar	923	74	9393	18143	79	6954	30	40%	6029	1281	2022	86%	76%	85%
Chandigarh	10	306	337	540	208	172	66	70%	115	138	69	92%	88%	88%
Chhatisgarh	233	125	3071	6725	116	2488	43	53%	2772	798	380	89%	83%	85%
D & N Haveli	3	156	52	90	141	29	45	57%	26	15	9	94%	87%	87%
Daman & Diu	2	422	50	81	177	24	52	66%	24	5	9	90%	91%	95%
Delhi	166	224	5016	9798	237	2783	67	71%	1798	2774	1478	90%	86%	86%
Goa	16	196	295	595	151	183	46	58%	148	152	57	89%	74%	74%
Gujarat	556	174	13946	19303	139	8413	61	76%	2770	2353	4131	92%	88%	88%
Haryana	234	157	4568	7494	128	2606	45	47%	1624	1136	1591	90%	85%	85%
Himachal Pradesh	65	216	1608	2698	166	946	58	61%	507	539	579	93%	88%	90%
Jammu & Kashmir	120	133	1410	2499	83	1063	36	37%	489	558	322	90%	89%	90%
Jharkhand	296	107	4783	8628	117	3834	52	69%	2719	635	750	91%	85%	90%
Karnataka	568	188	10138	16893	119	6331	45	59%	4034	3097	2399	84%	76%	78%
Kerala	339	189	3408	6238	74	2660	31	63%	1249	1512	661	85%	80%	83%
Lakshadweep	1	74	1	3	18	1	6	8%	2	0	0	100%	100%	100%
Madhya Pradesh	680	111	10808	19593	115	6982	41	51%	6492	2096	2888	88%	83%	86%
Maharashtra	1055	148	18811	34965	133	13132	50	62%	9306	5616	4016	90%	84%	86%
Manipur	26	150	322	1114	172	228	35	47%	454	225	63	87%	86%	86%
Meghalaya	25	134	500	1208	193	334	53	71%	259	325	147	86%	86%	87%
Mizoram	10	190	183	520	215	136	56	75%	136	164	41	97%	92%	92%
Nagaland	22	117	298	740	137	262	49	65%	197	121	90	90%	89%	91%
Orissa	395	134	6441	11706	119	5014	51	60%	3026	2046	980	87%	82%	86%
Puducherry	11	320	398	341	129	152	57	77%	53	86	48	91%	80%	80%
Punjab	263	139	4534	7586	115	2960	45	47%	1572	1357	1306	88%	82%	85%
Rajasthan	635	139	14515	24855	156	8638	54	68%	7783	2655	4703	92%	87%	89%
Sikkim	6	277	147	313	213	99	67	90%	59	87	45	91%	88%	88%
Tamil Nadu	658	220	11148	20790	126	7980	48	65%	5749	4202	2264	90%	83%	84%
Tripura	35	138	405	601	69	345	40	53%	102	92	48	87%	86%	92%
Uttar Pradesh	1874	131	33845	57918	124	23371	50	53%	18339	5390	8554	90%	84%	87%
Uttarakhand	94	162	1911	2840	121	1092	47	49%	707	406	527	92%	86%	87%
West Bengal	868	151	14389	24069	111	11241	52	69%	5026	3531	2734	90%	86%	87%
Grand Total	**11310**	**146**	**200300**	**346794**	**123**	**136805**	**48**	**48%**	**94746**	**47061**	**47706**	**90%**	**84%**	**86%**

1 Projected population based on census population of 2001 is used for calculation of case-detection rate. 1 lakh = 100,000 population

2 Smear positive patients diagnosed include new smear positive cases and smear positive retreatment cases

3 Total patients registered for treatment includes new sputum smear positive cases, new smear negative cases, new extra-pulmonary cases, smear positive retreatment cases and 'Others'

Other major initiatives

1. Internal evaluations undertaken by the state and national level during the fourth quarter have shown a need for continued intensive supervision and monitoring, and trainings of the field level staff. The states have been advised to follow-up on the recommendations of internal evaluation through periodic reviews and monitor action taken by the districts to improve performance. The programme emphasizes the need for supportive supervision and monitoring at all levels with continued 'zero tolerance' to falsification of data.

2. The national task force (NTF) CME-cum-workshop for the involvement of medical colleges was held at AIIMS, New Delhi from 29th to 31st October, 2007. The theme of the CME was 'Drug Resistant TB', and the "Chennai consensus meeting recommendations" on MDR & XDR TB (available on www.tbcindia.org) was endorsed by the NTF. During the meeting, it was re-emphasized, that prevention of drug resistant TB by implementing quality DOTS must remain the top-most priority. The implementation of the recommendations made during the workshop will further strengthen the involvement of medical colleges.

3. Trainings on Procurement and Drug Logistics Management were conducted for State Level officials (Dy.Dir./STOs/DTOs/ SDS-Pharmacists/MOs) by the Central TB Division. These trainings were held at the LRS Institute for the east zone on 4-5th October, north zone on 22-23rd November, South Zone – 11-12th December. With this the state level officials have been trained for the whole country. We urge that the State action plan to train the District level officials (DTO, DTC-Pharmacist and others) must be strictly adhered.

4. As part of EQA for sputum microscopy, the National Reference Laboratories (NRLs) undertook on-site evaluation visits to Chhattisgarh, Rajasthan, Goa, Kerala, Pondicherry and Mizoram. The central laboratory team visited to oversee the progress in culture and drug susceptibility testing facilities at Mahahrashtra, Rajasthan and Tamil Nadu. The Intermediate Reference Laboratory at Nagpur (Maharashtra) has completed proficiency testing with NTI and has achieved good concordance for Rifampicin and Isoniazid sensitivity testing. The IRLs of Gujarat, Andhra Pradesh and Delhi are under proficiency testing with their respective NRLs as a part of the ongoing accreditation process under RNTCP.

5. An implementation support mission of the World Bank and other donor agencies was undertaken from December 3-14, 2007. The mission was satisfied with the overall progress made by RNTCP and also in progress made in challenging areas like TB/HIV collaboration, collaboration with NRHM, PPM activities, monitoring and supervision. The mission stressed the need for more attention towards poor performing districts/ states which are still underperforming.

The programme has achieved the global targets of treatment success and is close to the global target for case detection. RNTCP is concerned about the high default rates and taking steps to address the issue. Partnership with the civil society organizations, private sectors and NGOs is one such step towards reducing default rates and providing supportive environment to the patients.

Case Report

ACCIDENTAL ISONIAZID POISONING - A REPORT

R.L. Agrawal[1], N.C. Dwivedi[2], Manju Agrawal[3], Sachin Jain[4] and Anand Agrawal[5]

(Article received on 20.2.2007. Accepted on 8.1.2008)

***Summary*:** Eight patients who had taken accidental overdose of Isoniazid were followed in relation to its clinical manifestations, EEG changes and management. All cases survived without any residual effect. **[*Indian J Tuberc 2008; 55: 94-96*]**

***Key Words*: I**soniazid, Neurological manifestations, Pyridoxin.

INTRODUCTION

Isoniazid is known to be least toxic, effective and economical so widely used as anti-tuberculosis drug and its common side effects are peripheral neuropathy[3], Toxic psychosis[5] and Hepatitis[4] with other anti-tuberculosis drugs. Its overdose taken either accidental or suicidal has been reported with wide clinical manifestations[6,8,11]. We report here eight patients who had taken overdose of Isoniazid accidentally with their clinical manifestations, EEG changes and its management.

CLINICAL RECORD

Accidental Isoniazid poisoning was reported among a group of eight male patients aged between 15 to 38 years admitted in chest ward, S.R.N. Hospital, Allahabad. This incidence took place when a nurse staff mistook 300 mg Isoniazid tablet as 100 mg Ethambutol. Thus the patient, who used to take 1200 mg Ethambutol, received 13 tablets equivalent to 3.9 gm of Isoniazid (13 x 300= 3900mg), including one tablet of Isoniazid, as their daily dose. Hence eight patients took a varying dose of Isoniazid from 2.1 gm to 3.9 gm as single dose for one day only.

A thorough physical examination was conducted and recorded. Necessary opinion of a neuro-physician was also obtained for management. Besides routine investigations certain special investigations such as liver function test, EEG changes etc., were carried out and repeated, whenever required.

Details of patients, who took overdose of Isoniazid, have been summarized in tabulated form. All patients had nausea or vomiting, vertigo, coma from grade I to grade III within half an hour to six hours after ingestion of Isoniazid. Total Isoniazid taken in dose varied from 2.1 gm to 3.9 gm as total or 64 to 83 mg (mean 74.2) /kg body weight. Only one patient developed intractable seizure while acidotic breathing was observed in two cases. Serum pH and estimation of Isoniazid level could not be done because of lack of facility. Inactivation status of patients could not be determined. EEG record of all patients showed abnormal generalised discharges as sharp and slow waves. Repeat EEG tracing was done after one month in all normal cases.

Gastric lavage, intravenous infusion and symptomatic treatment were given in all cases as general measures while dopamine infusion, intravenous diazepam, phenytoin, sodabicarb as intensive treatment was administered in two cases only. Recovery of patients' was uneventful.

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OBSERVATIONS IN GROUP OF CASES

Name	S.K.	N.C.	P.	R.N.	C.L.	L.	S.	B.P.
Age/sex	30, M	38,M	23,M	26,M	23,M	36,M	28,M	15,M
Dose of Isoniazid ingested in gm (mg/kg)	3.9 (81)	2.7 (79)	2.1 (66)	3.3 (83)	2.7 (71)	2.7 (73)	2.7 (77)	2.7 (64)
Symptoms first seen after Isoniazid ingestion (Hr.)	1/2	4	6	6	2	3	1/2	4
Symptoms Nausea/vomiting Vertigo Seizure Acidotic Breathing	 + + + +	 + + - -	 + + - -	 + + - +	 + + - -	 + + - -	 + + - -	 + + - -
Grades of coma	III	I	I	III	I	I	I	II
Sign Pupil reaction Tendon reflexes	Semidilated sluggish Diminished	 N Diminished	 N N	Semi-dilated sluggish Diminished	 N N	 N Diminished	 N Diminished	 N Diminished
L.F.T. E.E.G. Changes	WNL +	WNL +	WNL +	Not done +	WNL +	Not done +	WNL +	WNL +
Management (without Pyridoxin therapy)	Intensive care	Symptomatic	Symptomatic	Intensive care	Symptomatic	Symptomatic	Symptomatic	Symptomatic
Response after therapy (Hr.)	24	9	16	18	12	24	10	20

N= Normal, WNL = Within normal limit,+ =Present.

DISCUSSION

Accidental or intentional poisoning with Isoniazid may manifest within half to three hours[2] as intractable seizure, acidosis and coma[6,8,11]. Single high dose of pyridoxin was used as antidote to Isoniazid intoxication with good response[6,8,9,11]. Metabolic acidosis after acute Isoniazid poisoning results in cerebral oedema and coma. Seizure activity due to overdose of Isoniazid is believed to be related to decrease in GABA level in brain by the inhibiting effect of brain pyridoxal 5' phosphate, active form of vitamin B6[8,11].

Wason et al[11] reported five cases of their own with review of 41 cases from the literature. All their reported patients had seizure, coma and acidosis. Among this group of patients, all had vertigo and different stages of coma I to III , while two had acidotic breathing in which one had both acidosis and intractable seizure . Cent per cent seizure activity and acidosis in reported cases of Wason et al[11] may be explained by intake of very high dose of Isoniazid 100- 417 mg /kg body weight (mean 267). Prabhakaran[10] reported death with 0.5 gm of Isoniazid. Serious ill-effects were also observed by Ansari et al[1] after 0.6 gm of Isoniazid. Overall 7% mortality has been reported in the literature after its overdose.

No residual toxicity and neurological effects were recorded among present group of patients.

We regret for this mishappening and a sigh of relief was felt after recovery of all the patients. Action has been taken against the nurse staff.

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Case Report

PSORIASIFORM LUPUS VULGARIS

L. Padmavathy[1], L. Lakshmana Rao[2], N. Ethirajan[3] and M. Dhanlaklshmi[4]

(Article received on 24.7.2007. Revised version on 19.11. 2007. Accepted on 7.2.2008)

Summary: Tuberculosis is a major public health problem in both developing and developed countries. Cutaneous Tuberculosis constitutes a minor proportion of extra –pulmonary manifestations of Tuberculosis. Lupus Vulgaris (LV) is one of the clinical variants of Cutaneous Tuberculosis. A case of a large plaque type psoriasiform lesion of lupus vulgaris on the thigh, of 15 years' duration, in an 18-year-old girl is reported. This case highlights the ignorance level among the patients and consequent failure to avail proper anti-tuberculous treatment despite campaign in print and audio visual media.

[***Indian J Tuberc 2008; 55: 97-99***]

Key words: Lupus Vulgaris, Psoriasiform LV

INTRODUCTION

Tuberculosis, termed the "captain of the men of death" is a major public health problem. With effective anti-tuberculous therapy, there has been a decline in recent times. With the advent of HIV infection, there is a resurgence of tuberculosis. Cutaneous tuberculosis constitutes a minor proportion of extra-pulmonary Tuberculosis and Lupus Vulgaris is one of its clinical variants. A case of plaque type lesion of Lupus Vulgaris on the thigh in a 18-year-old girl is reported here. The ignorance of the patient and the chronic nature of the condition are highlighted.

CASE REPORT

A 12-year-old girl presented with the complaint of a hyperkeratotic verrucous plaque on the right gluteal region of nine years duration. There was a history of fever on and off, for a few months but no respiratory symptoms like cough. There was no contact history of tuberculosis among family members. She had chicken pox at three years of age and has since become totally blind in both eyes. Right eye showed a vascularised cornea and in left eye there was a corneal opacity.

Cutaneous examination revealed a 10" x 13" well defined indurated psoriasiform, scaly, plaque with areas of ulceration on the right thigh and gluteal region (Fig.1). She also had verruca vulgaris lesions on the left thigh. There was no inguinal lymphadenopathy. Before any further management, she absconded from the hospital and was untraceable for follow-up.

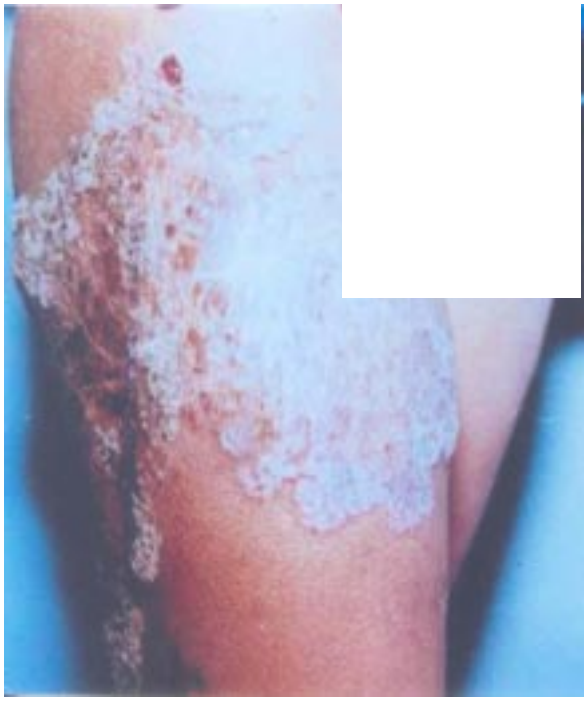

Fig.1: 10" x 13" well defined indurated psoriasiform, scaly, plaque with areas of ulceration on the right thigh and glufeal region

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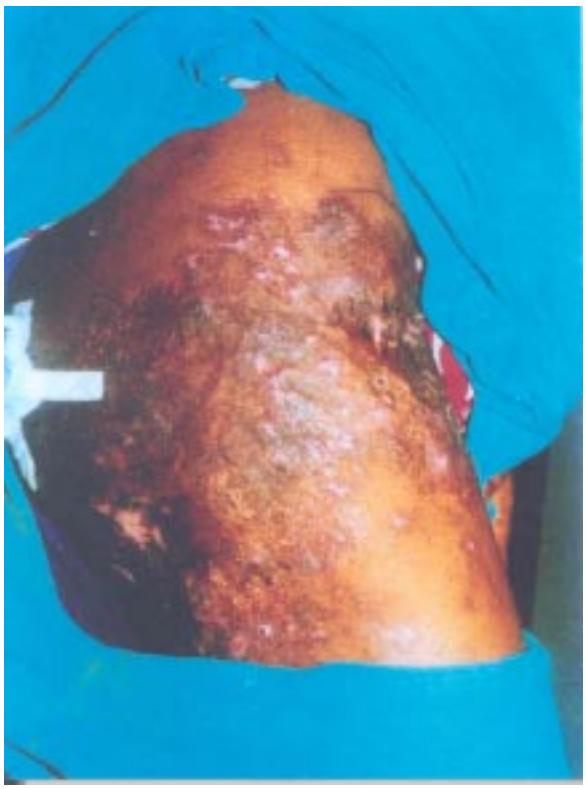

Fig. 2: Partially healed lesions with scars on the right thigh (after 6 years)

However, she resurfaced six years later, as an 18-year-old girl, with partially healed lesions scars on the right thigh and gluteal region. (Fig. 2). She also had lesions of scabies.

Except for an elevated ESR of 36 mm, her hematology and biochemical investigations were within normal limits. Roentgenogram of chest was normal. Other systems were normal clinically except for the eye changes. Mantoux test was positive. A biopsy from the gluteal region showed the typical non-caseating tuberculous granulomas composed of epithelioid cells, Langhan's giant cells and peripheral rim of lymphocytes. The epidermis showed hyperkeratosis, parakeratosis and acanthosis. Zero Tissue sections were negative for AFB by ZN stain.

Patient was started on standard anti-tuberculous therapy with category I drugs, i.e., INH 600mg, Rifampicin 450mg, Pyrazinamide 1500mg, Ethambutol 1200 mg thrice weekly and Pyridoxine 10mg, daily. The lesions began to heal well.

DISCUSSION

An ulceration that tore into the flesh, viz. was like the ravages of a wolf, probably fitted the clinical description and explains the word "lupus" " which means wolf. The commonness of this condition in earlier times accounts for the adjective - Vulgaris, in Lupus Vulgaris[1]. The earliest description of Lupus Vulgaris was by Erasmus Wilson in 1865. The other synonyms for this condition are "tuberculosis luposa" and "tuberculosis luposa cutis" [2].

Lupus Vulgaris is a progressive form of tuberculosis occurring in people with moderate or high degree of immunity and is more common in women[3]. The lesions of LV progress steadily and although spontaneous involution does occur, new lesions arise within old scars and without therapy complete healing is rarely observed[4]. The patient was unavaiible for follow-up for six years during which she had no anti-tuberculous treatment. However, there were symptoms of incomplete healing at her second visit clinically, while histopathology demonstrated tuberculous granulomas still.

The clinical variants of tuberculosis are many and include hypertrophic , plane, ulcerative, and scarring forms in addition to mucosal (nasal, oral and conjunctival) lesions[5,6]. Rarely psoriasiform mutilating ,vegetative and nodular lesions occur[3]. The patient had the psoriasiform lesions of LV, at her first visit.

Lupus Vulgaris originates from a tuberculous condition or a clinically inapparent focus elsewhere in the body by hematogenous, lymphatic or contiguous spread[4]. In view of the absence of a tuberculous focus, it could be presumed that in our patient , the primary focus is clinically inapparent as opined by Wolff and Tappeiner.

Lesions of Lupus Vulgaris are asymptomatic[4] which explains the long interval

between the initial and the latter consultations in our patient. In Western countries, face is reported to be the most common site of involvement[4] while in India, lower extremities, especially the buttocks are affected more commonly as in our patient. .The probable hypothesis is that the bacilli lying dormant for years are re-activated by trauma and non-specific inflammation[3].

Though tuberculosis is an easily treatable infective condition, the chronicity and the sequel as in our patient highlight lacunae in health delivery system. The importance of educating community about the nature of the disease and treatment options available, to achieve reduction in associated morbidity cannot be over emphasized.

ACKNOWLEDGEMENTS

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Case Report

OBSTRUCTING MASS LESION OF EPIGLOTTIS: IT CAN BE TUBERCULAR

Rajesh Gupta*, Sajay Fotedar, Pradeep Sansanwal[1], S.P.S. Yadav[2], Anupama Gupta[3], K.B. Gupta and Kuldeep Saini

(Received on 8.1.2008. Accepted on 4.3.2008)

***Summary*:** We report a case of 60-year old male who had difficulty in breathing as well as in swallowing. On examination, he was found to be having proliferative growth of epiglottis and right aryepiglottic fold mimicking neoplasm. So emergency tracheostomy was performed and biopsy taken. He was found to be having asymptomatic miliary mottling on routine x-ray chest PA view. Further on HRCT, it turned out to be lesion suggesting tubercular etiology. Histopathology (epiglottic biopsy) report confirmed the whole process as tubercular. The patient recovered promptly in due course with anti-tubercular treatment. Point remains to be seen that if we can avoid tracheostomy and its complications in such cases. **[*Indian J Tuberc 2008; 55: 100-103*]**

Key words: Epiglottis, Tuberculosis, Obstruction, Tracheostomy.

INTRODUCTION

Tuberculosis of larynx as such is rare.[1,2] However, tuberculosis of epiglottis presenting as mass lesion is an unusual phenomenon. It mimics and is frequently misdiagnosed as malignant laryngeal tumour especially in high risk patients i.e. old male, ch. Smoker.[3,4]

The commonest site involved in tuberculosis of larynx is posterior commissure; however, any part may be involved, including epiglottis. Epiglottic tuberculosis presenting with the respiratory tract obstruction is extremely rare.

On review of literature, few cases of laryngeal tubercular mass are reported.[1,2] Here, we present a case of epiglottitic growth presenting as acute airway obstruction needing tracheostomy, which subsequently was found to have tubercular etiology as a part of miliary tuberculosis.

CASE REPORT

A 60-year old male presented as an emergency case with difficulty in breathing as well as in swallowing with recent onset. On subsequent interrogation, he was found to be having hoarseness of voice for one year, cough with expectoration and decreased appetite for last two months. He denied any history of fever in near past. On examination, he was found to have proliferative growth of epiglottis and right aryepiglottic fold. Vocal cords appeared unaffected and showed normal mobility. The remaining findings on general physical and systemic examination were normal. He was a chronic smoker and non alcoholic. Patient was examined immediately and an emergency tracheostomy was performed. Simultaneous excision biopsy was taken and sent for histopathological examination.

Laboratory investigations were as follows: Hemoglobin 13 gm%, total leukocyte count 1200mm^3, polymorphs 75%, lymphocytes 24%, and eosinophils 2%. Other biochemical parameters were within normal range. Patient was non-reactive for serum HIV and sputum was negative for malignant cells on five occasions. Chest radiography showed miliary shadows in the bilateral lung fields (Fig. 1).

Pulmonology consultation was sought for lung lesions. HRCT of chest and neck was advised along with sputum for acid fast bacilli and Mantoux test.

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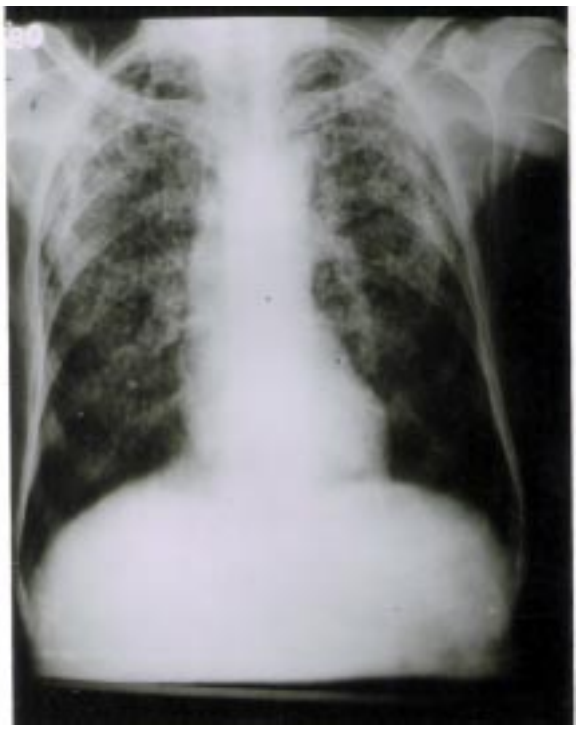

Fig. 1: Chest roentgenogram PA view showing bilateral miliary shadows.

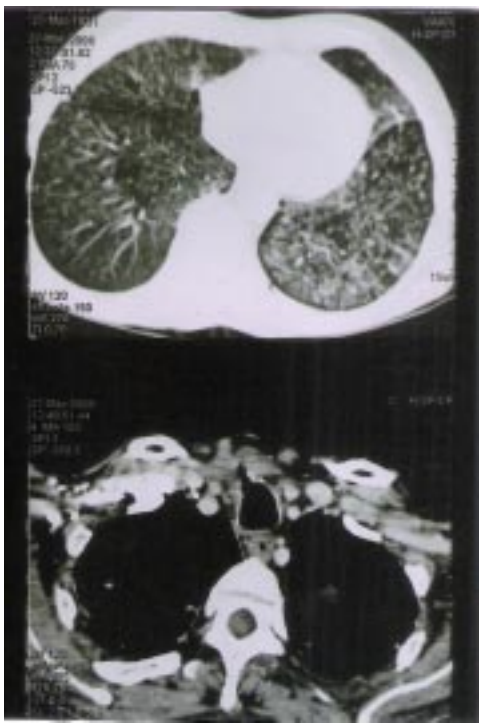

Fig. 2: HRCT of chest showing bilateral miliary shadows (top) and neck showing airway track of tracheostomy (bottom).

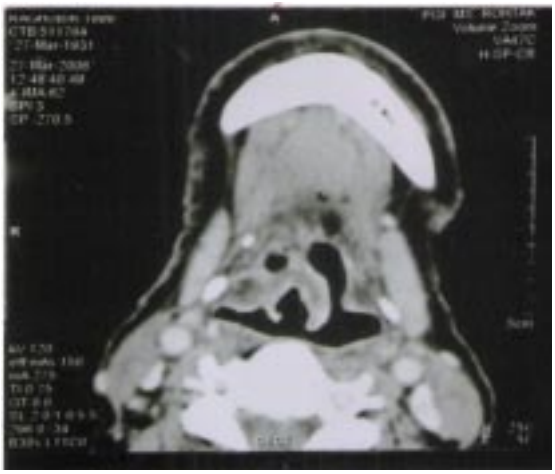

Fig. 3: HRCT of neck showing thickened epiglottis and right aryepiglottic fold.

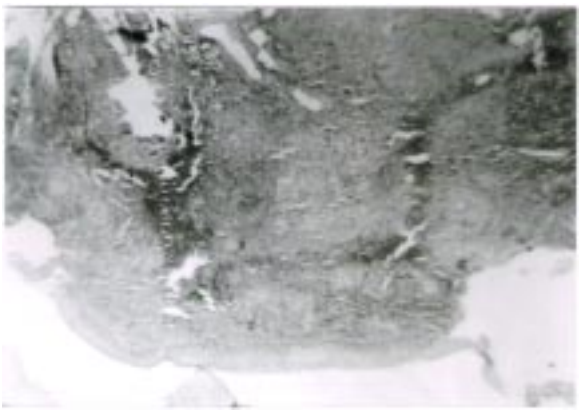

Fig. 4: Excision biopsy of epiglottis showing epitheliod giant cell granulomatous epiglottitis.

HRCT revealed bilateral miliary shadows (Fig. 2, top) with the involvement of epiglottis and right aryepiglottic fold (Fig. 3). Sputum for AFB was negative on three occasions and Mantoux test was positive -15mm. Histopathological examination revealed epithelioid cell and giant cell granulomatus epiglottitis consistent with tubercular etiology (Fig. 4). On the basis of clinical, radiological and histological findings, the diagnosis of miliary tuberculosis with epiglottic involvement was established. Patient improved markedly with anti-tubercular treatment and tracheostomy was decannulated in two weeks. Hoarseness subsided in three weeks.

DISCUSSION

The clinical presentations of patients with tuberculosis and carcinoma overlap to a significant extent and may be similar, i.e. including hoarseness, dysphagia, dypnoea and sore throat. Patient often gives history of weight loss, smoking and alcohol abuse.[2- 5]

Laryngeal tuberculosis can develop due to contaminated sputum, particularly in the interarytenoid region (direct spread). This leads to the formation of sub-mucosal tubercles which may later show caseation and ulcerate. This may also occur through hematogenous and lymphatic route with or without lung lesion.[6, 7]

Miliary tuberculosis results from massive hematogenous spread of tubercular bacilli. In children, it is often the consequence of recent primary infection, but in adults it may be either due to recent infection or re-activation of old disseminated foci. The unit lesion is usually yellowish granuloma 1 to 2mm in diameter that resembles the millet seed[8]. Miliary pattern on the chest radiograph is the hallmark of miliary tuberculosis. Sometimes it may manifest as coalescent opacities, presenting difficulty in the diagnosis. HRCT is useful in the assessment of such unusual presentation.[8-10]

HRCT is also proving to be useful tool in the diagnosis of various laryngeal lesions. Kim *et al* reported diffuse thickening of free margins of epiglottis, a characteristic and frequent finding in tuberculosis.[11]

In the modern chemotherapeutic era, incidence of laryngeal tuberculosis has decreased on the whole.[12,13] Soni and Chatterjee in a study of 500 patients of pulmonary tuberculosis reported the incidence of laryngeal involvement to be 4%.[14] In a series of 31 cases of tubercular laryngitis, Aggarwal and Bais described only one patient of tubercular epiglottitis[15]. Bull reported seven patients with laryngeal tuberculosis masquerading as carcinomas[16],and later, Coscaron et al reported one more case of misdiagnosed laryngeal tuberculosis[3], Smallman *et al* reviewed six cases of laryngeal tuberculosis in a 10-year period[17] ,stressing the point that the symptoms and signs often mimic carcinoma, chronic laryngitis or other granulomatous disease of the larynx.

For the diagnosis of granulomatous lesions of the epiglottis, tuberculosis, syphilis, sarcoidosis, Wegener's granulomatosis, cat-scratch disease, fungal infections (histoplasmosis, blastomycosis, coccidioidomycosis), and neoplastic lesions are to be considered as differentials. Cryptococcosis, actinomycosis, candidiasis, and leprosy are other chronic laryngeal infections.[18] Systemic diseases that affect the larynx are systemic lupus erythematosus, rheumatoid arthritis, relapsing polychondritis and amyloidosis should also be considered in the differential diagnosis.

The case has certain peculiar features of which foremost is mass lesion of epiglottis large enough to cause severe respiratory tract obstruction needing tracheostomy. The second is the presentation of severe form of tuberculosis i.e. miliary tuberculosis as obstructive epiglottic growth. The third is atypical nature of chronic disease in a 60-year old man, a chronic smoker, suggesting malignancy.

Though extremely rare, tuberculosis as a cause of upper respiratory obstruction should be kept in mind in epiglottic swellings and in cases presenting with hoarseness of voice and

painful dysphagia for early management. The Pulmonologists and other specialists should be aware of the tuberculosis of other organs of the body and the change in pattern of the disease. This may avoid operative intervention like tracheostomy and its complications.

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FORUM

Panel Discussion on MDR and XDR TB* (Moderator: Dr. V. K. Arora)

Moderator: DOTS was introduced in India in 1993 as a pilot project and large-scale expansion began in 1997, and by March 2006 whole country was covered under DOTS under Government of India's Revised National TB Control Programme (RNTCP). But at the same time CDC/WHO reported patterns of drug resistance to second line anti-TB drugs in addition to MDR-TB and termed it extensively drug resistant TB (XDR-TB). The potential destruction which can be caused by this virtually untreatable form of TB has been demonstrated in the KwaZulu Natal province of South Africa. In 2006, a deadly outbreak of XDR–TB occurred in the small town of Tugela Ferry in KwaZulu-Natal. Of 536 TB patients at the Church of Scotland Hospital, which serves a rural area with high HIV rates, some 221 patients were found to have MDR-TB and of these, 53 were diagnosed with XDR–TB. Fifty-two of these patients died almost instantaneously.

At a global meeting called by WHO in October 2006 XDR-TB was re-defined as MDR-TB with resistance to any one of the fluoroquinolones and one of the three injectable drugs (amikacin, kanamycin or capreomycin).

How much is the problem of MDR/XDR in India? Let us ask from our panelist, Dr. Singla.

Dr. R. Singla*:* There have been many studies about magnitude of MDR TB in India but methodology has not been uniform and laboratories are not accredited. For introduction of DOTS Plus in India, CTD decided to conduct DRS in six districts with uniform methodology and the data has revealed primary MDR upto 3% and acquired MDR upto 12%. In Gujarat, acquired drug resistance has been reported upto 17%. Regarding XDR, only TRC Laboratory is accredited for conducting susceptibility test against second line drugs. All other reports, like from Mumbai may not be reliable if lab is not reliable.

Moderator: *Now, let us know from Dr. Dhingra who has the experience of treating MDR TB, how much is the problem of MDR/XDR in their area?*

Dr. V. K. Dhingra: Review of our data from 2001-2006 shows that out of the 18,440 isolates examined 0.89% of all MDR cases were found to be XDR as per the latest definition, although our laboratory is not accredited.

Moderator: DRS surveys have been conducted in different parts of India. Let us ask Dr. Fraser Wares what is his view about the problem of MDR/XDR?

Dr. Wares : Globally it is estimated that in the year 2004, almost 4.25 lac MDR-TB cases emerged, out of which 87,000 were in India. Most of these cases are thought to have emerged outside of the RNTCP. However even a rate of 1% MDR-TB amongst new TB cases in India equates to a large number of cases in terms of absolute numbers. Thus the focus of activities should be on the prevention of the development of MDR-TB cases by bringing the maximum number of TB cases under the management of the RNTCP for treatment. Regarding XDR-TB, some data is expected soon on second line drug resistance patterns amongst those cases identified as having MDR-TB strains during the recent drug resistance surveillance survey in the State of Gujarat. Up to now, there is only one reliable report of an XDR-

The Panel Discussion was held during 62nd National Conference of TB Association of India (NATCON 2007) from 14th to 16th December 2007 in Delhi.

**This write up is based on the discussions held during the panel discussion, may not be the actual words spoken.*

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TB case from TRC, Chennai. It is certain that XDR-TB is here in India, it is just that we do not know the levels but it is felt to be relatively low.

Moderator: In conclusion 87,000 MDR cases, out of which 0.89 to 1.5% (8,000) XDR cases are expected in India. Is it a threat to TB Control Programme? Let us get the response from Dr. Prasad.

Dr. R. Prasad: In Lucknow, out of 68 MDR isolates, 5 (7.4%) were found to be of XDR TB. As MDR is a threat, XDR is also because management of TB is not proper in private sector. So if the MDR is not properly managed, it can become XDR TB which is a real threat.

Moderator: *What are your views on this point, Dr. Wares?*

Dr. Wares: If not a threat, it is definitely a challenge. Drug marketing surveys have shown that in the year 2006, 94 million dollars worth of ATT drugs were sold in India. 75% of this total was bought and used outside of the RNTCP, mainly in the private sector. The regimens used and TB management outside of the programme are often not best practice. There is no proper supervision and many patients do not complete treatment, leading to the potential of creation of MDR-TB. So prevention of MDR-TB is difficult until private sector health workers are fully involved in the programme's control activities.

In year 2006, around 8.4 million dollars worth of second line anti-TB drugs were sold and bought in India. Virtually 100% of this total was outside of the programme and given unsupervised. Hence it would appear that as of today in India, the vast majority of MDR-TB cases are treated outside of the programme, under poor management practices leading to the great potential for the development of XDR-TB cases, and making it a major public health challenge.

Moderator: *Are MDR and XDR strains equally infectious?*

Dr. Selvakumar: Definitely yes, especially in immuno-compromised persons. Evidence is in the form of South Africa outbreak where 51 out of 53 HIV patients develop XDR TB.

Moderator: By 2015, treatment access will be available to all MDR and XDR cases. Are we moving towards this target?

Dr. Wares: I think yes. The programme has plans to introduce DST and DOTS-Plus services across the whole country in the coming three years. By 2010, at least 24 State level laboratories will have culture and DST facilities up and running, linked to the establishment of at least 24 DOTS-Plus sites for the treatment of MDR-TB patients. But it is a huge challenge. The programme also wishes medical colleges and private hospital laboratories involved in its service provision to face this challenge. However, the major risk remains related to the treatment by many doctors of patients suffering from drug sensitive TB. If not treated properly, they can turn a disease which is curable with an inexpensive treatment to a disease which needs expensive treatment and with possible lower cure rates. In short, all health care workers who are providing treatment to a TB case have a public health responsibility to ensure adequate and complete treatment to ensure cure of such patients.

Moderator: So India's response has been on the positive side. Do you think it is so in clinician's point of view also?

Dr. Prasad: Yes, in the form of first DOTS and now DOTS-PLUS. But as clinician DOTS PLUS should be speeded up. Till DOTS-PLUS is introduced, these patients should be managed as per WHO guidelines and medical colleges, which are ready with their laboratories, should take up the responsibility of proper management of MDR cases.

Moderator: *What are the newer diagnostics tools for quick diagnosis?*

Dr.Selvakumar: At present there are 13,000 designated diagnostic centres where MDR/XDR TB cases are suspected. For them, 4 NRLs and 24 IRLs are not sufficient. Secondly, conventional DST takes long time leading to mortality in such

cases, so nucleic acid amplification tests should be used. Suspected samples should be transported from periphery to these specialized centers for diagnosis.

Moderator: In clinical practice, should we wait for DST report or start treatment on the basis of the previous history of ATT?

Dr. Singla: We cater two types of MDR cases. First is a Cat II failure case from the programme, where DST is available by the time patient completes Cat II treatment. So these patients can be put on DOT-PLUS regimen and followed up. Second type of cases is treatment failure cases outside the programme. There are two options for them. If patient is sick, the treatment schedule may be developed as per history of ATT and treatment started. Otherwise in such cases we can wait for DST results. If a patient can afford sensitivity by BACTEC system can be used.

Moderator*:* What about infection control policy measures for prevention of MDR cases?

Dr. Singla: Ideally segregation of such patients is the best. But practical it is difficult. Possible measures include cough hygiene, well ventilated wards, segregation of suspected cases of TB and HIV, and if possible engineering control in the form of special MDR wards with negative pressure exhaust and positive pressure ventilation, air exchanges, HEPA filters etc. In high risk areas such as operation theatres, bronchoscopy rooms etc. personal protection devices such as N 95 masks etc. should be considered.

Dr. Selvakumar: Infection control measures in the laboratory are also important for the protection of laboratory technicians in the form of proper ventilation and air expulsion through HIFA filters.

Moderator*:* What are the research projects you suggest for a resource constrained country like India?

Dr. Dhingra: Analysis of the available data of DST results of patients referred to a tertiary level laboratory.

Dr. Singla: Operational research whether two years daily DOTS or daily DOTS in intensive phase and intermittent DOTS in continuation phase.

Dr. Wares: Documentation of previous history of ATT in patients suspected of MDR or XDR TB to determine risk factors for the development of drug resistance.

Dr. Arora: Correlation of history of ATT *vs* DST among MDR cases.

Dr. Prasad: Modified DOTS PLUS with decreased supervision, especially during continuation phase, and less number of cultures during whole treatment period.

Dr. Selvakumar: Role of direct drug sensitivity for rifampicin on LJ Media.

V. K. Arora and K. K. Chopra

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Annexure

- *All the health care providers managing MDR TB cases need to adhere to the following guidelines issued as consensus statement on the problem, prevention and management of MDR and XDR TB cases by Central TB Division on the basis of consultative meeting in Chennai held in Sept 2007.*
- *Drug resistance may be suspected based on history of prior treatment (e.g. smear positive case after repeated treatment courses, Cat II failure etc.) and/or close exposure to a possible source case confirmed to have drug resistant TB.*
- *Drug susceptibility test results of the first line anti – TB drugs pyrazinamide, streptomycin and ethambutol should be interpreted with caution due to the poor reproducibility of these results even under optimal conditions.*
- *Drug susceptibility test (DST) results of the second line anti – TB drugs should be interpreted with caution due to limited capacity of laboratories, absence of quality assurance, and lack of standardized methodology.*
- *Preferably the standardized regimen as recommended in the national DOTS-Plus guidelines should be used [6(9) Km Ofx Eto Cs Z E/ 18 Ofx Cs Eto E]*
- *At least six months of Intensive Phase (IP) should be given followed by 18 months of Continuation Phase (CP).*
- *Smear examination should be conducted monthly during IP and at least quarterly during CP.*
- *Culture examination should be done at least at 4, 6, 12, 18, and 24 months of treatment.*
- *To reduce the risk of development of resistance to second line anti – TB drugs and promote optimal treatment outcomes, all efforts should be made to administer treatment under direct observation (DOT) over the entire course of treatment.*
- *Health care facilities/practitioners managing MDR-TB patients should maintain a systemic regimen, doses, duration, side effects, investigation results and treatment outcome for all patients initiated on second-line treatment.*

Status of smear positive TB patients after two-three years of DOTS iniiation.

Sadacharam and colleague[1] in their study have found mortality in 15% and relapse in 19% patients smear positive tuberculosis at two-three years after successful treatment under DOTS programme. It is imperative for a much needed study to assess the long term success rate of DOTS in India. We are concerned for the cause of higher long term mortality rate and relapse among successfully treated patients.

The messages derived from the present study might merit exploration in tuberculosis programme evaluation and improvement. Chang and co-workers[2] have reviewed 12,946 tuberculosis cases seen from 1998 to 2000 in Hong Kong, who had completed therapy within 12 months. Among these, they identified 113 (0.93%) cases that relapsed within 30 months, and each matched to two control subjects by sex, age, year of treatment initiation, clinic at which they were treated, and baseline sputum culture result. In conditional logistic regression analysis, relapse was statistically significantly associated with thrice-weekly (versus daily) therapy, and with the presence of cavitation at diagnosis. Prolongation of therapy significantly lowered the estimated odds of relapse[2]. Programme needs to evaluate the value of reductions in relapse and late mortality cases.

Can higher mortality be attributed to long diagnostic delays? Tuberculosis relapse and mortality can be increased if disease duration has been sufficient for cavity formation with associated lung damage, and chemotherapy cannot overcome this damage. We feel the programme needs to consider some changes in approach of individualization of regimens depends on associated factors like diabetes, extensive disease or cavitation who are slow to respond to anti-tubercular treatment[3]. The extensive disease may necessitate additional drugs/duration of treatment. Again these individuals must not discourage us from use of DOTS, but should remind us that sophisticated management based on case-specific situation is still needed. We are learning about the advances of host and bacterial genomics, that individuals may differ in their responses to therapy, and that these differences may teach us the implications for individualization of treatment. We think the time has come for considering the augmentation of Category-I regimen in special circumstances.

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Importance of Address and Contact Details in Extra-pulmonary Samples Suspected for Mycobacterial Infections.

Extra-pulmonary tuberculosis causes a lot of morbidity and mortality. The RNTCP does not include extra-pulmonary samples for diagnosis. However, once a patient is diagnosed of extra-pulmonary tuberculosis, they are enrolled into the RNTCP programme for treatment.

In our hospital we receive various samples like FNAC, pus, urine, gastric lavage, pleural fluid, ascetic fluid, tissues, stool, CSF. In our laboratory from February 2006- October 2007, we received a total of 4,403 samples. Of these, 3,908 were negative and 495 were positive by the Ziehl Neelsen (ZN) staining technique. In our laboratory, we receive samples from both indoor and OPD patients. The reports of indoor patients are despatched on second day of receiving the samples to the respective wards. However, OPD patients have to visit the laboratory to collect their reports. During this period, it was observed that a total of 607 reports (13.79%) were not collected by the patients or their relatives. Out of these 10.71% were positives reports.

These patients never came to know about their positive status. Smear sensitivity is very low in extra-pulmonary tuberculosis[1]. Therefore, if 10.71% of smear positive extra-pulmonary cases were just lost to treatment as they did not turn up to

	Samples	Received		Reports	Not	Collected
	Total	Neg (%)	Pos (%)	Total	Neg (%)	Pos (%)
Feb-Dec 2006	2245	1954 (87.04%)	291 (12.96%)	331 (14.74%)	295 (15.10%)	36 (12.37%)
Jan-Oct 2007	2158	1954 (90.55%)	204 (9.45%)	276 (12.79%)	259 (13.25%)	17 (8.33%)
Total follow-up	4403	3908 (88.76)	495 (11.24%)	607 (13.79%)	554 (14.18%)	53 (10.71%)

collect their reports, then such a problem needs to be addressed effectively. Smear positive pulmonary cases attract public health importance as they are infectious to others. RNTCP is based on microscopic investigation of pulmonary samples. There is no provision at present to include extra-pulmonary samples for diagnosis. Considering the high morbidity and mortality rate due to extra-pulmonary tuberculosis and with global pandemic of HIV/ AIDS, there is utmost importance to register patients suspected of extra-pulmonary tuberculosis with their complete address and contact details when the samples are sent for investigations. At present such an exercise is lacking in our hospital. Dedicated staff should be appointed to register such suspected patients and follow-up their track.

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uBox

The Bihar Tuberculosis Association, in association with The Prajnopaya Foundation, a charitable organization, had organized a four-day training programme at Dhanarua, District Patna from 7th to 10th January, 2008. During the period, a six-member team of students and researchers from Massachusets Institute of Technology, USA, had visited to demonstrate their innovations - uBox (an intelligent pill dispenser and data collection device) and uPhone (a cell phone based data collection device).

The Bihar TB Association says that usage of these innovative devices will have a great long-term impact on TB treatment delivery. The Bihar TB Association intends to launch the first pilot phase of this programme in March 2008 in the districts of Madhubani, Samastipur and Nawada and later in whole of Bihar.

uBox: This is easy to smart pill box. Drugs can be stored in this box for a particular number of days and the box is then closed. An electronic chip will subsequently monitor release of dose on each occasion and is capable of recording date and time of delivery of dose and can also remind DOTS worker when next dose is due. Thus this will help in ensuring compliance, adherence and tracking defaults.

uPhone: This is a cell phone based medical collection device and maintains the medical date of patients and adherence record. Thus it can be used to monitor individual patients' medical history.

Editor's Comment:

Both of these devices claim to improve patient compliance and address default. However, their applicability under field conditions and their replicability and scalability still need to be demonstrated. **Representatives of Central TB Division, Directorate General of Health Services, New Delhi, also attended this demonstration. Their opinion is that these devices would further burden DOTS provider with added work and would escalate cost of service delivery and thus may not be feasible for the programme.**

ABSTRACTS

High resolution CT in miliary tuberculosis of the lung : Correlation with Pulmonary Function Tests and Gas Exchange Parameters in North Indian patients

S.N.J. Pipavath, S.K. Sharma, S. Sinha, S. Mukhopadhyay and M.S. Gulati. *Indian J Med Res* 2007; **126**: 193-198.

High resolution computed tomography (HRCT) scans are known to be helpful in early diagnosis and management of patients with miliary tuberculosis (MTB). We made an attempt in this study to identify patterns of pulmonary MTB on HRCT and to correlate the HRCT disease extent with pulmonary function tests (PFT) and gas exchange analysis (GEA). A total of 16 non-HIV patients with MTB underwent HRCT of the chest, PFT and GEA. All the investigations in these patients were completed within 20 days of presentation. Evidence of TB was diagnosed by biopsy from lymph nodes (3/16), organ biopsy [skin, liver, bone marrow and lung (transbronchial) (6/16)]. In one patient, fundoscopy revealed choroid tubercles. In six patients, diagnosis was confirmed by clinical/ radiological improvement following anti-tuberculosis therapy. Radiological patterns of involvement on HRCT of the lungs were studied and disease extent was estimated in each case by consensus between two radiologists using specially devised visual scoring system. Disease extent was correlated with PFT and GEA. Spearman rank correlation was used for statistical analysis.

Findings on HRCT in MTB included miliary nodularity (16/16), alveolar lesions such as ground glass attenuation and/or consolidation (5/16), lymphadenopathy (8/16), peribronchovascular interstitial thickening (1/16), emphysema (1/16), pleural pathology (2/16), and pericardial effusion (2/16). A significant correlation was noted between disease extent score and forced vital capacity (FVC) (r = -0.76; *P=0.003)*, forced expiratory volume in one second (FEV_1(r = -0.74; *P* = 0.005), total lung capacity (TLC) (r = -0.66; *P* = 0.037), oxygen saturation in arterial blood (SaO_2 (r = -0.69, *P* = 0.01), diffusion capacity of the lung (DLco) (r = -0.8; *P* = 0.02). Our findings showed that HRCT reliably diagnosed MTB, and thus could help in predicting derangement of pulmonary function tests and GEA in these patients.

Sputum Conversion at the end of intensive phase of Category I treatment of Pulmonary Tuberculosis patients with Diabetes Mellitus or HIV infection: An analysis of risk factors

V.V. Banu Rekha, Rani Balasubramanian, Soumya Swaminathan, Rajeswari Ramachandran, Fathima Rahman, V. Sundaram, K. Thyagarajan, N. Selvakumar, A.R. Adhilakshmi, Sheik Iliayas and P.R. Narayanan. *Indian J Med Res* 2007; **126:** 452-458.

New smear-positive pulmonary tuberculosis (PTB) patients in the Revised National Tuberculosis Control Programme (RNTCP) are treated with a six-month short-course chemotherapy (SCC) regimen irrespective of co-morbid conditions. We undertook this retrospective analysis to compare sputum conversion rates (smear, culture) at the end of intensive phase (IP) of Category-I regimen among patients admitted to concurrent controlled clinical trials: pulmonary tuberculosis alone (PTB) or with type 2 diabetes mellitus (DM-TB) or HIV infection (HIV-TB), and to identify the risk factors influencing sputum conversion.

In this retrospective analysis sputum conversion rates at the end of intensive phase (IP) in three concurrent studies undertaken among PTB, DM-TB and HIV-TB patients, during 1998 - 2002 at the Tuberculosis Research Centre (TRC), Chennai, were compared. Sputum smears were examined by fluorescent microscopy. HIV infected patients did not receive anti-retroviral treatment (ART). Patients with DM were treated with oral hypoglycaemic drugs or insulin (sc). The study population included 98, 92 and 88 patients in the PTB, DM-TB and HIV-TB studies. At the end of IP,

the smear conversion (58, 61, and 62%) and culture conversion (86, 88 and 92%) rates were similar in the three groups respectively. The variables associated with lack of sputum smear or culture conversion were age >45 yr, higher pre-treatment smear and culture grading, and extent of the radiographic involvement.

Comparative study of Bactec MGIT 960 AST and conventional proportion method using Ogawa medium for the drug susceptibility testing of *Mycobacterium Tuberculosis* to Isoniazid.
Satoshi Mitarai et al. *Kekkaku* 2007; **82**: 449-454

The aim was to evaluate the accuracy of drug susceptibility testing to Isoniazid with BACTEC MGIT 960 (MGIT AST) comparing with the standard proportion method using Ogawa medium. A total of 1,109 *M. tuberculosis* strains, which were selected from the collection of RYOKEN drug resistance survey in 2002, were selected and subjected to the susceptibility testing to Isoniazid using MGIT AST and 1% Ogawa standard methods. The results from MGIT AST were compared with the judicial diagnosis by Ogawa. The sensitivity to detect drug resistance, the specificity for susceptible strain, the efficiency of overall agreement, and kappa co-efficient were calculated to evaluate the performance. The treatment process, outcome and prognosis were analysed for the patients on whom the tests showed discrepant results.

Compared with the judicial results, the sensitivity, specificity, efficiency, and kappa co-efficient of MGIT AST were 100%, 97.1%, 97.3%, and 0.798, respectively. The strains, which showed discrepant results between MGIT AST and Ogawa, were all susceptible by Ogawa and resistant by MGIT AST. A total of 11 out of 30 discrepant cases were followed clinically and no relapse cases were identified, irrespective of the modification of the treatment regimen. As for the proportion of primary INH drug resistance in the present study, it was 5.3% with MGIT AST but was 2.7% with Ogawa, and the difference was statistically significant (p= 0.005).

The discrepancies on the results of drug susceptibility testing of *M. tuberculosis* strains to Isoniazid between MGIT AST and 1% Ogawa proportion method have been reported. In the present study, the sensitivity, specificity, and overall efficiency of MGIT AST on the prevalent strains in Japan were all beyond 95%, and considered sufficient as the anti-tuberculosis drug susceptibility testing (AST), though 2.7% of discrepancy was observed: Even for the discrepant cases, there was no difference in the treatment outcome and prognosis. Thus, MGIT AST was confirmed as a reliable AST method comparable to Ogawa standard. However, MGIT AST might increase the proportion of INH resistance if it was used as a major AST method, compared with Ogawa.

Features of Bronchial Tuberculosis
Atusuhisa Tamura et al. *Kekkaku* 2007; **82**: 647-654

The aim of this study was to clarify the features of bronchial tuberculosis. We analyzed the clinico-pathological data from 103 out of 4467 (2.3%) cases of culture positive tuberculosis admitted from 1993 to 2004 in which bronchial tuberculosis was confirmed by bronchofiberscopy.

There were 62 women and 41 men, and 53 of them were less than 50 years old. The most common symptom, namely cough, was observed in 70 cases, while 79 cases showed III 1 to III 2 on roentgenographic examination, and 81 cases were smear-positive for acid-fast bacilli in the sputum. Regarding the bronchofiberscopic findings, ulcers were detected in 60 cases, and the major site of bronchial tuberculosis was in the left main bronchus (35 cases). The number of cases in which the time span from the onset of symptoms to diagnosis took over three months was 29, and 26 of them were "doctor's delay" cases which had a history of medical consultation resulting in diagnosis and treatment of other diseases, such as bronchial asthma (seven cases). There were 41 cases in which the second bronchofiberscopic findings have been reviewed, and regardless of the length of the span from the onset to diagnosis, the first bronchofiberscopy mostly revealed ulcer within one month after the start of treatment for tuberculosis,

and three months after the start of treatment, many patients developed fibrous scars. Between 1999 to 2004, the first bronchofiberscopies were usually performed within two weeks to one month after the start of the treatment in contrast to the cases admitted between 1993 to 1998 in which bronchofribroscopy was mainly performed before the start of the treatment. However, there were no differences in the findings due to the timing of bronchofiberscopy.

The clinical characteristics of bronchial tuberculosis have not changed, and the delay of diagnosis of bronchial tuberculosis due to doctor's delay also continues to be an important issue today. In patients showing positive sputum smear for mycobacteria, the timing of brohchofiber-scopy, although required upon medical examination, is considered to be more appropriately performed from two weeks to one month after the start of treatment from the view point of nosocomial tuberculosis infection control strategy.

Essay Competition For Medical Students-2008

The Tuberculosis Association of India awards every year a cash prize of Rs. 500/- to a final year medical student in India for an original essay on tuberculosis. The subject selected for the year 2008 competition is '**Smoking and Tuberculosis**'. The essay should be written in English, typed double spaced, on foolscap size paper and should not exceed 15 pages (approximately 3,000 words, including tables, diagrams, etc.). Four copies of the typescript should be forwarded through the Dean or Principal of a College/University to reach the Secretary-General, Tuberculosis Association of India, 3 Red Cross Road, New Delhi-110 001, before 30th June, 2008 along with a certificate that the author is a final year medical student.

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