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Editorial

SMOKING AND TUBERCULOSIS

[*Indian J Tuberc 2012; 59: 125-129*]

Tobacco smoking and Tuberculosis are the two major health problems, especially in developing countries. As per estimates, deaths from tobacco consumption will be around 8.4 million in 2020, almost double to that estimated in 1990.[1] These mortality figures are not only contributed by pulmonary diseases like lung cancer and chronic obstructive pulmonary disease but cardiovascular diseases like stroke and coronary heart disease are also attributable.

The shift in smoking paradigm from industrialized world to developing world coincides with the increased prevalence of Tuberculosis in these regions. About 17% of smoking population lives in India.[2,3] At least one-third of the smokers belong to middle age group, and as per surveys, it is in this age group pulmonary tuberculosis is most prevalent. Males are affected two-four times more than females.[4]

In 1992, about a third of world population was infected with *Mycobacterium tuberculosis*, the World Health Organization (WHO) declared tuberculosis a 'global emergency'.[5] The progression of infection of *Mycobacterium tuberculosis* to active disease is strongly associated with human immunodeficiency virus (HIV) co-infection.[6] With the emergence of multidrug-resistance tuberculosis, a different set of challenges and problems for clinicians and public health programme has set in.

Smoking unfortunately has been received as social acceptance, despite it being a major health hazard. The socio-economic conditions, including poverty, overcrowding, poor ventilation and rooms with no natural light, poor nutrition and alcohol abuse have been associated with smoking and are also known risk factors for tuberculosis infection. Potentially, smoking is one of the most modifiable exposures, but smoking alone as a contributor for morbidity and mortality due to tuberculosis has not been very well validated.

Importantly, it is not only the smoker who is at the risk of tuberculosis, but also studies have validated the role of passive smoking, second hand smoke and environmental tobacco smoke exposure (ETS)as a contributory factor for active tuberculosis.[7,8]

Tuberculosis and Epidemiological Triad

The risk factors for tuberculosis consist of epidemiological triad of agent, host and environment. The agent being the tubercle bacilli, a susceptible person as a host and an environment which allows the bacilli to survive and transfer from one host to another. Smoking can act as a risk factor by increasing the susceptibility of human host. Smoking causes cough in the patient which allows the transit of tubercle bacilli into environment from infectious host. The bacilli are then inhaled by another susceptible host leading to pulmonary tuberculosis.

Smoker's lung: Pathology and Pathophysiology

Smoking almost affects all the organs in human body, the maximal damage is borne by the lungs. Smoking is a very well recognized causative factor for lung carcinoma and chronic obstructive pulmonary disease. The studies have shown that common cold, bacterial pneumonia, spontaneous pneumothorax, interstitial lung diseases (RB-ILD, IPF), pulmonary haemorrhagic syndrome have either incidence or severity definitely or possibly increased by smoking.[9]

Smoking impairs the host immune defences right from the beginning. Smoking impairs the clearance of secretions present on tracheobronchial mucosa. This is the first line of defence which aids in clearance of inhaled particles. Thus, by impairing the clearance, it allows the tubercle bacilli to escape the defence and propels it to reach alveoli.[10] Pulmonary alveolar macrophages constitute early defence mechanism against the tubercle bacilli. Studies have shown that smoking alters the function of these macrophages and thus impairing their ability to clear the bacilli from airways. The macrophages from smokers were bigger in size, had abnormal surface morphology which led to impaired antigen-presenting function.[11] The alveolar macrophages of smokers had reduced phagocytic activity and low levels of pro-inflammatory cytokines.[12]

There is evidence of imbalance between oxidant and anti-oxidant levels in smokers. This imbalance leads to increased oxidative stress on lung tissues primarily by oxidants contained in cigarette smoke and the decreased anti-oxidant mechanism of aging macrophages.[9]

Recently, a novel mechanism suggesting the role of nicotinic cholinergic receptors on macrophages has been proposed. Nicotine in cigarette smoke acts on these receptors and decrease the production of tumour necrosis factor, thus impairing the killing ability of macrophages.[13]

A prospective trial suggested that smoking accelerates the decline in lung function and cessation of smoking reduces the rate of decline. In a population, women are more susceptible to decline in lung function caused by smoking.[14] Smoking during pregnancy leads to poorer lung function in children as compared to non-smoking pregnant mothers.[15]

The above mentioned interactions among smoking and pulmonary host defence support a causal association between smoking and the increased risk of acquiring tuberculosis or clinical progression from infection to disease.

Smoking and Tuberculosis infection (Tuberculin test)

The infection of tubercle bacilli is characterized by tuberculin reactivity (despite the known factors affecting the test like cross reactivity and exposure to non-tuberculous bacteria).

Studies have shown conflicting results on whether tuberculin reactivity is affected by smoking. One of the earliest studies in this regard by Kuemmerer and Comstock in 1967[16] noted that tuberculin reactions were greater in families where both parents were smokers. The assessment of other socio-economic factors suggested that education, overcrowding, immigration and residence in urban places were significantly associated with positivity of tuberculin test.

A study in 1993 by Nisar and colleagues[17] showed that smoking was associated with increased Heaf grade reaction. The reaction was stronger in current smokers than in ex-smokers. The results

of this study also showed stronger reaction of smokers (current and ex) when compared against non-smokers.

Den boon *et al*[18] conducted his study in socially homogenous group in high prevalent tuberculosis areas. His results showed that tuberculin reactivity is related to smoking, especially if cumulative dose is of more than 15 pack-years.

Smoking and Tuberculosis disease

The first ever study for association in active smoking and pulmonary tuberculosis was done in UK by Lowe *et al*[19] in 1956. This was a case-control study and the results suggested that in patients above 30 years of age with pulmonary tuberculosis of both sexes had a 'highly significant deficiency of non-smokers and light smokers and an excess of moderate and heavy smokers'.

In 1966, Don and Hill reported an observation 'the relationship between smoking and mortality from pulmonary tuberculosis is distinct, but with a disease so influenced by social factors, more precise data are needed to justify a direct cause and effect hypothesis.' This hypothesis prompted Adelstein and Rimington in 1967 to design a longitudinal study analysing pulmonary tuberculosis and smoking on the basis of mass miniature radiography.[20] The results of the study showed that rate of tuberculosis rises in accordance with the number of cigarettes smoked.

Other studies[21,5] also showed the higher relative risk of tuberculosis in smokers and also a more severe and disseminated pattern of tuberculosis in smokers.[22]

Recently, a study in India by Kolappan and Gopi[23] concluded a positive association between pulmonary tuberculosis and tobacco smoking. The study also showed a strong dose-response relationship with smoking of >20 cigarettes/day having a very high odds ratio.

Passive smoking and Tuberculosis

Passive smoking was not, in general, associated with *M. tuberculosis* infection. However, recent studies have indicated the role of passive smoking and environmental tobacco smoke (ETS) exposure as a risk factor for tuberculosis.

The study by den Boon *et al*[7] in South Africa suggested that passive smoking may increase the risk of acquiring tuberculosis infection, given household infection to adult index case. The results were alarming as in developing countries with high burden of tuberculosis; there is rapid increase in smoking prevalence in household.

A prospective cohort study in Hong Kong by Leung *et al*[24] demonstrated a significant and independent association between passive smoking and pulmonary tuberculosis.

A study[25] in India reported a relative risk of 2.68 (95% CI: 1.52-4.71) for *M. tuberculosis* infection in children less than five years of age who lived in a household with an index case of pulmonary tuberculosis and were exposed to ETS compared with children not exposed to ETS.

The pathological and physiological mechanisms of lung injury caused by passive smoking are similar to that caused by active smoking.

Conclusion

A review of literature suggests that smoking should be considered as an important risk factor for development of tuberculosis. There is enough evidence that shows a direct relationship between active smoking and incidence of pulmonary tuberculosis. Some studies even show a dose-response curve for the number of cigarettes smoked to risk of developing pulmonary tuberculosis. Exposure to ETS in children was found to be associated with an increased risk of developing pulmonary tuberculosis immediately following infection. Greater tuberculin reactivity in a dose dependent manner was observed amongst current smokers.

Cough in smokers is attributed to smoking and this often results in a diagnostic delay in a patient with tuberculosis who smokes. Further prospective studies are required to strengthen the available data and also highlight this noxious association.

Smoking in any form should strongly be discouraged right from teenage years so as to avoid nicotine addiction and other harmful effects of smoking.

Raj Kumar* and D. Behera**

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Editorial

NEW STRATEGIES OF TB CONTROL IN INDIA : ARE WE ON THE RIGHT TRACK?

[*Indian J Tuberc 2012; 59: 130-134*]

The control of tuberculosis still remains elusive as the epidemic of tuberculosis (TB), fuelled by human immunodeficiency virus (HIV) co-infection and increased in resistance to currently available anti-mycobacterial drugs, continues to play havoc in many countries, particularly low-income countries. India is the highest TB burden country with an annual incidence of 2.3 (2.0-2.5) million cases at 185 (167 – 205) per 100,000 population and an estimated prevalence of 3.1 (2.0-4.6) million cases with a rate of 256 (161-373) per 100,000 population, even if the figures are yet to be correctly calculated.[1] The number of annual deaths is estimated at 3,20,000 (210,000-470,000) at a rate of 26 (17-39) per 100,000 population. Nearly 2.31 million population in India are living with HIV, with ~ 0.9 million co-infected and ~5% of TB patients estimated to be HIV + , although the figures vary from region to region. Around 3% of all new cases and between 12-17% of re-treatment cases of tuberculosis in India are MDR-TB and there are reports of XDR-TB occurring here and there with a high prevalence of fluoroquinolone resistance reported from many centres.[2-5] Although tremendous improvement has occurred in the control of TB in India, much more is desired to be achieved. Since the inception of Revised National Tuberculosis Control Programme (RNTCP), more than 44 million suspects have been examined, more than 13 million patients have been put on treatment and more than 2.3 million lives have been saved.[2] Stop TB Strategy is one of the key recommendations of the World Health Organization for expansion and enhancement of TB control programmes for both drug-susceptible and drug-resistant disease and better delivery of HIV management in collaboration with TB programmes. Besides TB-HIV collaboration, management of drug resistant TB, TB in children and elderly, participation of NGOs including medical colleges and operational research in some of the crucial areas. The stop TB strategy advocates pursuing quality DOTS expansion and enhancement, addressing TB/HIV and MDR-TB, contributing to health system strengthening, engaging all care providers, empowering patients and communities and enabling and promoting research (diagnosis, treatment, vaccine). These developments are crucially dependent on research on new tools[6] and will require political commitment, funding support and collaboration among experts to expedite the process.[7]

The Central TB Division has revised its strategy from the current 85/70 to 90/90 during the 12th five year plan wherein it envisages to record at least 90% of all TB cases and to have a success rate of at least 90% of cases. The greatest challenge for the programme will be to tap the vast yet uncontrolled private sector. It is the need of the hour that all TB cases be notifiable that need to be followed by everyone. Other high risk groups like subjects with diabetes mellitus, immunosuppressed individuals like HIV, malignancy, subjects taking anti-cancer chemotherapy and other immunosuppressive drugs, elderly individuals, patients who had history of tuberculosis in the past or those who have evidence of old-healing scars on chest skiagram, certain occupations, subjects detected to have radiological abnormalities while investigated for other reasons, smokers, patients with chronic renal failure, chronic liver failure, malnutrition, close contacts of tuberculosis including family members, alcoholics are some of the new suspects for tuberculosis. Besides, subjects with PUO, unexplained weight loss, suspected extra-pulmonary tuberculosis need to be brought to the fold of suspects if we have to increase our case detection. Although all these new suspects may not add a very large number to the currently adopted strategy of two weeks' cough, no doubt they will increase case detection. However, it should be taken into account that this will add hugely to the existing infrastructure, which is worth trying if we have to detect more cases. Of course, the strategy needs proof whether it is really going to help through operational research. The other important

new tools that should have priority for TB control include diagnostics, drugs, vaccines and biomarkers.[8] These developments must be accompanied by enhanced programmatic preparedness to adopt the new tools rapidly. Unfortunately, the diagnosis of new cases of tuberculosis still relies on sputum microscopy, the sensitivity of which is around 50%. Newer tools that have emerged recently are the LED- microscopy for such cases but it is yet to be seen what impact this makes in the overall case detection. The other deficiencies of the diagnostic tools currently available result in under-diagnosis or delay in the diagnosis of TB, particularly among drug-resistant cases, with increased morbidity and mortality among patients due to non-treatment till the results are available, and transmission of infection to the community.[9-11] Unfortunately, the country and the scientific community, as a whole, has failed to develop quality-assured accredited laboratories in sufficient numbers, even if TB is our great problem. Instead, many laboratories had adopted the easy path of serological diagnostics for commercial purposes, which are not only inaccurate, but also misleading. Correctly, the Government has now banned these tests following WHO warning. Newer diagnostic tools for MDR-TB that include liquid culture, Line Probe Assays and Gene-X'pert tests need rapid evaluation and adaptation. However, over-diagnosis of TB will lead to unnecessary treatment, with its associated risks, no benefit and wastage of resources.[11] The benefits of some of the rapid diagnosis of TB including the drug-resistant forms of MDR and XDR-TB will only be assessed with the translation of findings into practice, and addressing obstacles to implementation.[9,12] It is important to estimate their impact on health care services, and it will be necessary to monitor and evaluate their use through operational and health systems research.

While India is rapidly expanding the DOTS-Plus strategy to handle the issue of MDR/XDR-TB, there is still concern of universal coverage because of dissociation between plan and real achievements, particularly in terms of establishing new accredited laboratories. It is planned to have 43 laboratories which are to be established and strengthened with enhanced sputum processing capacity (staff, centrifuges, bio-safety cabinets), solid culture and DST capacity in all laboratory units, Line Probe Assay (LPA) in all laboratories, and liquid culture in 33 laboratories. This will enhance the annual DST capacity to 2,20,000 sputum samples annually at the end of 2013. Besides these, other private sector and medical colleges will be engaged and encouraged to be involved in these activities. Other strategies that will be adopted include strengthened human resource capacity, DOTS Plus and TB HIV supervisor in every district, additional staff at laboratories and DOTS Plus sites, 120 DOTS Plus sites across the country (1/10 million population) with up-gradation to national airborne infection control standards, advocating with Indian Drug Manufacturers with Global Drug Facility (GDF) support to adhere to WHO Prequalification and GDF quality assurance systems, to develop second line drugs (SLD) production plans with national drug demand in view, and to integrated national on-line electronic recording and reporting system (E-TB Manager Brazil Model being adapted by MSH) with advocacy for rational use of anti-TB drugs (like flouoroquinolones in respiratory cases).

It is envisaged that by 2012, access to laboratory-based quality-assured MDR-TB diagnosis and treatment will be achieved for all smear positive re-treatment TB cases and new cases who have failed an initial first-line drug treatment. By 2015, access will be possible to MDR-TB diagnosis and treatment for all smear positive TB (new and re-treatment) cases registered under RNTCP. RNTCP plans to initiate at least 30,000 MDR cases on treatment annually by 2013. Thus, still there will be a huge deficit of coverage to all new MDR-TB cases. However, there are many challenges to these plans both related to diagnosis and treatment issues related to MDR-TB. There is delay in establishment of accredited state-level laboratories due to a host of reasons like sub-optimal functioning of the accredited laboratories, non-availability of trained manpower, dedicated regular staff in addition to the contractual posts, uninterrupted power supply, diagnostic delay with conventional method (three-four months turn-around time), and special requirements for introduction of newer rapid diagnostics- laboratory infrastructure and training. Treatment related issues include long duration, toxic, and expensive treatment, (~2,100 US $ per patient course), daily ambulatory

DOT with six-nine months of injectables, availability of DOTS-Plus in-patient sites (one per 10 million population), extensive training, supervision and monitoring needed at all levels, ensuring treatment adherence and timely follow-up, and uninterrupted supply of second line drugs. Other important issues include unsupervised and uncontrolled private sector in the management of tuberculosis. In 2006, substantial quantity of first line and almost 100% of second line drugs were sold and used outside of RNTCP. It is well documented that management of TB patients outside of RNTCP is often poor, leading to risk of failure of treatment and development of drug resistance. This large unregulated private sector, conflict of interest, and easy availability of anti-TB drugs are important hindrances to a successful programme. One has to take steps to promote rational use of anti-TB drugs in the form of "Chennai Consensus Statement" which was developed and disseminated by the Central TB Division, by involving Indian Medical Association on behalf of RNTCP interacting with Medical Council of India for guidelines to all healthcare providers on rational use of anti TB drugs, by interactions with office of DCGI (Drug Controller General of India) to draft guidelines for the regulation of anti-TB drugs, especially second-line drugs, and encouragement of additional pre-qualified drug manufacturers. The other issue that needs urgent attention is the infection control practices in Indian Hospitals and health care facilities. It is usually considered synonymous with waste management, which actually is not. Recently, national guidelines on Airborne Infection control in context of TB have been developed that need to be used in actual practice. There is usually overcrowding/lack of space at health facilities, lack of awareness and commitment of hospital administrators. To overcome these problems, various steps undertaken include the constitution of National Airborne Infection Control Committee, and "National guidelines for airborne infection control" for all healthcare facilities are developed and pilot-tested. Further, provision of support to upgrade infection control measures at DOTS-Plus site indoor facilities, Intermediate Reference laboratories, Collaboration with AIDS control programme to ensure infection control measures at ICTCs and ART centres and encouraging Medical Colleges (through Task Force mechanism) to develop and implement infection control measures are other important steps taken by the programme.

As of January 2012, the total number of accredited laboratories is 35 with solid culture and DST in 29, LPA in 18 and facilities for liquid culture are available in three for the programme (CTD – personal data). By that date, all 35 states have Programmatic Management of Drug resistant Tuberculosis (PMDT) services in some districts with variable access and scaling up and 508 million (43%) population have access to services. 11 /35 (31%) States-UTs achieved 100% state-wide coverage. 260/662 (40%) districts have access to services and 65/662 (10%) districts switched to MDR TB Suspect Criteria B (All smear +ve re-treatment pulmonary TB cases at diagnosis and any smear +ve follow up of new or re-treatment cases in addition to Criteria A i.e. all failures of new TB cases (CAT I), smear +ve re-treatment cases who remain smear +ve at four months onwards in CAT II, and all pulmonary tuberculosis cases who are contacts of known MDR TB case). 50 DOTS Plus Sites are functional in the country and nearly 38,155 MDR-TB suspects had been examined by January 2012, of which 10,263 (27%) were MDR-TB and of this number, 6994 (68%) were initiated on treatment. This is not desirable as still nearly a third of the patients could not receive therapy despite being diagnosed. Of course, these are only initial problems that could be taken care of as one goes along. The treatment outcome of the small numbers is gradually improving from a success of 43% in the initial cohorts to about 51% in later cohorts. One should remember that these patients were waiting for variable periods of time before the PMDT services could take up and they had exhibited high level of second line drug resistance (Ofloxacin ~ 24%) and also they were heavily treatment experienced cohorts. The outcome is expected to improve further subsequently.

As new drugs and vaccines are being developed/ becoming currently discovered and available, they need to be evaluated in our setting. For such activities, the country must be prepared for clinical trials, change in some of our drug-trial policies without compromising the ethical and legal issues and the good

clinical practices. The overall attitude of our clinicians and others involved in drug/vaccine trials need to be positive following prescribed guidelines. These new agents are absolutely necessary and are the needs of the hour to shorten and simplify treatment for drug-susceptible disease, to improve the treatment of drug-resistant cases and to refine treatment in special populations, such as people living with HIV, children and elderly patients.[13] It must be realized that new drug development is a long and laborious process, very costly and needs thorough and meticulous scrutiny of their efficacy.[14,15]

Although some advocate the treatment of latent infection, particularly in contacts of drug-resistant TB cases, for this country, we have still some reservation because of high INH-resistance. There is some renewed interest and discussion regarding the World Health Organization's recommended 'Three I's for HIV-TB' (isoniazid preventive treatment [IPT], infection control for TB and intensified case-finding [ICF] for TB).[16] Use of vaccines is another approach that could provide a new paradigm for the control of TB. Besides efficacy issues, other concerns include cost and safety of the vaccine for those who are latently infected or immunocompromised (especially for those living with HIV). New approaches have enabled development of candidate vaccines, now in early phases of clinical trials. Thus, many challenges remain for the development and utility of a new vaccine.[17,18]

One of the most important and perplexing issues in the understanding of tuberculosis is our incomplete understanding of the host protective immune response against *M. tuberculosis,* why some persons develop tuberculosis, the pharmacogenomics of anti-tuberculosis drugs and the optimal end-points for clinical efficacy in vaccine trials. Attention has focused on putative genes and transcriptomes through genomics and proteomics, etc. Assessing multiple biomarkers may be more useful than testing a single biomarker.[19,20]

Even if challenges remain, there are positive signs in the horizon and we are clearly closer to obtaining some of the new tools we need.[21,22] While for the present, we have to follow the currently available strategies through our managerial and administrative skills, newer developments and discoveries must be rapidly adopted after quick but adequate evaluation, so that we can achieve the millennium development goals of TB control.

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Original Article

RELATIONSHIP BETWEEN SPUTUM SMEAR GRADING AND SMEAR CONVERSION RATE AND TREATMENT OUTCOME IN THE PATIENTS OF PULMONARY TUBERCULOSIS UNDERGOING DOTS- A PROSPECTIVE COHORT STUDY

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Summary

***Background*:** The Smear Conversion Rate (SCR) is an operational indicator for the Directly Observed Treatment Short-course (DOTS) strategy of Revised National Tuberculosis Control Programme (RNTCP) in India. The present study was undertaken to determine the relationship between sputum smear grading and smear conversion rate among the Category I smear positive pulmonary tuberculosis patients undergoing DOTS.

***Methods*:** A prospective cohort study was conducted among the Category I smear positive pulmonary tuberculosis patients registered under DOTS at GTB, Karawal Nagar and Shahdara Chest Clinics of Delhi. Sample size for the present study was calculated on the basis of a similar study of a retrospective design conducted at LRS Institute of Tuberculosis and Respiratory diseases New Delhi, India using statistical software Epi Info version 6. Accordingly, a total of 338 sputum smear positive patients with 169 each in the High Positive Cohort (pre-treatment sputum grading 3+) and Low Positive Cohort (pre-treatment sputum grading 2+, 1+ and Scanty) were followed periodically at two months (end of Intensive Phase) , at three months (after one month extension of Intensive Phase), at two months of Continuation Phase and then at the end of the treatment to record the sputum AFB result and treatment outcome as per the RNTCP guidelines. Data was analyzed using SPSS Version -15.

Results: After two months (end of the intensive phase), SCR was 57.9% (98 of 169) among the High Positive and 71.6% (121 of 169) in the Low positive cohort (p -0.008). After three months (one month's extension of intensive phase), cumulative SCR was 85.2%(144 of 169)) in the High Positive and 92.3%(156 of 169) in the Low Positive cohort (p-0.03). Cure rate was 82.8%(140 of 169) in the High Positive and 84.6%(143 of 169) in the Low Positive cohort. Default rate was 3% (five of 169) in the High Positive and 5.3% (nine of 169) in the Low Positive cohort. Failure rate was 11.2% (19 of 169) in the High positive and 6.5% in the Low positive Cohort (11 of 169). Only one patient (0.6%) in each High and Low Positive cohort died during course of treatment (p -0.631). Treatment outcome was further compared among the patients according to their sputum status achieved at two and three months of the treatment after ignoring their initial sputum status. The cure rates for the patients who converted at two months was 90.9% (199 of 219) and for those who did not convert at two months, was 74.3% (84 0f 113) (p -0.000). Similarly, the cure rate for the patients who converted at three months was 84% (68 of 81) and for those who did not convert at three months was 55.2% (16 of 29) (p-0.01).

***Interpretation*:** Patients with higher grades of sputum positivity at the beginning of the treatment have significantly lower SCR at the end of intensive phase and even after extending the intensive phase for one month. Hence, they are likely to remain infectious for a longer duration and continue to transmit infection in the community. Therefore, these patients demand to have more stringent self-precautionary measures to break the chain of infection in the community. The SCR at two months and three months as an operational indicator should be given more importance rather than being practised only as a documentation and academic exercise. The patient should be investigated for the possible co-morbid conditions and drug resistance which could be a cause for the persistent sputum smear positivity at two and three months and hence poor treatment outcome. **[*Indian J Tuberc 2012; 59:* 135-140]**

***Key words* :** DOTS, Pulmonary Tuberculosis, Sputum Smear Grading, Smear Conversion Rate, Treatment Outcome

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INTRODUCTION

The Directly Observed Treatment Short-course (DOTS) strategy along with the other components of the Stop TB strategy, implemented under the Revised National Tuberculosis Control Programme (RNTCP), is a comprehensive package for TB control in India. Sputum AFB microscopy remains the corner stone for the success of DOTS strategy worldwide as it plays a fundamental role in case detection, diagnosis, categorization and follow up. In the DOTS programme, smear is graded according to WHO recommendations for RNTCP as 3+,2+,1+,scanty or negative based on the number of AFB seen in the sputum smear[1].

Change in the bacteriological status of sputum of patients from initial AFB positive to AFB negative after treatment is referred to as sputum smear conversion. Smear Conversion Rate (SCR) at two months of intensive phase (IP) and at three months of IP (extended IP) is a significant operational indicator as it shows the capacity of the programme to maintain the patients on treatment. It also provides an objective evidence for the patient's response to therapy and hence the treatment outcome[2].

The present study was undertaken with an objective, to determine the relationship between pre-treatment sputum smear grading (as 3+,2+,1+ and scanty) and SCR and its relative importance in predicting the treatment outcome of the tuberculosis patients registered under DOTS.

MATERIAL AND METHODS

A prospective cohort study was carried out among Category I pulmonary smear positive patients registered at the DOTS centres of GTB, Karawal Nagar and Shahdara Chest Clinics in the East and North –East Delhi[3]. Sample size for the present study was calculated on the basis of a similar study of a retrospective design conducted at LRS Institute of Tuberculosis and Respiratory diseases, New Delhi, India using statistical software Epi Info version 6. The patients with pre-treatment sputum grading 3+ were placed in High Positive Cohort (HP Cohort) and those with pre-treatment sputum grading as 2+, 1+ and scanty were placed in Low Positive Cohort (LP Cohort) for analysis. Accordingly, a total of 338 Category I patients with 169 each in HP Cohort and LP cohort were enrolled in the study within one month of their registration at their respective DOT Centre during November 2006 to October 2007.

Sputum results and treatment outcome were recorded from laboratory records and patient's treatment cards.

All the study subjects were interviewed using a pre-designed, pre-tested semi-structured interview schedule within one month of their registration at the DOT centres. Subsequent visits to the DOT centres were made to collect the information on sputum results at two months of IP, at three months IP (extended IP), at two months of Continuation Phase(CP) and then at completion of the treatment. The outcome variables of the study, SCR and Treatment Outcome were defined as per operational guidelines under the RNTCP.

Data was analyzed using SPSS version 15. Chi square test for the categorical variable and Student t-test for the continuous variable were applied. P- value below 0.05 was considered to be statistically significant.

RESULTS

Socio-demographic information such as age, sex, and socio-economic status (according to Modified Kuppuswamy Scale -2007) is summarized in Table-1. The mean age of the patients in HP Cohort was 29.8 years (SD$\pm$13.2) and that for the LP Cohort was 32.09 (SD$\pm$15.4) years. Majority of the patients in both the cohorts was from the most productive age group of life i.e between 20-41 years of age. The percentage of males was slightly more than the female patients in both the study cohorts. With respect to socio-economic status , 75.1% patients of HP Cohort and 66.3% patients of LP cohort fall in the upper

Table 1: Socio-demographic details of study subjects of High Positive and Low Positive Cohorts

Characteristics	High Positive Cohort (N=169) n(%)	Low Positive Cohort (N=169) n(%)	p- Value
1. Age (in years)			
<20	39(23.1)	42(24.3)	0.380 (χ^2-3.07;df-3)
20-41	99(58.6)	85(50.3)	
41-60	27(15.9)	36(21.9)	
>60	4(2.4)	6(3.5)	
2. Gender			
Male	95(56.2)	100(59.2)	0.582 (χ^2-0.303;df-1`)
Female	74(43.8)	69(40.8)	
3. Socio Economic Status (According to Modified Kuppuswamy Scale-2007)			
Upper	0(0.0)	0(0.0)	0.189 (χ^2-4.77;df-3)
Upper middle	5(0.3)	9(5.3)	
Lower middle	26(15.4)	28916.6)	
Upper Lower	127(75.10	112(66.3)	
Lowe lower	11(6.5)	20(11.8)	

Table 2: Smear Conversion Rate in the High Positive Cohort *vs*. Low Positive Cohort at two months of IP and at three months of IP (extended IP)

Patients initiated on DOTS	High Positive Cohort (N=169)	Low Positive Cohort (N=169)	p - Value
Smear Conversion Rate at 2 Months of IP n(%)	98(57.9)	121(71.6)	0.008 (χ2 - 6.86 ; df-1)
Smear Conversion Rate at 3 months of IP (cumulative) n(%)	144 (85.2)	156 (92.3)	0.03 (χ2 - 4.27; df-1)

Table 3: Treatment Outcome of High Positive Cohort *vs*. Low Positive Cohort

Patients initiated on DOTS	Cured n(%)	Treatment completed n(%)	Defaulted n(%)	Failure n(%)	Transferred Out n(%)	Died n(%)
High positive cohort (N=169)	140(82.8)	1(0.6)	5 (3.0)	19 11.2)	3 (1.8)	1(0.6)
Low Positive cohort (N=169)	143(84.6)	1(0.6)	9(5.3)	11(6.5)	4(2.4)	1(0.6)
Total (N=338)	283(83.7)	2(0.6)	14(4.1)	30(8.9)	7(2.1)	2(0.6)
P-value	0.631 (χ^2-3.45;df-5)					

lower category according to Modified Kuppuswamy Scale. As shown in Table-1, the present study did not reveal any statistically significant difference in the socio-demographic profile of study cohorts.

Being a prospective cohort study, the number of the patients varied at each stage of the treatment due to loss to follow up as default, death and transfer out. In the HP Cohort, five patients defaulted (one in IP, one in extended IP and three in CP). One patient died and three patients were transferred out during CP. Fourteen patients were labelled as failures because of persistently positive sputum at two months completion of CP, therefore shifted to the Category II. Five patients of HP cohort were found to be persistently sputum positive even at the end of treatment and therefore labelled as

Table 4: Treatment Outcome of Converted Cohort *vs*. Not converted Cohort at two months (ignoring pretreatment sputum smear status)

Patients initiated on DOTS	Cured n(%)	Treatment completed n(%)	Defaulted n(%)	Failure n(%)	Transferred Out n(%)	Died n(%)
Converted at two months(N=219)[a]	199(90.9)	2(0.9)	6(2.7)	9(4.1)	2(0.9)	1(0.5)
Not converted at two months(N=113)[b]	84(74.3)	0(0.0)	4(3.5)	21(18.6)	4(3.5)	0(0.0)
Total (N=332)	283(85.2)	2(0.6)	10(3)	30(18.6)	6(1.8)	1(0.3)
P-value	0.000 (χ2-24.2;df-5)					

Note: Table presents the treatment outcome of patients with regard to their sputum status achieved at two months. Therefore, only those patients whose sputum result was available at the two months were analyzed for their treatment outcome ignoring their initial sputum AFB status. Died, defaulted and transferred out were not included in the analysis. Thus, at the end of two months, total patients (combined HP and LP cohort) Converted were(98+121=219)[a] and Not converted were (70+43=113)[b].

Table 5: Treatment Outcome of Converted cohort *vs*. Not converted cohort at three months i.e. after one month's extension of Intensive Phase (ignoring pretreatment sputum status)

Patients initiated on DOTS	Cured n(%)	Defaulted n(%)	Failure n(%)	Transferred Out n(%)
Converted at three months (N=81)[c]	68(84)	1(1.2)	11(13.6)	1(1.2)
Not converted at three months(N=29)[d]	16(55.2)	1(3.4)	10(34.5)	2(6.9)
Total (N=110)	84(76.4)	2(1.8)	21(19.1)	3(2.7)
P-value	0.01 (χ2-0.2;df-3)			

Note: Table presents the treatment outcome of patients with regard to their sputum status achieved at three months. Therefore, only those patients whose sputum result was available at the three months, were analyzed for their treatment outcome ignoring their initial sputum AFB status. Died, defaulted and transferred out were not included in the analysis. At the end of three months, total patients (combined HP and LP cohort) Converted were (46+35=81)[c] and Not converted were (23+6=29)[d]

failures. So, at the end of treatment, 146 patients remained in the HP cohort. In the LP cohort, nine patients defaulted (three in IP, one in Extended IP and five in CP), one patient died and four patients were transferred out. Eight patients were labelled as failures because of persistently positive sputum at two months' completion of CP, therefore shifted to the Category II. So, at the end of treatment, 147 patients remained in the LP cohort.

As shown in Table-2, SCR after two months of IP showed statistically significant difference between the two cohorts (HP Cohort-57.9% and LP Cohort-71.6%; p value 0.008). The cumulative SCR after extended IP was also significantly different between the two cohorts (HP Cohort-85.2% and LP Cohort-92.3%s; p value 0.03).

Treatment outcome as shown in Table-3, did not show any significant difference between the two cohorts (p value-0.631) but, comparatively, higher proportion of patients were failures (11.2%; 19 of 169) in the HP Cohort than in the LP Cohort (6.5%;11of 169)**.**

In Tables 4 and 5, treatment outcome was further compared among the patients according to their sputum status achieved after two and three months of treatment after ignoring their initial sputum status. For this purpose, patients were again divided as Converted and Non-converted cohorts at two months and similarly at three months. It may be noted that only patients whose sputum result was available at two and three months, were analyzed for their treatment outcome. Died, defaulted and transferred out were not included in the analysis. It was found that the cure rate was significantly lower for the patients who failed to achieve smear conversion at two and three months. This finding further reiterates that SCR at two and three months is a very strong determinant of treatment outcome of patients.

DISCUSSION

Pre-treatment sputum smear grading is a direct measure of number of bacilli present in a smear and thus severity of disease which may affect the smear conversion and final treatment outcome. There are studies available which highlighted the importance of initial sputum smear grading and revealed that higher grades of smear positivity result in delayed smear conversion poor treatment outcome.

The present study revealed that the patients of the HP Cohort achieved lower conversion and cure rates as compared to the patients in LP Cohort. Similar findings were reported in a retrospective study by Singla R. *et.al*. at L.R.S Institute of Tuberculosis and Respiratory Diseases, New Delhi. The study reported SCR among the patients graded as 3+ and rest of the patients (combined graded 1+ and 2+) at the end of the IP as 62.2% and 76.8% respectively (p<0.001) and at the end of three months as 81.3 % and 89.5% respectively (p<0.001). The cure rate among 3+ and in 1+ and 2+ patients were 76.6% and 85.1% respectively (p< 0.001) and failure rates were 7.7% and 4.5% respectively (p<0.001). However, the present study did not show statistically significant difference in the cure rate and failure rate among the HP and LP Cohort[3].

Lienhardt *et al* reported sputum conversion at the end of two months in patients with initial sputum smears 1+,2+ and 3+ to be 96.2%, 85.8% and 81.8%, respectively. They further observed that the cure rate also decreased with a higher initial bacillary load[4].

Another study from Saudi Arabia reported numerous bacilli on pre-treatment sputum smear examination as an independent risk factor associated with persistent sputum smear positivity at the end of two months of treatment using DOT under national programme conditions[5].

Similarly, in a refugee camp in Thailand under DOTS, Rieder *et al* observed that sputum conversion at the end of two months of treatment among patients with initial weakly positive sputum to be 90.9%. It was 77.9% and 61.7% among patients with initial moderately positive and strongly positive sputum smear results, respectively[6].

Findings from the present study reiterate the fact that the patients who have higher grades of sputum positivity at the beginning of treatment have significantly lower conversion rates at two and at three months even though the overall treatment outcome did not show any significant difference with respect to sputum grading. The importance of SCR as an operational indicator can be further understood by the fact that the patients who remain smear positive (not converted) at two and three months, achieve significantly lower cure rates in comparison to those who get converted at two and three months.

So, the pre-treatment sputum smear grading can help pinpoint a group of patients who may require an extension of intensive phase of treatment more often and their treatment outcome is likely to be worse than that of others. SCR at two and three months can help a physician in deciding the necessary investigations to be done at the earliest to detect other co-morbid conditions and go for sputum culture and sensitivity to rule out drug resistance, if any. The pre-treatment higher bacillary load and consistent smear positive status at two and three months of IP is found to be a significant risk factor for developing Multi Drug Resistant Tuberculosis (MDR TB) according to a study done in Eritrea[8]. Similarly the study conducted at the All India Institute of Medical Sciences by J.N Pande *et al* reported the pre-treatment higher bacillary load to be a risk factor for MDR TB.[9] Understandably, the delayed and poor SCR of patients with higher grades at the beginning of treatment continue to transmit infection in the community for a longer duration of time and hence contribute to the prevalence pool of infective tuberculosis in the community. These patients need to be motivated to take self-precautionary methods to prevent the spread of infection in the community.

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Original Article

SAME DAY SPUTUM SMEAR MICROSCOPY APPROACH FOR THE DIAGNOSIS OF PULMONARY TUBERCULOSIS IN A MICROSCOPY CENTRE AT RAJAHMUNDRY

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Summary

Background: Sputum smear microscopy is the initial and rapid diagnostic technique for tuberculosis. This requires two (spot and morning SM) sputum sample examinations over two days. Collection of two spot samples (SS_2) on the same day would reduce the number of visits, time, money and early initiation of treatment.

Methods: We evaluated same day approach (SS_2) against standard (SM) for the diagnosis of pulmonary tuberculosis.

Results: Out of 658 participants, same day approach could identify 62 cases, whereas standard approach could identify 64 cases. Both the approaches are equally effective ($p>0.05$).

Conclusion: The diagnosis of pulmonary tuberculosis is possible in one day by examining two spot samples. **[*Indian J Tuberc 2012; 59: 141-144*]**

Key words: Sputum smear microscopy (ssm), Standard (SM) approach, Same day (SS_2) approach, Tuberculosis (TB)

INTRODUCTION

Tuberculosis (TB) is a major public health problem. There are world wide 9.3 million new cases and 1.7 million deaths per year[1]. Among these, 90% of cases occur in low and middle income countries.[1] In these high burden countries, infrastructure for the diagnosis of infectious diseases is not adequately resourced. The only diagnostic technique for TB, suitable to peripheral levels of health services, is serial sputum smear microscopy with Ziehl Neelsen (ZN) staining.

Most of the national TB control programmes collect specimen using Spot Morning Spot (SMS) scheme i.e. spot sample at the time of the first visit to the hospital; morning sample is collected at home on the morning of the following day and spot sample is collected again when the patient brings the morning sample. This scheme requires at least two visits and the patient often abandons the diagnostic procedure.[2-4] The greatest disadvantage is that such defaulting patients continue to move in the community and finally by the time we are able to diagnose them, they have already contributed to the spread of disease in the community.

In SMS scheme, majority of patients with smear positive pulmonary TB (PTB) are identified by the first two sputum specimens [5]. World Health Organization (WHO) and also Revised National Tuberculosis Control Programme (RNTCP)[6] have recently changed its policies in this respect, reducing the minimum number of sputum specimens to be examined for each patient from three to two i.e. Spot Morning (SM) scheme. This SM scheme results in reduction of laboratory workload with the potential of improving the quality of sputum microscopy [7]. Case detection may thus be expected to increase in locations where the number of new cases would be detected through improved quality of microscopy.

The SM scheme of TB diagnosis still requires two visits, which is same as the minimum required time by the SMS scheme. In addition, the SM and SMS schemes still examine a substantial proportion of samples on the second day of the diagnostic process. If the total process could be completed on the "same day", that is, if all or

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majority of sputum sample collections could be made on the first day itself and results made available on the same day, it would reduce the number of visits required and the patient drop-out.

In this study, we described the yield of same day diagnostic scheme, in which second spot specimen (referred here as S_2) was collected one hour after the first spot specimen. So in the current study, the mode of sputum collection was SS_2M. In addition, we compared the two schemes {standard (SM) *Vs* same day (SS_2)} in order to access as to which scheme could detect maximum number of the smear positive samples.

MATERIAL AND METHODS

The study was conducted in the Department of Microbiology, GSL Medical College, Rajahmundry, Andhra Pradesh from January to July 2011. This study was approved by the Institutional Ethics Committee. Informed written consent was taken from all the participants. Individuals with ≥ 2 weeks of cough and age of 14 years and above were included in the study. After collecting the clinical history, the patient was explained the importance of submitting sputum rather than saliva. This was done by describing as well as visually showing the difference between the two.

Subsequently, it was demonstrated[8] that by taking three deep breaths, followed by a deep cough, a good quality sputum could be brought out from the lungs.

Finally, patients were explained to provide 5ml of the sputum sample. This was shown by marking the required level on a demonstration sputum container. The participants were requested to give three sputum samples as per SS_2M scheme. After collecting S and S_2 samples, individuals were provided with pre-labelled sample containers for the collection of morning (M) sample at home.

Specimens were assessed macroscopically and the findings were recorded. New unscratched slides were labelled with study numbers and they were used for smear preparation. The smears were stained by ZN staining. ZN staining technique and smear grading were done as per RNTCP technical manual guidelines.[9] The study numbers were covered with wrap around stickers before microscopy. With this, the lab technicians (LTs) were blinded to smear results, and the bias was avoided. Stickers were removed by different LT before entering the results in the study book. As part of internal quality control (IQC), all the positive slides and randomly, 50% of negative slides were screened by the microbiologist.

RESULTS

During the study period, a total of 702 patients were enrolled, male female ratio was 1.7:1. Owing to the non-compliance of study protocol, analysis was possible for 658 participants. The first two smears from standard approach (SM scheme) identified Acid Fast Bacilli (AFB) in 64 patients, whereas same day scheme (SS_2) could identify 62 cases. Therefore, SS_2 scheme missed two patients. There was, however, no statistical difference between the two schemes ($p>0.05$).

DISCUSSION

Despite numerous technical advances, microscopy remains the cornerstone of TB diagnosis, particularly in developing countries.[10] WHO recommends continuous use of sputum smear microscopy (ssm) for diagnosis and treatment monitoring, even in areas served by new technologies such as TB culture, Line Probe assays and the Gene Xpert assay. As per WHO and the RNTCP, PTB patient is defined as an individual with at least one sputum smear (SM scheme) positive for AFB or culture positive for tubercle bacilli. In limited resource countries like India, culture facility is not readily available, so majority of PTB cases are diagnosed based on ssm only.

Owing to low sensitivity of ssm, the diagnosis of TB requires repeated sputum examinations on several days [11]. Currently, RNTCP follows two days (SM scheme) approach. With this SM approach also, patients are spending significant amount of money on travel and food besides loss of daily wage in order to reach the TB microscopy

centre and 40 to 50% of these patients are daily labourers. The major obstacle with ssm is patient dropouts. In the current study, patients were continuously motivated by the study team and in spite of this, the dropouts were 6.3% i.e., 44 out of 702. Under routine conditions, this number is much more.[2, 12, 13]

In a multi-country randomized study[14] on the diagnosis of TB, the participants were requested to submit sputum sample either by SMS or SSM scheme. In this study, more number of patients in SSM scheme submitted their first two sputum samples compared to the participants in SMS scheme and no statistical difference has been shown with regard to sputum smear positivity. In one study, S.Hirao[15] reported sputum positivity as 20% in SS_2 scheme and 21% in SM scheme, and the difference was again statistically not significant. In our study also, as far as the schemes were concerned, the results with both schemes were similar, 9.43% and 9.72% respectively, and the difference was also not statistically significant ($p>0.05$). The false negativity would not have occurred if all the slides were screened by the microbiologist.[16]

Of course, ethically and ideally, one should not miss out on any case but if we weigh this loss of positivity against the advantage of returning the patients and preventing dropouts, we feel that this scheme is justified. In our study, the two cases that we missed in SS_2 scheme were also referred for anti TB treatment as per standard SM approach. It has been observed that whether it is under field conditions or in the micrsocpy centre, not enough time is spent in motivating the patient to bring out good quality sputum sample, which is the mainstay of the whole RNTCP programme. In our study, with some extra effort, we could get good quality sputum in most cases which definitely contributed to the increase in efficiency of the smear microscopy. Sputum sample collected in the morning is 10% more sensitive compared to spot sample because of higher bacillary load.[17] Hence, majority of the clinicians are reluctant to rely on negative result of spot specimen. However, **as per our study, if two spot samples are properly collected on the same day, we can do away with morning samples as the difference is not statistically significant. Further, this same day diagnostic approach for PTB can help to initiate therapy on the same day and can save time as well as resources of the patients.**

The approach used in the study needs to be validated by studies done on a larger sample size. If similar results are obtained, sputum specimen collection scheme may be revised from the present approach of collecting the spot and morning samples to two spot (SS_2) samples on the same day.

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Original Article

IMPLEMENTATION OF RNTCP IN A PRIVATE MEDICAL COLLEGE: FIVE YEARS' EXPERIENCE

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Summary

Background: Revised National Tuberculosis Control Programme (RNTCP) recognizes the need for involvement of all sectors, public and private, to create an epidemiological impact on Tuberculosis control .The private health sector in the country is an important source of care, even with the availability of public health services and Directly Observed Treatment Strategy (DOTS).The data regarding Private-Private mix in our country is lacking.

Aim: To evaluate the contribution of {private health sector (Private Medical Colleges and Private practioners (PP)} in TB case-detection, diagnosis and treatment outcomes in Delhi NCR , Ghaziabad, India.

Methodology: We analyzed the TB registers from May 2006-Dec 2010 from our institution and recruited the patients in our study, irrespective of the source. We strengthened the referral by promoting educational intra and inter departmental activities and awareness programme with more stress on retrieval action by contact tracing and counselling. We made a list of PP in our drainage area and regularly met them and tried to understand the barriers in referring cases to DOTS centre. During the study, we tried to maintain the flow of information working as a single window information system. We regularly passed on the information of follow up of patients to private practioners referred to us by them to generate confidence in them. During the study, no incentive was offered to any patient. Various indicators and data were collected annually and analyzed statistically.

Statistics: Retrospective, Descriptive Analysis

Results: There was a substantial increase of 116.3% in the total patients referred from all sources to Santosh Hospital. The proportion of extra-pulmonary cases was 29.1% to 34.4% of all total cases from the year 2006 to 2010. During subsequent years, we found a significant increase in referral from Private Practioners that was the result of our activities performed in private set up. It was 12.5%, 21.2%, 30.8%, 27.3%, and 29% during 2006, 2007, 2008, 2009 and 2010 respectively. The outcome in our study was in accordance with the outcome at national level under RNTCP.

Conclusion: Because of extensive educational activities, single window information system and referring the patients back to private sector after completion of treatment increased the confidence amongst the private physicians. These results strengthen the Private - Private Collaboration and show that a stronger link can be developed between medical college and private setup, leading to implementation of successful Private-Private Strategy. **[*Indian J Tuberc 2012; 59:* 145-150]**

Key words: Tuberculosis, Private-Private Mix, DOTS, RNTCP.

INTRODUCTION

Public private mix (PPM) has been recognized as an important component in the RNTCP. The aim of this coordination is to effectively link the national TB programme and all public and private health care providers presently out of realms of national TB programme efforts so as to provide standardized treatment to all TB patients in the country.[1,2]

The programme has consistently maintained the treatment success rate >85% and NSP case detection rate (CDR) close to the global target of 70%. In 2009, RNTCP has achieved the NSP CDR of 72% and treatment success rate of 87% which is in line with the global targets for TB control. While we are diagnosing around 80 smear positive cases per lakh population annually, we are not able to put them all on treatment.[2] In other words, these infectious cases diagnosed in DMCs are still away

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from our effective proven DOTS treatment under RNTCP; and this quantity is not small and definitely more than 10%. Cumulatively, it turns out to be more than five lakhs in the last five years. We have not been able to reduce this big gap in these years. This necessitates the urgency to strengthen referral feedback mechanism and efforts for prompt initiation of treatment.[3,4]

Thus, there is an urgent need for involvement of all spheres of medical services in India at all levels because of the long duration of treatment, requiring social support network to facilitate adherence to ambulatory treatment. Also DOT services in urban slums require involvement of private and NGO sectors to reach out to special groups like migrants and slum dwellers and in view of running of the newer initiatives, like DOTS plus, TB- HIV collaboration to improve the access of DOTS for the TB patients.[4-7] Here, in this study, we evaluated the functioning and efficacy of programme at Designated Microscopy-cum-DOT Centre (DMC) situated at a Private Medical College which is probably one of the studies of this type. We also wanted to evaluate the effect of continuing medical education to the private set up and their transformation in the outcome.

METHODOLOGY AND RESULTS

In our study we are presenting five year data (from January 2006- December 2010) of DMC at Santosh Medical College, Ghaziabad, Uttar Pradesh. Although, there are many studies carried out regarding Private-Private collaboration to assess the impact of case detection by PPs in NTP and to improve the treatment outcome in private sector through practice of DOTS, the present paper aims to highlight the participation of a private medical college in this regard.

We have our DMC functioning from the year 2006 and working regularly. In this study, all patients of tuberculosis, irrespective of site, who were registered in TB register, were enrolled in the study. The total number of patients were 86,132,144,176 and 186 in the year 2006, 2007, 2008, 2009 and 2010 respectively. During the subsequent years, we were working on the strengthening of our referral system by promoting educational intra and inter departmental activities and awareness programme with more stress on retrieval action by contact tracing and counselling. We made a list of private practioners in our drainage area and regularly met them and talked of hurdles. We

Table: Patient Characteristics of the study group

Year	Sex		Total Patients	Site / Positivity		
	Male (%)	Female (%)		Sputa Positive	Sputa Negative	Extra-pulmonary
2006	52(60.5%)	34(39.5%)	86(11.9%)	35(40.7%)	26(30.2%)	25(29.1%)
2007	70(53%)	62(47%)	132(18.2%)	50(37.8%)	38(28.8%)	44(33.4%)
2008	76(52.8%)	68(47.2%)	144(19.9%)	54(37.5%)	42(29.1%)	48(33.4%)
2009	90(51.8%)	86(48.8%)	176(24.3%)	62(35.2%)	54(30.7%)	60(34.1%)
2010	94(51.6%)	92(49.4%)	186(25.7%)	66(35.5%)	56(30.1%)	64(34.4%)
	382(52.7%)	342(42.3%)	724(100%)	267(36.9%)	216(29.8%)	241(33.3%)

regularly passed on the follow up of patients to private practioners referred to us by them to generate confidence in them. We also distributed the educational material to the private physicians and did an awareness camp in Santosh Hospital, Ghaziabad, U.P. annually.

In our study, male outnumbers the female, although there was an increase in number of females in subsequent years. It was 39.5%, 47%, 47.2%, 48.8% and 49.4% in the year 2006, 2007, 2008, 2009 and 2010 respectively. In this study, we have found almost equal number of patients from sputa positive, negative and extra-pulmonary category. In fact, the proportion of extra-pulmonary cases were increased by a mild fraction during subsequent years. It was 29.1%, 33.4%, 33.4%, 34.1%and 34.4% in the year 2006, 2007, 2008, 2009 and 2010 respectively (Table). Talking of sputa positive and negative cases, they were of around constant proportion during subsequent years. It was 40.7%, 37.8%, 37.5%, 35.2%, 35.5% and 30.2%, 28.8%, 29.1%, 30.7%, 30.1% for sputa positive and sputa negative cases for the year 2006, 2007, 2008, 2009 and 2010 respectively. We witnessed the increase in the number of extra-pulmonary cases as we had more referred patients from various departments of Medical College. In our set up, we have referral from the hospital itself, from government institution and private practioners. During subsequent years, we found a significant increase in referral from private practioners, that was the result of our activities performed in private set up (Figure 1). It was 12.5%, 21.2%, 30.8%, 27.3%, and 29% during 2006, 2007, 2008, 2009 and 2010 respectively. The outcome in our study was satisfactory and in accordance with the national outcome. The cure rate was 85.7%, 88%, 88.9%, 90.3% and 92.4% during subsequent years. In our study, cases completing treatment but not cured was also satisfactory. It was 94.1%, 96.3%, 97.8%, 100%, and 100% during 2006, 2007, 2008, 2009 and 2010 respectively. We found a decline in default rate; it was 2.3% (two patients) in 2006, 0.8% (one patient) in 2007 and no default in 2008, 2009 and 2010. Similar trends were seen in failure cases, which were

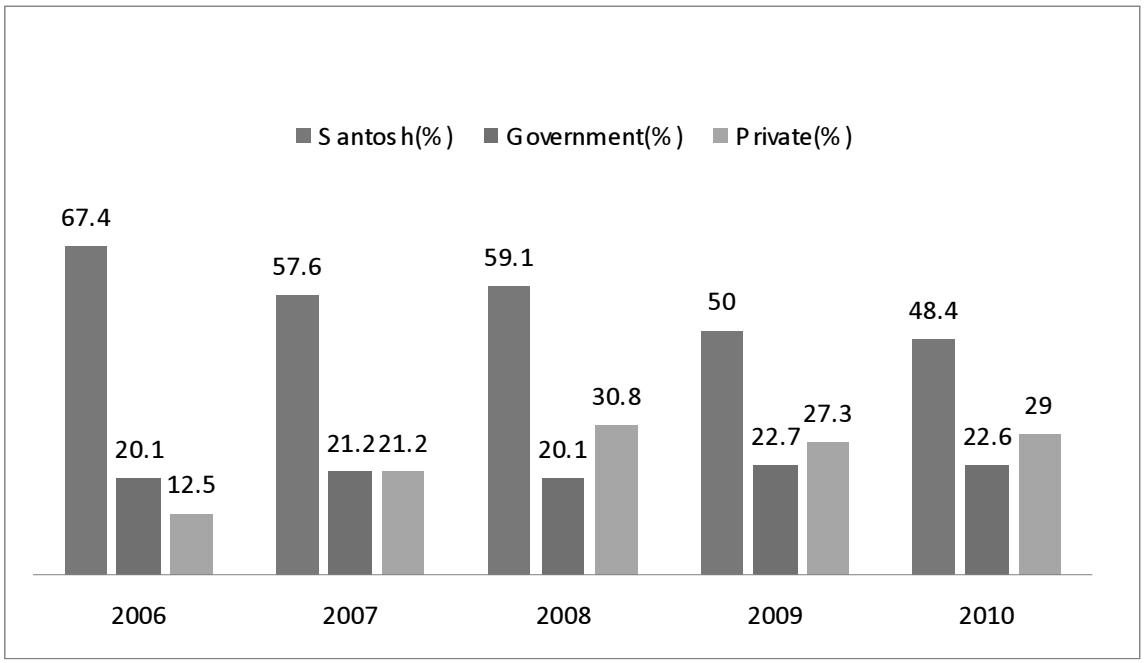

Figure 1: Referral pattern of patients to Santosh hospital

3.5% (three patients), 3.0% (three patients), 2.1% (three patients), 1.2% (two patients) and 1.1% (two patients) during 2006, 2007, 2008, 2009 and 2010 respectively (Figure 2).

DISCUSSION

In our study, the total number of patients were 86,132,144,176 and 186 in the year 2006, 2007, 2008, 2009 and 2010 respectively.[2] We had witnessed the increasing trend of patients enrolled in our study, which was the result of continued efforts of the Department of TB and Respiratory Diseases. We strengthened the referral by promoting educational intra and inter departmental activities and awareness programme with more stress on retrieval action by contact tracing and counselling. We made a list of private practioners in our drainage area and regularly met them and talked of hurdles. We regularly passed on the follow up of patients to private practioners referred to us by them to generate confidence in them. We also put the camp in the nearby slums every quarter for increasing the referral to our hospital.

Indian Governments are deliberately promoting private providers, acknowledging their *de facto* role in increasingly pluralistic health systems and using them to alleviate their own funding constraints. More than 2500 NGOs, 19,000 private practitioners, 150 corporate hospitals and 273 medical colleges are implementing RNTCP. The RNTCP PPM IMA project supported by round-6 of the GFATM has completed two years. The project is being implemented in 167 districts in five states of Andhra Pradesh, Maharashtra, Haryana, Punjab, Uttar Pradesh and UT of Chandigarh. The project is being extended to 10 more states, under the Rolling Continuation Channel (RCC) project of GFATM. IMA is involved in 16 states (167 districts) which are playing a pivotal role in sensitizing and training IMA members and thus strengthening the TB cure. Medical colleges facilitate in continuing education, research and maintaining the adequate statistics. At present out of 307 medical colleges, 282 are implementing DOTS-RNTCP. From 1st July, 2009 to 30th June, 2010, around 6,11,683 TB suspects examined for diagnosis, 92,071 smear positive TB cases were diagnosed and 13,3803 were put on treatment under RNTCP.

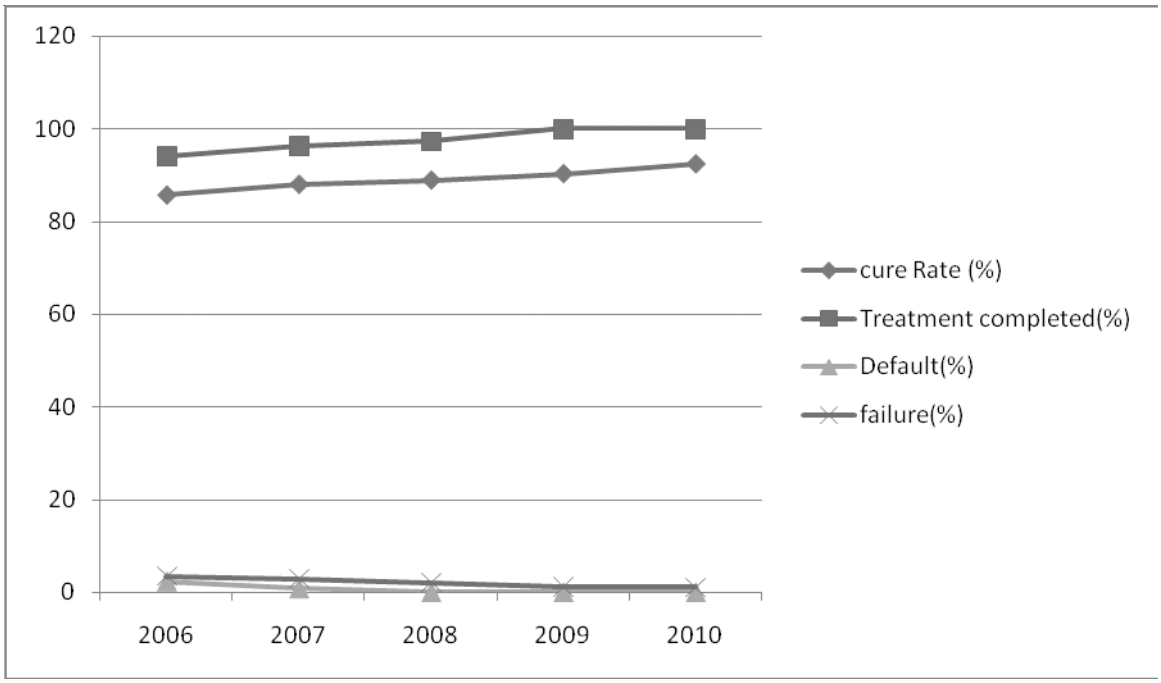

Figure 2: Outcome from the Santosh Hospital

Feasibility of improved TB case detection through involvement of PPs has also been recently reported from Ho Chi Minh City (HCMC), Vietnam, where increasing referral, testing and case detection by PPs was observed over the study-period. As compared to the previous year, detection of new sputum smear positive cases in PPM districts increased by 18%, while a slight decrease was noticed in control districts. Further, case-detection in HCMC as a whole was found to increase by seven percent.[8]

There is growing evidence of Private-Private Mix in the society for smooth implementation of programme which is also gearing up and reaching out to most terrain of people. The NGOs and private providers are often closer to and more trusted by patients and perform an active role in health promotion in the community. As the role of the private sector has grown, the need to regulate its activities has become increasingly apparent. Managing the private health care in Indian setup is inevitably a difficult task, and always the responsibility of government. It requires, in part, careful thought about the most appropriate instruments of regulation in a particular context and for a particular action and goal.

In addressing private/private mix issues, it will be particularly important to see what private-sector actors can bring to the policy development process - whether it be information and technical skills to help make decisions, or power or resources to allow for smooth implementation of policy.[12,13] At the same time, the government clearly needs to manage the less desirable features that the private sector may bring to policy development. Chief among these, especially in the for-profit sector, is divergent interests. Thus, the reform leader must weigh up the positive and negative characteristics of each private-sector actor in relation to the development of the policy. The critical question is whether, and how, the policy process can utilize the positive characteristics of private sector actors while avoiding the negative effect. This will only be possible if careful attention is given to the way in which policy is to be developed.[13,14]

CONCLUSION

This study indicates that case detection under DOTS can be increased considerably through formal involvement of PPs in RNTCP, though the incremental cases were predominantly sputum negative cases. The smooth co-ordination of private medical colleges and private physicians can be facilitated by increasing awareness among private physicians, un-interrupted flow of information, referring back the patients after cure for follow up to them and without offering any incentive to them. Private medical colleges can act as a single window for providing information and taking the responsibility of treatment initiation until cure.

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RESEARCH PROPOSALS F0R FINANCIAL ASSISTANCE

Worthwhile suitable proposals on operational research in tuberculosis are invited in prescribed application (available on our website www.tbassnindia.org) for consideration by the Research Committee of the Tuberculosis Association of India for financial assistance.

Kindly keep in mind that:

(1) The proposal must be approved by the head of the institution/ department and then forwarded to the Tuberculosis Association of India through its respective affiliated State TB Associations with the recommendations of both the respective affiliated State TB Associations and the head of the institution/department.

(2) As per the terms and conditions laid down by the Research Committee, the research proposal needs the clearance of the Ethical Committee of the Institution where the research will be carried out.

Six copies of the applications/research proposals, complete in all respects, should be submitted to:

The Secretary General,
Tuberculosis Association of India,
3, Red Cross Road,
New Delhi-110 001
Phones: 23715217, 23711303

E-mail: tbassnindia@yahoo.co.in

Original Article

PASSIVE SMOKING, INDOOR AIR POLLUTION AND CHILDHOOD TUBERCULOSIS: A CASE CONTROL STUDY

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(Received on 22.3.2012; Accepted after revision on 18.5.2012)

Summary

***Background*:** Passive smoking and biomass fuel use most probably are more harmful to children than adults for two reasons. The first one is children's respiratory and immune systems are not fully developed. Secondly, they spend more time at home and are, therefore, likely to experience more intense and prolonged smoke exposure.

***Aims*:** This study was planned to find out if there is any association between childhood tuberculosis and exposure to passive smoking and biomass fuel.

***Methods*:** A hospital-based case control study was done. All registered consecutive newly diagnosed pediatric tuberculosis cases (0-14 years) from the outpatient department of a tertiary care hospital were recruited as cases. Age and sex matched controls were recruited from a public general hospital of the same locality. A semi-structured, pre-coded interview schedule was administered to parents or legal caregivers of all subjects after obtaining informed written consent.

***Results*:** A total of 200 cases and 200 controls were recruited in the study period. The factors which were significantly associated with development of tuberculosis were education of the mother, (OR 1.411, 95% CI 0.888-2.243, p-0.001), a family member having tuberculosis in the last two years and residing in the same house (OR 2.797, 95% CI 1.353-5.789; p-0.004), being a passive smoker (OR 1.725, 95% CI 1.142-2.605; p-0.009). No association between biomass cooking fuel use and development of tuberculosis was found.

***Conclusion*:** Passive smoking is associated with development of childhood tuberculosis. This requires health education programmes and medical antitobacco advice and services. **[*Indian J Tuberc 2012; 59: 151-155*]**

Key words: Passive smoking, Childhood tuberculosis

BACKGROUND

The extent of childhood tuberculosis (TB) is unknown and is estimated to constitute between 6% and 11% out of all incident cases, with the majority of cases occurring in high TB burden countries.[1] India contributes approximately 21% to the global incidence of TB and has the highest burden in the world.[2] In 2008, at the national level, 79,779 patients (6% of the new cases) registered under RNTCP were aged less than 15 years.[3]

The association between tuberculosis and smoking was reported as early as in the year 1918. Passive smokers are also exposed to similar toxic substances as active smokers but concentration level is different for both the groups. However, only limited data are available to support the association between passive smoking and TB, among children specially from India. Another area of concern is indoor air pollution specially from use of biomass fuel.

Passive smoking and biomass fuel use may have more harmful effects in children when compared to adults as children's respiratory and immune systems are not fully developed.[5,6] Children spend more time at home and so are more likely to experience more intense and prolonged smoke exposure if adult household members smoke[7] or if biomass is used as cooking medium.

We planned this study to find out if there is any association between childhood tuberculosis and exposure to passive smoking and biomass fuel.

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METHODOLOGY

A case control study was done to find out the mentioned objectives. Cases were recruited from the pediatric Pulmonology Department of Lala Ram Swaroop Institute of Tuberculosis and Chest Diseases, a tertiary care respiratory disease institute in North India. Age and sex matched controls were recruited from out patient department of a public general hospital of the same locality. The cases were diagnosed based on the criteria set by country's National Programme, Revised National Tuberculosis Control Programme (RNTCP) which is as per standard WHO criteria.[8,9] All registered consecutive newly diagnosed pediatric tuberculosis cases (0-14 years) were recruited as cases. Children, not having any current or past history of diagnosed tuberculosis, history suggestive of ATT intake, any signs and symptoms suggestive of tuberculosis or any lower respiratory tract illness were recruited as controls. Cases and controls were recruited for over a period of six months starting from 1st July 2009 to 31st December, 2009. A study subject was considered as a passive smoker when exposed to tobacco smoke in the household at a regular basis at the time of appearance of symptoms. A semi-structured, pre-coded interview schedule was administered to parents or legal caregivers of all subjects, both cases and controls after obtaining informed written consent. The interview schedule had different parts like socio-demographic profile of the study subjects, passive smoking as well as assessment of exposure to biomass fuel, etc. It was decided that children who are active smokers, if any, would be excluded from the analysis. Socio-economic status was ascertained with the help of modified Kuppuswamy's scale[10] for residents of urban and Uday-pareek's scale[11] for rural areas. The criterion used for defining overcrowding was number of persons residing per room. The study was approved by the institute's ethics committee.

To find out association between two variables, chi-square test was computed. Data were entered in MS excel sheet and analysis was done using SPSS-16. The statistical analysis comprised calculating proportions, means and applying chisquare tests to check for the significance of difference between the cases and controls regarding passive smoking and exposure to biomass fuel. The level of significance was set as 0.05. Initially, bivariate analysis was conducted with the presence/ absence of tuberculosis as the dependent outcome. Subsequently, logistic regression analysis was performed to analyze the significant factors associated with the disease tuberculosis status of the subjects.

RESULTS

A total of 200 cases and 200 controls were recruited in the study period. None of the study subjects was reported as an active smoker.

Among the cases, there were more females (119 cases 59%) than males. Majority of the children were aged > 5yrs of age, only 18 (9%) being in the age group of 0-5 years. Minimum age of the cases was one year. A little more than half i.e. 105 (52.5%) of the patients diagnosed were extra-pulmonary tuberculosis (EP-TB) and among them the most common type was tuberculosis of peripheral lymph node , followed by pleural TB (9%) and abdominal (5%). Among all pulmonary tuberculosis, 53.7% were sputum positive pulmonary TB cases.

Using bivariate analysis (Table-1), the cases and controls seemed comparable except education of the mother (p-.001). A little less than half (48%) of the mothers in the case group was illiterate compared to 33% in the control group. The other factors which were significantly associated with development of tuberculosis were a family member having tuberculosis in the last two years and residing in the same house (OR 2.797, 95% CI 1.353-5.789; p-0.004), being a passive smoker (OR 1.725, 95% CI 1.142-2.605; p-0.009). The OR was 2.502 (95% CI 1.353-4.628) for the number of cigerettes smoked per day upto 10 compared to no cigarettes smoked (p-0.024), however the strength of association did not increase with the increased number of cigarettes smoked. The reason might be that the number of subjects in other strata was too small. No statistical significant association was found between occurrence of tuberculosis and other factors (p >0.05).

Table 1: Factors associated with development of tuberculosis

Characteristics and Category	Cases (n=200) n(%)	Controls (n=200) n(%)	Chi-square value	P value	OR (95% CI)
Resident					
Rural	46 (23)	31 (15.5)	3.619	0.057	0.614 (0.371-1.018)
urban	154 (77)	169 (84.5)			
Age					
0-5	18 (9)	24 (12)	4.961	0.084	1.00
6-10	56 (28)	72 (36)			1.037(0.513-2.096)
11-14	126 (63)	104 (52)			1.615(0.832-3.138)
Socioeconomic status					
Lower	45 (22.5)	54 (27)	3.181	0.204	1.250(0.571-2.736)
Middle	141 (70.5)	125 (62.5)			1.692 (0.825-3.469)
Upper	14 (7)	21 (10.5)			1.00
Education mother					
Illiterate	96 (48)	67 (33.5)	13.802	0.001	0.550 (0.326-0.930)
5^{th}	38 (19)	68 (34)			1.411 (0.888-2.243)
> 5th	66 (33)	60 (32.5)			1.00
Education father					
Illiterate	68 (34)	78 (39)	3.339	0.342	1.312 (0.698-2.466)
5^{th}	27 (13.5)	33 (16.5)			0.748 (0.406-1.378)
10^{th}	33 (16.5)	23 (11.5)			0.797 (0.498-1.275)
> 10	72 (36)	66 (33)			1.00
Immunization with					
BCG	123 (61.5)	117 (58.5)	0.136	0.712	1.081 (0.715-1.634)
Yes	71 (35.5)	73 (36.5)			
no	6 (3)	10 (5)			
Uncertain					
Family member having tuberculosis in last 2 years			8.211	0.004	2.797 (1.353-5.789)
Yes	28 (14)	11 (5.5)			
No	172 (86)	189 (94.5)			
Overcrowding					
Yes	148 (74)	132 (66)	3.04	0.081	1.466 (0.953-2.255)
No	52 (26)	68 (32)			
Family _smoker					
Yes	85 (42.5)	60 (30)	6.761	0.009	1.725 (1.142-2.605)
No	115 (57.5)	140 (70)			
No of cigarette/day					
0	115 (57.5)	140 (70)	9.416	0.024	1.00
1-10	37 (18.5)	18 (9)			2.502 (1.353-4.628)
11-25	33 (16.5)	29 (14.5)			1.385 (0.794-2.417)
>25	15 (7.5)	13 (6.5)			1.405 (0.642-3.072)
Place of cooking					
In a room used for living and sleeping	103 (51.5)	89 (44.5)	4.040	0.257	0.952 (0.537-1.688)
					0.731 (0.425-1.259)
Separate room used as kitchen	64 (32)	72 (36)			1.00
Open place	33 (16.5)	39 (19.5)			
Type of fuel used					
LP gas	116 (58)	129 (64.5)	1.780	0.218	0.760(0.508-1.138)
biomass fuels	84 (42)	71 (35.5)			

*** The category "uncertain" was not taken into consideration for tests for statistical significance*

A multiple logistic regression analysis was done (Table-2) including the factors which were significant in bi-variate analysis like education of mother, being a passive smoker and family member having tuberculosis in the past two years. The variable number of cigarettes smoked per day was not included in the final model as no change in dose response was found among different strata. The factors which were significant are being a passive smoker (OR 1.752 95% CI 1.154-2.662; p 0.009) and family member having tuberculosis in the past two years (OR 2.804, 95% CI 1.343-5.850; p 0.006).

Table 2: Result of multiple logistic regression

Characteristics and Category	P value	OR (95% CI)
Education mother		
Illiterate	0.177	1.327(0.929-1.489)
5th		
> 5th		
Family member having tuberculosis in last 2 years		
Yes	0.006	2.804 (1.343-5.850)
No		
Passive Smoking		
Yes	0.009	1.752 (1.154-2.662)
No		

DISCUSSION

This study is one of the very few studies that try to find out an association between passive smoking and clinical tuberculosis in patients less than 15 years of age. Though the underlying biological mechanism is still not very clear, some other studies also found an positive association between passive smoking and clinical tuberculosis.[13-16] Ariyothai N *et al*[14] reported having exposure to passive smoking and risk of developing tuberculosis among adults as strong (OR = 4.62, 95% CI = 1.68-14.98). Alert *et al*[16] also reported passive smoking as a risk factor for Pulmonary Tuberculosis among children (OR: 5.29; 95% CI: 2.33-12.82; P < 0.00005).Results from our study also corroborated these former reports. A recent meta-analysis reported that the risk of TB among children exposed to passive smoking was significantly higher than it was among adults (p-0.002).[17] Alert *et al*[16] also reported increased TB risk with the number of cigarettes smoked by the household members per day. However, we could not find a dose response association stratified on exposure intensity. A probable reason might be very few number of study subjects in each stratum.

We did not get a positive association between biomass cooking fuel use and TB disease like the study done by Shetty *et al*[18] in South India while other studies[19-21] have found a positive association. A case control study done by Behera *et al*[22] also did not find any association between type of fuel use and tuberculosis. Sixty-one percent of the current study population have used a non-biomass fuel like Liquid petroleum Gas (LPG) as cooking medium and more than 80% of study population were residents of urban area. These characteristics might have some effect on study findings related to biomass fuel use and need further exploration.

The present study being case control in nature has all the limitations of such a study. Recall bias may distort the associations, as cases may have better recall than control subjects. Ability to recall also could have affected the result of dose relationship association as our study was not supported by any laboratory tests as a biomarker. Temporality also could not be ascertained which is a major inherent limitation of case control study design.

Majority of the patients had EP-TB and sputum smear-negative PTB, there are possibilities of misclassification of TB diagnosis. However, with the study done in a tertiary care hospital and the cases diagnosed by a pediatrician following diagnostic algorithm for pediatric TB suspects as per RNTCP guidelines, it is believed that misclassification, if any, is likely to be insignificant.

Selection of controls from those attending other clinics has limitations of having the potential of exposure to TB. This was ruled out when the potential control subject was screened for TB.

Furthermore, the sample size in our study may not have been large enough to have sufficient

power to detect significant differences in the sub-group.

CONCLUSION

Passive smoking is associated with development of tuberculosis in patients less than 15 years of age. Health Education Programmes and tobacco cessation intervention services should be utilized under National Tobacco Control Programme. Further studies, especially follow-up in nature, need to be conducted to establish temporality of passive smoking and development of clinical tuberculosis.

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Original Article

INHIBITORY EFFECT OF ISONIAZID AND ORLISTAT COMBINATION ON MYCOBACTERIAL ES-31 SERINE PROTEASE *IN VITRO* AND ON THE GROWTH OF *M.TB* BACILLI IN AXENIC CULTURE[@]

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Summary

***Background*:** Isoniazid and orlistat were reported to have inhibitory effect on mycobacterial ES-31 serine protease *in vitro* and bacterial cell growth in axenic culture.

***Aim*:** To study the cumulative effect and understand drug - drug interaction, if any, when isoniazid and orlistat used in combination.

***Material and Methods*:** Inhibition of mycobacterial ES-31 serine protease by different combinations of orlistat and isoniazid together and individually were studied using azocasein assay. Inhibition of secretion of excretory secretory ES-31 antigen in Sautan culture medium was studied under axenic condition and growth of *M.tuberculosis* H_{37}Ra bacilli by CFU count on LJ-medium.

***Results*:** Orlistat and isoniazid both showed inhibitory activity of ES-31 serine protease in *in vitro* as well as *in vivo*. Individually, isoniazid showed 90% inhibition at 200 ng/ml while orlistat at 250 ng/ml showed 65% inhibition of mycobacterial ES-31 serine protease *in vitro*. A combination of orlistat (250 ng/ml) and isoniazid (200 ng/ml) showed 86% inhibition *in vitro* while 73% inhibition was observed by orlistat (25 ng/ml) and isoniazid (200 ng/ml) on bacterial growth in axenic culture.

***Conclusion*:** Significant inhibition by orlistat suggests that it could be tried in patients with intolerance to isoniazid or in those already developed isoniazid resistance. It may also be explored in the suspected TB patients as initial medication in place of antibiotics for clinical relief. **[*Indian J Tuberc 2012; 59:* 156-161]**

Key words: Mycobacterial ES-31, Serine protease, Orlistat, Isoniazid, Inhibition

INTRODUCTION

Drug resistance is becoming a serious problem in tuberculosis control in recent times. Resistance to the first line anti-tuberculosis drugs is observed in the clinical isolates soon after their introduction[1]. Most *M. tuberculosis (M.tb)* clinical isolates are resistant to more than one anti-TB drug and to isoniazid[2]. Isoniazid is shown to target 2-trans-enoyl carrier protein reductase i.e. InhA. Interaction of isoniazid with InhA prevents the biosynthesis of mycolic acid, a major lipid in mycobacterial envelope[3].

Excretory-secretory-31 (SEVA TB ES-31) antigen with protease and lipase activities, isolated from culture filtrate of *Mtb* H_{37}Ra bacilli, has been shown to be a biomarker in the diagnosis of pulmonary and extra-pulmonary tuberculosis[4]. Earlier study from this laboratory reported inhibitory activity by serine protease and metallo-inhibitors like Pefabloc, 3,4-dichlorocoumarin, Phenyl Methyl Sulphonyl Fluoride (PMSF), Ethylene Diamine tetracetic acid (EDTA), 1,10 phenanthroline and first line anti-TB drug i.e. isoniazid on mycobacterial ES-31 serine protease *in vitro* and also on *M. tb* bacilli growth in axenic culture and in infected human

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macrophages[5]. Anti-obesity drug, orlistat was also shown to inhibit ES-31 serine protease *in vitro* and in axenic culture[6]. Further, it was reported that orlistat (a lipase inhibitor) and PMSF, EDTA (metallo- and serine protease inhibitors) inhibit the protease as well as lipase activities of ES-31 serine protease confirming the presence of common catalytic triad at active site similar to subtilisin[7].

It will be of interest to study whether we can replace isoniazid or decrease its dose in the presence of Orlistat. In this communication, we report the inhibitory effect of combination of isoniazid and Orlistat on mycobacterial ES-31 serine protease *in vitro* and in axenic culture.

MATERIAL AND METHODS

Isolation of mycobacterial ES-31 serine protease

Culture filtrate protein was obtained from *M. tuberculosis* H_{37}Ra bacilli grown in thyroxine-supplemented Sauton medium for 10 days as described earlier[8]. ES-31 serine protease was isolated from culture filtrate protein by affinity chromatography using anti ES-31 serine protease antibody raised in goat. Briefly, monospecific anti ES-31 serine protease antibody was coupled to the Sepharose 4B column (1 cm) and *M. tuberculosis* culture filtrate protein (1 mg) was applied to the column and washed with 0.01 M phosphate buffer saline (PBS pH 7.2). Bound ES-31 serine protease was eluted with Glycine-HCl buffer (pH 2.5) and neutralized with Tris-HCl buffer (pH 8.6).

Isolation of anti ES-31 serine protease antibody from anti-DSS IgG raised in goat

Detergent Soluble Sonicate (DSS) antigen was prepared from *M. tuberculosis* H_{37}Ra bacilli. Briefly, bacilli were inactivated by 5% phenol in 0.5 M phosphate buffer (PBS, pH 7.2) and incubated with Sodium Dodecyl Sulphate (SDS) extraction buffer. The supernatant was dialysed against 0.01M PBS, pH 7.2 and used as an antigen source[9]. Anti-DSS IgG antibodies were raised in goat by immunization with 500 µg protein/ml DSS antigen with 1 ml Freund's incomplete adjuvant on days 0, 20, 33 and 45. Immune sera were collected on days 32, 44, 57, 60 and thereafter fortnightly and anti-DSS IgG was isolated by 33% saturation with ammonium sulphate under ice, followed by diethyl amino ethyl-cellulose ion exchange column chromatography as described earlier[9]. Anti ES-31 serine protease antibody was isolated from anti-DSS IgG by affinity chromatography using ES-31 serine protease coupled Sepharose-4B column[10].

Study of orlistat and isoniazid on protease activity of mycobacterial ES-31 serine protease in vitro

The effect of orlistat and isoniazid on the proteolytic activity of ES-31 was studied using azocasein as protease substrate as described earlier[5]. In brief, incubation mixture of 100µg ES-31 protein in 5ml of 0.5M Sodium Bicarbonate buffer (ph 8.3) was incubated at 37^0C for six hours. Further, 1ml aliquot solution was removed and added to 4 ml of trichloroacetic acid (5%). After mixing and filtration using 0.45 µm syringe filters, again 1ml aliquot was removed and 3ml of 500 mM NaOH solution was added to the solution. Decrease in absorbance of the orange color was measured at 440 nm using a spectrophotometer (Ultra-spec, Elico Ltd, India). To study the effect of protease inhibitors on mycobacterial ES-31 serine protease, enzyme (100 µg) was incubated in the presence of drugs at different concentrations from half to 50 times of its therapeutic dose of Orlistat and from 1/4th to the critical concentration of Isoniazid[11] at 37^0C for one hour followed by azocasein assay of protease activity[5]. Azocasein assay without protease inhibitor incubation served as control.

Peroxidase Sandwich ELISA for Quantitation of secretory ES-31 serine protease in culture filtrate

To study the effect of orlistat and isoniazid on the secretion of mycobacterial serine protease (SEVA TB ES-31 antigen) in axenic culture, the concentration of ES-31 antigen was determined in culture filtrate by sandwich peroxidase ELISA using anti ES-31 antibody[4]. In brief, the wells of microtitre plate (NUNC) were sensitized with optimal concentration of anti ES-31 antibody 50 µg/100µl/ well in 0.06 M carbonate buffer pH 9.6 overnight at 4^0C followed by blocking with BSA (1%) for two

hours at 37^{0}C. The plate was washed twice with PBS containing 0.05% Tween 20 (PBS/T) followed by addition of 100 µl of culture filtrate and incubated for one hour at 37^{0}C, followed by three washes. Then the wells were exposed to 1:1000 diluted goat anti ES-31 antibody IgG Peroxidase conjugate for one hour at 37^{0}C, followed by three washes. The colour was developed using TMB substrate and reaction stopped using 50µl stop solution (2N H_2SO_4). The optical density was measured at 450 nm with ELISA reader. Assay was done in triplicate. Using the standard graph with purified ES-31 antigen, concentration of ES-31 antigen (ng) was determined in culture filtrate.

Study of orlistat and Isniazid on M. tuberculosis bacilli in axenic culture: (in vivo)

The effect of orlistat and isoniazid was studied on secretion of ES-31 serine protease by *M. tuberculosis* H_{37}Ra bacilli culture in Sauton medium. For the preparation of experimental cell suspension as the inoculum, *M. tuberculosis* H_{37}Ra bacilli were grown in thyroxine supplemented Sauton medium at 37^0 C for 10 days. Rapidly growing cells were harvested by centrifugation at 4000 g at 4^{0}C for 20 minutes. The first-line anti-TB drugs, isoniazid and orlistat, were added individually and at different concentrations of combination of both the drugs to the Sauton medium (100 ml) with inoculums, in sterile 250 ml Erlenmeyer flasks and kept for incubation at 37^{0}C for 10 days. Culture medium with inoculum but without inhibitor served as control. At the end of 10th day, the concentration of ES-31 serine protease was determined in culture filtrate by sandwich peroxidase ELISA using anti ES-31 serine protease antibody as described earlier[4]. To measure the number of colony forming units (CFU/ml), the bacterial suspension in the Sauton medium was dispersed and serially diluted to a final dilution of 10^2. A loopful of diluted bacterial suspension (0.005ml) was inoculated on LJ agar. After incubation for two weeks, CFU were counted.

RESULTS

Orlistat alone at 250 ng/ml, 25 ng/ml, 10 ng/ml, 5 ng/ml and 2.5 ng/ml showed 65%, 60%, 35%, 33% and 30% inhibition of mycobacterial ES-31 serine protease respectively while isoniazid alone at 200 ng/ml, 100 ng/ml and 50 ng/ml showed inhibition of 90%, 61% and 41% *in vitro* respectively. Orlistat at maximum concentration of 250 ng/ml showed 65% inhibition of mycobacterial ES-31 serine protease while isoniazid showed inhibition of 89% at its critical concentration of 200 ng/ml *in vitro*. Combining these two drugs, ES-31 serine protease showed inhibition of 86% at orlistat 250 ng/ml and isoniazid 200 ng/ml and 81% at orlistat at 25 ng/ml and isoniazid 200 ng/ml (Table 1). Orlistat, up to 25 ng/ml showed *in vitro* inhibition below 40% and therefore it was not considered for axenic culture study. For axenic culture study, orlistat at additional concentration of 125 ng/ml i.e. in between 25 ng/ml

Table 1: Study of inhibition of ES-31 serine protease at different concentrations of Orlisat and Isoniazid *in-vitro*

Drug (ng/ml)	**Activity of ES-31 serine protease (Units/mg protein)$^{\#}$ (% inhibition)**					
Orlistat → / ↓ Isoniazid	0	2.5	5	10	25	250
0	*C16.7 (0)	11.7 (30)	11.1 (33)	11 (35)	6.7 (60)	**5.8 (65)**
50	9.72 (41)	-	-	-	-	-
100	6.52 (61)	-	-	-	6.5 (61)	5.58 (67)
200	**1.63 (90)**	-	-	-	**3.02 (81)**	**2.22 (86)**

* Control contained 100 µg ES-31 antigen without any inhibitor

$^{\#}$ Unit of activity represents A_{440} × 1000/mg protein/min.

- Not Done

Table 2A: Study of inhibition of ES-31 serine protease secretion in the culture filtrate at different concentrations of Orlisat and Isoniazid.

Drug(ng/ml)	**Concentration of ES-31 (ng/ml) in culture filtrate (% inhibition of secretion)[#]**			
Orlistat →	0	25	125	250
↓ Isoniazid				
0	*C 70 (0)	40 (42.9)	34 (51.4)	**29 (58.6)**
100	38 (54.7)	33 (52.85)	**25 (64.2)**	**22 (68.6)**
200	**12 (82.9)**	**22 (68.6)**	49 (44.9)	31 (55.7)

Table 2B: Study of inhibition of ES-31 serine protease at different concentrations of Orlisat and Isoniazid in axenic culture

Drug(ng/ml)	**CFU count (% growth inhibition) [$]**			
Orlistat →	0	25	125	250
↓ Isoniazid				
0	*C 16.6×10^5(0)	6.6×10^5 (60)	**5.6×10^5 (66)**	6.8×10^5 (59)
100	6.8×10^5 (59)	7.8×10^5 (53)	8.6×10^5 (48)	7×10^5 (58)
200	**2.4×10^5 (85)**	**4.4×10^5(73)**	**4.6×10^5 (72)**	**4.8×10^5 (71)**

* *Control contained 100 µg ES-31 antigen without any inhibitor.*

[#] *Quantitation of mycobacterial ES-31 serine protease in the culture fluid was done by peroxidase sandwich ELISA. Amount of anti ES-31 serine protease antibody coated: 50 µg/well and Goat anti-ES-31 serine protease antibody IgG peroxidase conjugate dilution: 1:1000 were used.*

[$] $CFU/ml\ of\ culture = \frac{number\ of\ colonies \times dilution\ factor}{volume\ of\ culture\ suspension}$

Dilution factor = 10^2 and Volume of culture suspension = 0.005ml.

and 250 ng/ml were explored. Inhibition of secretion of ES-31 serine protease was observed to be maximum i.e. 68.6% at two drug combinations i.e. orlistat at 25 ng/ml and isoniazid at 200 ng/ml as well as at orlistat 250 ng/ml and isoniazid at 100 ng/ml (Table 2A). In axenic culture, orlistat at 125 ng/ml showed 66% and isoniazid at 200 ng/ml showed 85% inhibition. In combination, maximum inhibition of 73% was observed with orlistat at 25 ng/ml and isoniazid at 200 ng/ml (Table 2B).

DISCUSSION

Resistant strains of *Mycobacterium tuberculosis* that are insensitive to first-line antitubercular drugs have emerged worldwide causing concern for the successful control of the tuberculosis - world's leading cause of death from a single infectious agent. *Mycobacterium tuberculosis(M.tb)* and other pathogenic mycobacteria secrete a large number of proteins into their extracellular milieu when growing either axenically or within phagosomes of host cells. These extra-cellular proteins are worthy targets for development of new drugs, as observed in the case of secreted *M.tb* Glutamine synthetase[12].

Mycobacterial ES-31 serine protease is secreted in culture medium and inhibition of this enzyme by serine protease inhibitors or specific

antibody inhibited the secretion and affected growth of bacilli in axenic culture as well as in infected macrophages, showing drug target potential of this enzyme. Interestingly, maximum inhibition (95%) of mycobacterial ES-31 serine protease was observed by isoniazid, one of the antitubercular drugs[5].

Isoniazid is a prodrug activated by *M.tb* KatG. The activated isoniazid acts as an inhibitor of acyl carrier protein reductase, inhA and prevents biosynthesis of mycolic acids, major lipids of mycobacterial envelope[13,14]. High level of isoniazid resistance is caused by mutation in KatG gene, which diminishes the activation of isoniazid. However, low level resistance is caused by point mutation in the regulatory region of inhA operon[15]. If frequent laboratory testings are not followed to the patients under anti-TB drugs, the patient is at risk of developing severe life-threatening complications[16]. Isoniazid along with first-line anti-tuberculosis drugs are metabolised by liver. Many of the metabolites of isoniazid are suggested as hepatotoxic. Thus, isoniazid and pyrazinamide cause hepatic dysfunction[17,18]. Mycobacterial ES-31 serine protease (similar to chymotrypsin or subtilisin serine protease) shows lipase activity and the catalytic activity was 100% inhibited by orlistat, a lipase inhibitor and an anti-obesity drug[7]. The binding of orlistat to the active site of pancreatic lipase leads to some conformational changes abolishing its activity. Orlistat inhibits >90% enzyme activity of gastric lipase, carboxylester lipase and phospholipase A2 *in vitro* and *in vivo*. Along with mild intestinal adverse effects, orlistat also causes diarrhoea, flatulence, oily spotting and fecal incontinence but it has no effect on gastric or pancreatic secretion and gastric emptying time[19].

Our aim was to find out whether there is any synergistic effect of using both the drugs, isoniazid and orlistat or whether the latter can be substituted for isoniazid, in treatment of tuberculosis. The study showed that orlistat at a concentration of 125 ng/ml inhibited bacterial growth by 66% in axenic culture, while addition of isoniazid (200 ng/ml) showed 72% inhibition. Isoniazid alone at 200 ng/ml inhibited bacterial growth by 85%. This is confirmed further by similar effect of these drugs on mycobacterial ES-31 serine protease *in vitro* (Table 1) and its secretion in culture medium (Table 2A). When orlistat was combined with isoniazid, there was no synergistic effect. However, inhibition was decreased suggesting that there is competitive inhibition between orlistat and isoniazid for the same active site of ES-31 as reported earlier[7]. **This study suggests that orlistat may be substituted for Isoniazid in Isoniazid resistant tuberculosis. Further, orlistat may be tried in place of antibiotic trial in suspected TB patients.**

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Case Series

FINE NEEDLE ASPIRATION CYTOLOGY IN BREAST TUBERCULOSIS : DIAGNOSTIC DIFFICULTIES – STUDY OF ELEVEN CASES

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Summary

***Background*:** Incidence of extra-pulmonary tuberculosis is on the rise. Tuberculosis of breast is rare and have multifaceted clinical presentation, often mimicking carcinoma and pyogenic breast abscess.

***Aim*:** To study morphologic variations and diagnostic difficulties of breast tuberculosis on fine needle aspiration cytology (FNAC).

***Methods*:** FNAC smears of breast tuberculosis were studied by Leishman's Stain and categorized into four groups. Cytology smears were also studied for presence of Acid Fast Bacilli (AFB) by Ziehl Neelsen (ZN) stain. Histology of excised tissue was studied by Hematoxylin and Eosin stain (H& E).

***Results*:** Out of 11 cases, 10 were females and 1 was male. Group1 (n=2) showed epithelioid granulomas with necrosis. Group2 (n=2) showed epithelioid granulomas without necrosis. Group3 (n=3) showed necrosis with a few scattered epithelioid histiocytes. Group4 (n=4) showed necrosis with numerous neutrophilic inflammatory cells. In four cases, caseous necrosis could be identified on cytology smears. AFB were found in five cases on FNAC smears. Histology confirmed diagnosis of tuberculosis in all cases.

***Conclusion*:** In developing countries like India, based on clinical history and other features, FNAC smears showing epithelioid granulomas with or without necrosis should be considered as breast tuberculosis as demonstration of AFB is not mandatory. Identification of caseous necrosis alone is diagnostic of breast tuberculosis in cytology smears. **[*Indian J Tuberc 2012; 59:* 162-167]**

Key words: Fine needle aspiration (FNA), Acid Fast Bacilli (AFB), Ziehl Neelsen (ZN), Breast Tuberculosis

INTRODUCTION

Tuberculosis is a worldwide problem and it is estimated that approximately 8-10 millions of new cases and two-three millions of death from tuberculosis occur every year.[1] Extra-pulmonary tuberculosis is on the rise world over. Tuberculosis of breast is relatively a rare occurrence with reported incidence varying from 3-4.5% in developing countries like India.[2]

Because of its multifaceted presentation, clinicians may confuse tuberculous mastitis with either breast abscess or carcinoma. First case of mammary tuberculosis was recorded by Sir Astley Copper in 1829 and he called it 'scrofulous swelling of bosom'.[3] FNAC is very well established as a diagnostic modality for elucidating the aetiology of breast lumps and definitive therapy is instituted on the basis of FNAC results.[4] Few reports dealing with the cytomorphology of breast tuberculosis are published.[5,6] Experience with FNAC diagnosis of breast tuberculosis in 11 cases is reported.

MATERIAL AND METHODS

Eleven cases were included in the study from January 2005 to December 2010 in which diagnosis of tuberculous mastitis was established by FNAC and culture examination / or Histopathology. Ten cases presented as painless slowly growing palpable lump and one case as tender lump in the breast. Three cases had axillary lymphadenopathy on same side of breast lesion. FNAC was requested for diagnosis. Aspirates were obtained using 22 gauge needle connected to 10 ml of disposable syringe. Three slides were stained using Leishman stain and two slides were subsequently stained by ZN stain for presence of AFB. Culture examination was possible in five cases only due to inadequate

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quantity of aspirate in remaining cases. In all, 11 cases' biopsy tissue was sent for histological examination. Tissue was fixed in 10% formalin. Paraffin blocks were prepared and four micrometer thick sections were cut. Sections were stained with H&E and ZN stain. Grocott-Gomori methanamine silver stains were done to rule out fungal infections. Diagnosis of tuberculous mastitis in histology sections was based on following criteria:

(1) Characteristic histologic features of tuberculosis such as epithelioid granulomas, caseous necrosis with or without AFB.

(2) Extensive suppurative necrosis and occasional caseating epithelioid granuloma with or without AFB.

RESULTS

Clinical presentation, cytological and histological profile of 11 cases are summarized in Table. Out of 11 cases, 10 were females and 1 was male. The commonest age group affected was 21-40 years (n=8), followed by 61-70 years (n = 3). Right breast was involved in eight cases and left breast in three cases. One patient was lactating. Definitive diagnosis of breast tuberculosis was established on FNAC in five cases based on morphologic features and presence of AFB, while the diagnosis of remaining six cases was suggested on FNAC smears. All cases were subsequently confirmed on histologic examination as tuberculosis.

Morphologic features observed in all cases were classified into four groups based on cytology:

Group 1: Epithelioid granulomas with necrosis. (n=2)

Group 2: Epithelioid granulomas without necrosis. (n=2) (Figure 1)

Group 3: Only necrosis with a few scattered epithelioid histiocytes. (n=3)

Group 4: Necrosis with a few scattered epithelioid histiocytes and numerous neutrophilic inflammatory cells (n=4).

Overall, necrosis was seen in nine cases out of which four cases (two each in Groups 1 and 3) showed caseous necrosis which was typically

Table: Clinical, Cytological and Histopathological Profile of 11 cases

Case no.	Age	Sex	Clinical presentation			F.N.A.C.						Histopathological Findings	
			Ste	Symptom	Location	Group	Granuloma	Epithelioid Histiocyte	Necrosis	Neutrophils	AFB	H&E	AFB
1	40	F	Rt.	Painless lump	-	I	+	+	Caseous	-	-	CEG	-
2	22	F	Lt.	Painful lump	+	II	+	+	-	-	-	CEG	-
3	35	M	Lt.	Painless lump	-	IV	-	Occasional	+	+	+	SN CEG	+
4	40	F	Rt.	Painless lump	-	III	-	+	+	-	+	CEG	+
5	70	F	Rt.	Painful lump	-	I	+	+	Caseous	-	-	CEG	-
6	29	F	Rt.	Hard lump	-	IV	-	Occasional	+	+	-	SN CEG	-
7	30	F	Lt.	Painless lump	-	IV	-	Occasional	+	+	+	SN CEG	+
8	75	F	Rt.	Painless lump	-	II	+	+	-	-	-	CEG	-
9	65	F	Rt.	Hard lump	-	III	-	+	Caseous	-	-	CEG	+
10	40	F	Rt.	Painless lump	-	IV	-	Occasional	+	+	+	SN CEG	+
11	40	F	Rt.	Painless lump	-	III	-	+	Caseous	-	+	CEG	+

Abbreviations: CEG = Caseating epithelioid granuloma
SN = Suppurative necrosis

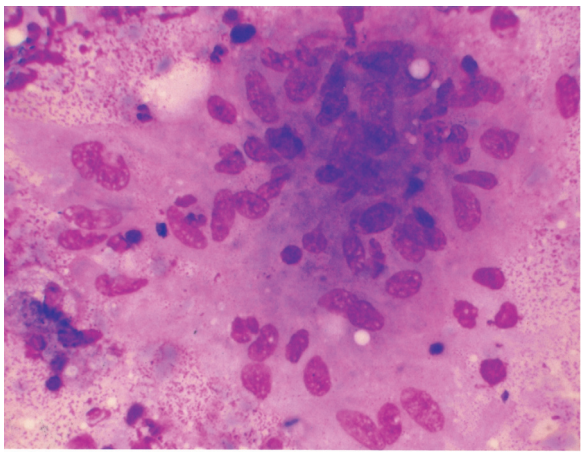

Figure 1: Photomicrograph showing epithelioid granulomas.(Leishman, x 400)

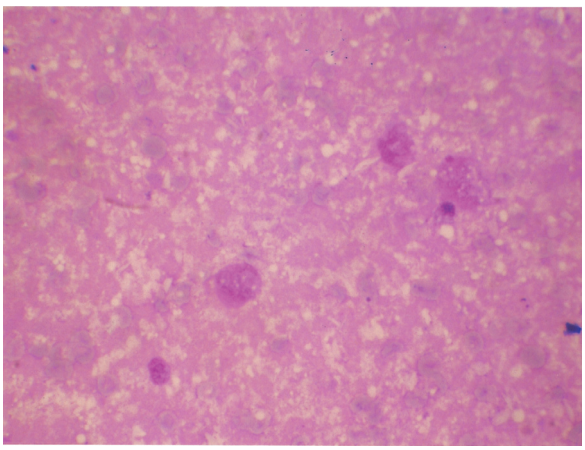

Figure 2: Photomicrograph showing acellular, granular caseous necrosis. (Leishman, x 400)

acellular, granular with loss of cellular details (Figure 2). In remaining five cases, necrosis was in the form of necrotic debris and fragments of necrotic epithelial cells (Figure 3). ZN stain was done in all cases. AFB were found in five cases (45%), two cases in Group 3 and three cases in Group 4. FNAC smears from draining axillary lymph nodes were available in one case (Group 4) who was male. Morphology of ductal cells in all cases was unequivocally benign except in one case (Group 4) where occasional ductal epithelial cells showed large atypical nuclei. Histology of all 11 cases was studied. Group1 (n=2), Group 2(n=2) and Group3(n=3) showed characteristic histologic features of tuberculosis. Group 4 (n=4) cases showed extensive suppurative necrosis with occasional areas showing epithelioid granuloma, caseous necrosis and Langhan's giant cells. AFB positivity on histology sections was seen in six cases.

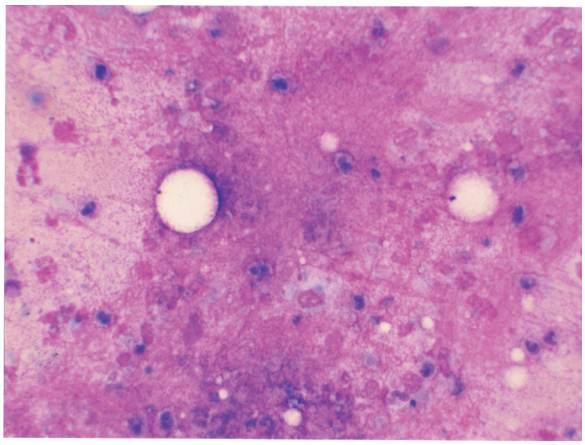

Figure 3: Photomicrograph showing necrotic debris and fragments of necrotic epithelial cells (Leishman, x 400)

DISCUSSION

Breast is not a common site for tuberculosis even in countries like India where it is rampant. It is more common in females than males and can present as a breast mass. It usually affects women in the reproductive age group, 21-40 yrs.[2,7,8] This fact was brought out in the present study as 10 were females and one was male and maximum cases (n=8) in the age group 21–40 years followed by age group 61-70 years (n=3). Constitutional symptoms like fever, weight loss and night sweats were present in two cases only. Though lactation makes the breast more vulnerable to tuberculosis,[7] only one case in our study was lactating. Primary tubercular infection of the breast may occur through skin abrasions or through duct openings of the nipple, but it is generally believed that infection of

the breast is secondary to tuberculous focus elsewhere in the body which may not be clinically or radiologically apparent. Such a focus could be pulmonary or a lymph node in cervical, mediastinal, paratracheal, intestinal, mammary or axillary region. Involvement in such cases can be hematogenous or by retrograde lymphatic extension[9]. None of our cases showed clinical or X ray findings suggestive of pulmonary tuberculosis probably unable to detect true nidus of the disease. Vassilakos P has cautioned against making diagnosis of primary disease, just because clinicians are unable to detect the true nidus of the disease in most of the cases.[10]

Breast tuberculosis has three clinical types – nodular, sclerosing and disseminated.[2,7] The commonest variant is nodular. It presents as well circumscribed slowly growing painless lump and at later stages it becomes ulcerated and produces multiple discharging sinuses. Sclerosing variant affects older women and presents as painless, hard, slowly growing mass and may cause nipple retraction. Disseminated variant presents as indurated and painless lump with frequent involvement of axillary lymph nodes and often develops numerous discharging sinuses. Most common clinical presentation in our study was also nodular in seven cases followed by sclerosing and disseminated in two cases each. Mammography remains inconclusive in breast tuberculosis[2,7] which happened in our cases. Out of 11 cases, mammography was done in six cases. Five cases showed dense round to oval mass with indistinct margins. One case revealed increased asymmetric density with speculated margins and axillary lymphadenopathy. Diagnosis of inflammatory changes was given in five cases and benign neoplastic lesion was offered in one case. Traditionally, biopsy with histologic examination has been used to establish diagnosis. In recent Years, FNAC has been found to be of great utility in diagnosing breast tuberculosis. Though mycobacterial culture is a gold standard for diagnosis, it is often negative due to sparse viable organisms. Due to inadequate quantity of aspirate, culture examination was done in five cases only, out of which one case was AFB positive on cytology smears. Two cases showed growth of AFB on Lowenstein Jensen medium. Polymerase chain reaction (PCR) is rapid and specific but have low sensitivity in AFB negative cases. Some reports delineating cytomorphology of breast tuberculosis reported that diagnosis can be given confidently with epithelioid cell granulomas, necrosis and AFB in cytology smears. Some reports even state that with epithelioid cell granulomas and caseous necrosis in FNAC smears, AFB positivity is not mandatory.[11-13]

The commonest cause of caseation in developing countries like India is Tuberculosis. Detection of caseous material in cytology smears depends on experience of the pathologists. It is typically seen as acellular, granular material with loss of cellular details.[14] In our study, four cases (two each in Groups 1 and 3) showed caseous necrosis on cytology smears enabling in to make a definitive diagnosis of tuberculosis. We feel that typical morphology of caseous necrosis with or without epithelioid granulomas in the absence of AFB on cytology smears is sufficient to make a definitive diagnosis of Tuberculosis. Overall, AFB positivity in cytology smears was quite high in our study (five cases) where necrosis was more in Group 3 (n=2) and Group 4 (n=3). On histology, it was slightly higher (six cases) which was attributed to the quantity of the samples.

Breast tuberculosis though much more common in females, it can occur in males. One case was male and showed the same cytologic findings as in female breast.

Group 1 cases (n=2) faced no difficulties in diagnosing on cytology smears since commonest cause of caseating granulomas in developing country like india is tuberculosis.

Group 2 cases (n=2) showed only epithelioid granulaomas, such cases need to be carefully distinguished from other causes of granulomatous mastitis especially duct ectasia, fat necrosis and mycotic mastitis.

In a recent report published by Raj K Gupta in New Zealand,[15] who studied 12 cases of granulomatous mastitis showing non-caseating

granulomas, multinucleate giant cells, lymphocytes, plasma cells and variable number of neutrophils were seen on FNAC smears. Since no primary aetiological agent was found on microbiologic or cytohistologic study, an autoimmune pathogenesis was suggested. Failure to demonstrate necrosis on FNAC smears does not exclude tuberculosis in view of small sample harvested and examined. Detection of AFB is not mandatory since their number must be 10,00 to 100,000/ml of the material to be detected by light microscopy. Both the cases in Group 2 were AFB negative on cytology smears and subsequently confirmed on histology as tuberculosis. Fungi were not detected on fungal stains and both the cases responded well to standard antituberculous treatment. Some authors emphasize that in the absence of well formed epithelioid granulomas, presence of epithelioid histiocytes is the single most common indicator of granulomatous inflammation. Strong suspicion of tuberculous pathology is required which happened in Group 3 cases. Out of three cases, two cases showed AFB on cytology smears which enabled definitive diagnosis. Though third case was AFB negative on cytology smears, it enabled strong suspicion of tuberculosis. Group 4 cases (n=4) where abscess like picture dominated by neutrophilic inflammatory cells was seen on FNAC smears , three cases showed AFB on smears. In remaining one case, scattered epithelioid histiocytes were seen which enabled strong suspicion of tuberculosis (Figure 4).

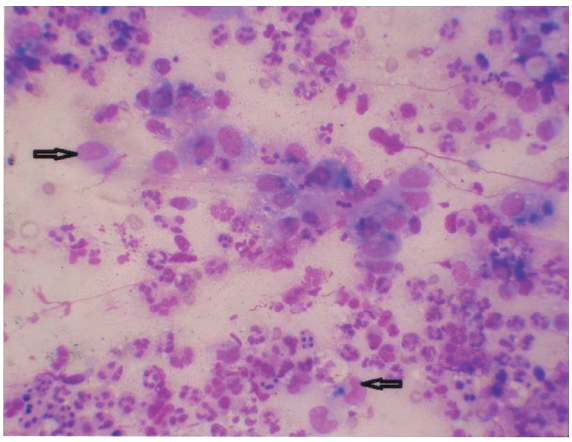

Figure 4: Photomicrograph showing neutrophils and scattered epithelioid cells. (arrow) (Leishman, x 400)

Out of 11 cases, eight cases received standard antitubercular treatment for six months (Category I) as per Revised National Tuberculosis Control Programme (RNTCP). Initial intensive phase with four drugs (Isoniazid, Rifampicin, Pyrizinamide and Ethambutol) for two months was given. Out of eight cases, six cases revealed clinical improvement. They were then treated with continuation phase for four months using two drugs (Isoniazid and Rifampicin). Two cases in which little improvement was seen clinically, category III atitubercular treatment was given. One case was lost for follow up.

The significance of breast tuberculosis is due to its rare occurrence and mistaken identity with breast cancer and pyogenic breast abscess. With the spread of AIDS worldwide and re-emergence of tuberculosis in developing countries like India, breast may become a major site of extra-pulmonary tuberculosis next to lymph node.

CONCLUSION

All our cases of epithelioid granuloma with or without necrosis and negative AFB on FNAC were confirmed as tuberculous mastitis on histology. Hence, we conclude that in developing countries like India, based on clinical history and features of epithelioid granulomas with or without necrosis and AFB negative, FNAC smears may be given a therapeutic trial of standard antitubercular drugs. Demonstration of AFB is not mandatory on FNAC explained by the fact that extrapulmonary sites usually contain only a few organisms. Cases in which abscess like picture is dominated by acute inflammatory cells, epithelioid histiocytes should be carefully searched for strong suspicion of tuberculosis. We also conclude that only typical morphology of caseous necrosis on cytological smears alone is sufficient to make definitive diagnosis breast tuberculosis.

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Case Report

SPINAL TUBERCULOSIS IN AN INFANT ASSOCIATED WITH MATERNAL URINARY TUBERCULOSIS.

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Summary: A ten-month-old infant who presented with regression of milestones and seizures was noted to have a gibbus deformity in the upper thoracic region. She was diagnosed to have spine and central nervous system tuberculosis by culture of pus from the paravertebral abcess which showed a growth of *Mycobacterium tuberculosis*. The mother, who had been having recurrent episodes of Urinary tract infection, was diagnosed to have Urinary TB proven by culture. Spinal tuberculosis, though rare, can be encountered in infancy and should be kept in mind while treating infants presenting with related symptoms. **[*Indian J Tuberc 2012; 59:* 168-170]**

Key words: Spinal tuberculosis, Spine TB, Infants, Maternal Urinary Tuberculosis

INTRODUCTION

Tuberculosis involving the spine is an infrequently encountered condition in infants. To date, there are a few cases reported in literature . A ten-month-old infant with spine and central nervous system tuberculosis with an unusual association of maternal urinary tuberculosis is reported.

CASE REPORT

A ten-month-old infant was referred with concerns of regression of milestones and seizures. She was the first child born to non-consanguinous parents of upper middle class strata and had been conceived spontaneously. During the antenatal period, her mother had five episodes of symptomatic urinary tract infection for which she was treated with antibiotics. The baby was born by lower segment caesarean section with a birth weight of 2.6 kg. Till five months of age, her development and weight gain had been appropriate. She had received all vaccines including BCG at birth as per recommendations. At six months of age, she was noted to have decreased movements of the lower limbs for which MRI brain was done which was normal. The following month, the mother noted that she was less alert and was unable to follow objects. At this point, she was diagnosed to have biotinidase deficiency and was started on biotin supplements. Her condition however worsened gradually and at about ten months of age, she developed refractory seizures and was referred for further management.

On examination, she was emaciated, dehydrated and having generalized tonic clonic seizures. GCS was 5/15, respiratory rate 64/min, heart rate 106/min and weight was 4.5 kg. A gibbus deformity was noted in the upper thoracic region. CNS examination revealed a right-sided divergent squint, hypertonia and exaggerated reflexes in all four limbs and bilateral ankle clonus. Liver was palpable 6 cm below right costal margin and spleen was palpable 5 cm below left costal margin. Bilateral crackles were heard in the chest. She was ventilated in view of status epilepticus.

Investigations showed Hb 10.4 gm%, TLC 10,900/cu mm, with differential count as N 45%, E 1% L 45%, M 5% and ESR of 50 mm at one hour. Serum electrolytes, liver function test and serum creatinine were normal. HIV Elisa was negative. Serum immunoglobulins were within normal limits. X-ray of the spine and chest showed destruction of T3 and T4 vertebrae with gibbus deformity and

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right-sided pleural effusion. Ultrasound abdomen showed calcific foci in the spleen and liver. MRI of the cervicothoracic spine and brain showed exuberant enhancing basal exudates and parenchymal foci, communicating hydrocephalus, destruction of multiple upper and mid thoracic vertebrae with resultant gibbus and pre and paravertebral collection having epidural extension (Fig.). Smear of the CT guided aspirate of the paravertebral abscess showed numerous acid fast bacilli. Child was started on anti-tuberculous treatment (ATT) as per Indian Academy of Pediatrics (IAP) recommendations. Culture of the pus in Lowenstein Jensen medium subsequently grew *Mycobacterium tuberculosis* H, sensitive to first line ATT.

Screening of contacts for Tuberculosis (TB) was done. Mother's urine smear showed numerous acid fast bacilli and urine culture confirmed growth of *Mycobacterium tuberculosis*. She was found to have right lower ureteric stricture and left-sided poorly functioning kidney for which she initially underwent bilateral DJ stunting and later on left-sided nephrectomy. Her chest x-ray showed right-sided apical streaky opacities suggestive of probable sequealae of old infection. The mother too was started on ATT.

The child's seizures stopped and she became afebrile. Immediate neurosurgical intervention was withheld in view of poor general condition and extensive damage to the vertebrae. At follow up, she was offered surgery, however parents were reluctant to further active intervention in view of severe neurological sequelae which had already occurred. At follow up after three years, she had no recurrence of TB. She however had sequelae in the form of spastic quadriparesis, visual insufficiency and seizure disorder.

DISCUSSION

Spinal tuberculosis, which is commonly known to occur in adults, is an extreme rarity in infants although other forms of extrapulmonary tuberculosis are more common in infants and children. It is seen in 5% of children with tuberculosis and usually results from a secondary spread of bacilli

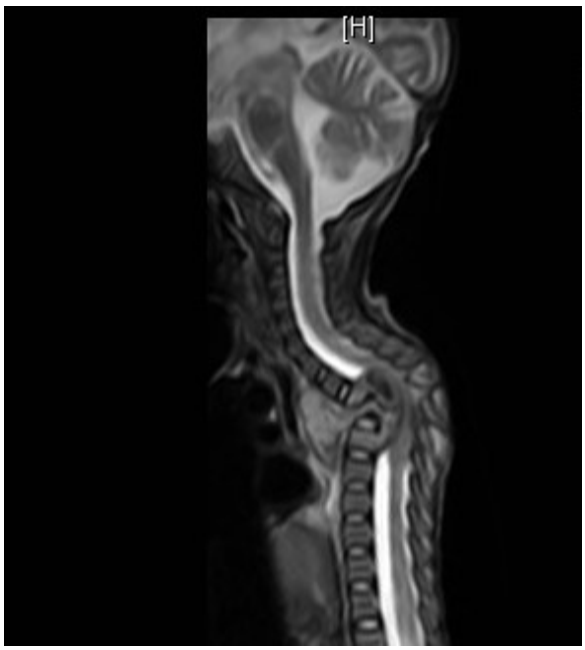

Figure: T2 sagital section of spine showing destruction of multiple upper and mid thoracic vertebrae with resultant gibbus and pre and para vertebral collection having epidural extension.

from a primary focus by hematogenous dissemination *via* the paravertebral plexus of Batson[1,2]. It is extremely unusual to encounter this entity in infancy as the minimum time required for osteoarticular TB to be manifested is one year after the primary infection (range 1-3years)[3]. Among the pediatric spinal tuberculosis cases reported by Bailey, *et al*[4]. (100 cases), Andronikou, *et al*[5]. (53 cases) and Mushkin and Kovalenko[6] (32 cases), there were no infants**.**

TB spine is an insidious disease in children. Diagnosis of spinal TB in infancy is often delayed as presentation is usually late due to the less dramatic effects of paraplegia or quadriplegia as compared to older age groups[7]. It is mandatory to make a careful clinical and radiological examination in those infants with weakness or paralysis of extremities.

This child is a rare case of culture proven spine TB presenting with symptoms initially at six months of age with an extremely unusual association

of culture proven maternal urinary TB which has not been reported so far to the best of our knowledge. Unfortunately, this child was brought to us very late after the onset of the symptoms and suffered serious neurological sequelae. TB in the mother too was diagnosed quite late and she too suffered from severe complications.

Whether this child could have acquired tuberculosis congenitally is a question that remains unanswered as endometrial biopsy had not been done for the mother and other criteria put forward by Cantwell for diagnosis of congenital TB too have not been fulfilled [8]. The fact that this child was asymptomatic and thriving well till five months of age makes this diagnosis unlikely although there have been reports of congenital TB presenting as late as four months.

A high index of suspicion, prompt diagnosis and treatment is crucial for favourable outcome of spinal TB. Late diagnosis and treatment of spinal TB seriously increases the mortality and morbidity.

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Case Report

EXAGGERATED MANTOUX REACTION IN A CASE OF LATENT TUBERCULOSIS INFECTION (LTBI)

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***Summary*:** A 42-year-old female presented with a history of receiving PPD on right forearm intradermally before two days. Patient started having itching and irritation within a few hours and pain, oedema and vesicles formation by next day at the injection site. On examination, the whole right forearm was oedematous with induration of size 50mm x 50mm around the site of injection. Tubercular infection was suspected and the patient was subjected to further investigation but nothing, including physical examination, hemogram, fundus examination, chest X-ray, USG abdomen and CT thorax, was found suggestive of tuberculosis, leading to a diagnosis of LTBI. **[*Indian J Tuberc 2012; 59:* 171-173]**

***Key words*:** Mantoux Test, LTBI, Exaggerated Mantoux Test

INTRODUCTION

Tuberculin Skin Test (TST) is mainly used to detect infection with tubercle bacilli. The Mantoux test {also known as the Mantoux screening test, Tuberculin Sensitivity Test, Pirquet test, or Purified Protein Derivative (PPD) test} is one of the two major tuberculin skin tests used in the world, largely replacing multiple-puncture tests such as the Tine test.

Tuberculin is a glycerol extract of the tubercle bacillus. PPD tuberculin is a precipitate of non-species-specific molecules obtained from filtrates of sterilized, concentrated cultures. It was first described by Robert Koch in 1890. The test is named after Charles Mantoux, a French physician who built on the work of Koch and Clemens von Pirquet to create his test in 1907.

A standard dose of PPD (1 Tuberculin unit in India) is injected intradermally between the layers of dermis and read 48 to 72 hours later[1]. A person who has been exposed to *Mycobacterium tuberculosis* is expected to respond with delayed type hypersensitivity (DTH) to the TST.

Exaggerated response to PPD has been documented[2] and so are false positive and false negative responses but a case of exaggerated response to standard dose of PPD in a case of LTBI is presented which, in our knowledge, has not been reported till date.

CLINICAL RECORD

A 42-year-old female patient, averagely built and nourished presented in the OPD of Pulmonary Medicine Department of Dr. RPGMC Tanda on 10.3.2011 with a history of patient's 2½ years' old grandson diagnosed as having smear negative pulmonary TB on 1.3.2011 and because of that, all close relatives were screened for TB. For this purpose, the patient received 5TU of PPD (SPAN's tuberculin PPD 10 TU/0.1 ml calibrated against Batch RT 23 manufactured by Statens Serum Institute, Denmark) on mid volar surface of right arm on 8.3.2011 intradermally. The patient started having itching and irritation over the site of injection and stretching sensation within a few hours. By next day, the patient developed pain, oedema and vesicles at the injection site that kept increasing till the patient presented in the OPD after 48 hours. Pain was so severe on movement of the arm that patient presented in the OPD with sling in the arm and was scared of moving it.

On examination, the whole right forearm was oedematous with vesicles at the injection site

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with some of them coalescing (Figure). Induration in the area of 50mm x 50mm around the site of injection was present which was painful on palpation. Local axillary glands were found normal. Tubercular infection was suspected and the patient was subjected to further investigation. But nothing was found suggestive of tuberculosis. On physical examination, there was no lymphadenopathy and Hemogram, fundus examination, chest X-ray, USG abdomen for lymphadenopathy and CT thorax all were found within normal limits.

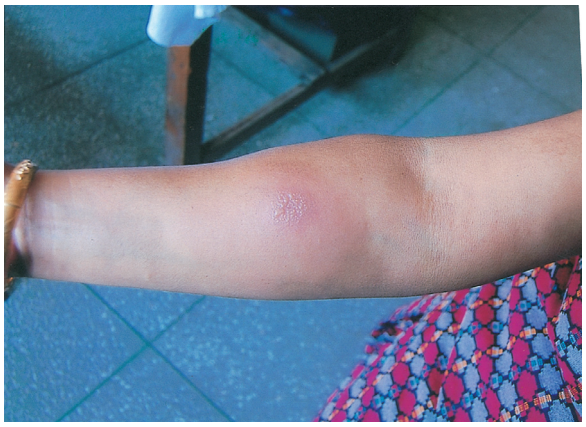

Figure: Exaggerated Mantoux Reaction in a Case of LTBI

The patient was reassured and not prescribed any medicine. Swelling gradually subsided and on next visit of patient, after 15 days, there was no swelling or induration over the arm, only slight hyperpigmentation and descaling marks were visible. Chest X-Ray was repeated on 30.05.2011 and was found to be normal. So patient was kept under observation.

DISCUSSION

The characteristic features of reaction to Mantoux test i.e. DTH include a delayed course, reaching a peak more than 24 hours after injection and an induration (palpable raised hardened area) with occasional vesiculation[3]. The reaction is read by marking the transverse margin of induration with a ballpoint pen and measuring the maximum transverse diameter in millimeters with a transparent ruler. If there is no induration, the result is recorded as "0 mm". Erythema (redness) is not measured[4].

Results of Mantoux test must be interpreted very carefully. Some subjects with heavy exposure to mycobacterial antigen show an immediate erythematous reaction with itching and irritation that peaks at 6-8 hours and disappears by 24 hours, this is not DTH[4]. Another important consideration in interpreting the results is false positivity. Due to the test's low specificity, many positive reactions in low-risk individuals are false-positives. The most important reason for a false positive result is infection by non-tuberculous mycobacteria or previous administration of BCG vaccine. However, the sensitivity induced by environmental mycobacteria results in smaller reactions to tuberculin than from true infection with *Mycobacterium tuberculosis*. Similarly, BCG induced sensitivity to tuberculin is also weaker than sensitivity induced by infection with tubercle bacilli[5]. False positives can also occur when the injected area is touched, causing swelling and itching. Considering all these and many more factors and based on experience gained by many studies, general guidelines have been issued for interpreting the results of tuberculin tests[4]. But almost all reactions with induration of 15 mm or more in size may be attributable to infection with tubercle bacilli, irrespective of BCG vaccination.

Present case study is unique in two ways, one is that the reaction is so much exaggerated and this exaggeration is not because of atopic nature of the individual as there is induration that lasted for a sufficiently long duration to rule out non-specific sensitivity and second, this seemingly true reaction is observed in a case of probably LTBI as is evident from all the tests performed to find the site of infection and such a long uneventful period (Tuberculin Positivity in absence of Clinical and Radiological features is the hallmark of LTBI)[6]. Though, some reports and text books mention a

rare possibility of exaggerated response to TST in some extremely sensitive individuals who are heavily infected but no information is available in literature or web about exaggerated reaction in LTBI that is presented. On the contrary, it has been found that TB morbidity increases with the size of induration[7]. Even if the dose of PPD in the present case is more than recommended for a developing country with huge prevalence of tuberculosis like India but this is not of much significance for explaining the exaggerated false positive response as many studies discuss the use of higher doses to decrease false negative cases[8,9] and this dose is being used in this area routinely. Besides, other family members of the case subject were administered the same dose from the same vial.

CONCLUSION

Mantoux has to be interpreted carefully and one should also be aware of unusual presentations like this.

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Indian J Tuberc 2012; 59: 174-176

UPDATES ON REVISED NATIONAL TB CONTROL PROGRAMME*

RNTCP has achieved and sustained its achievements in terms of twin objectives of case detection of more than 70% treatment cure rate of more than 85% amongst new smear positive TB cases, since more than five years. Decreasing Annual Risk of TB Infection (ARTI) from more than 1.7% in the last decade to 1.5% in 2002-03 to 1.1% in 2007-08 is indicative of reduction in transmission of Tuberculosis in the community.

However, it is important to accelerate TB control efforts and RNTCP has aimed at Universal access to TB care in the country. Programme has re-emphasized on early diagnosis with accredited diagnostics and complete regular treatment with quality drugs as delayed / inappropriate diagnosis and irregular and incomplete treatment of TB may contribute to complications, disease spread and emergence of Drug Resistant TB. Therefore for an effective TB control, to further reduce TB transmission and to address the problems of emergence of Drug Resistant TB, it is essential to have complete information on all TB cases. Keeping this crucial national and public interest in view, the Government of India has issued an order for TB notification (given in the following pages). Accordingly, all healthcare providers / clinical establishments run or managed by the Government (including local authorities), private or NGO sectors and/or individual practitioners will be required to notify TB cases to the local health authorities i.e. District Health Officer / Chief Medical Officer of a district and Municipal Health Officer of a Municipal Corporation / Municipality. Health care providers can notify such cases to the authorities by sending the details of patients in hard copy or by emails.

Patients diagnosed and / or treated in private sector will be benefited with extension of supportive services like counselling, treatment adherence and default retrieval.

Case-based web-based system has been developed by RNTCP in collaboration with NIC for real-time monitoring of TB cases currently diagnosed and treated under Programme. This system will be extended for TB notification for direct uploading of such information by the registered health care providers for convenience. It may be noted that the information regarding the identity of notified TB cases by any provider will not be disclosed in the public domain & will remain only with the provider. However, the general public will be able to know state or district-wise number of cases notified in a given period. Thus, confidentiality of each patient will be maintained at all levels. Overall, mandatory notification will assist in establishing effective and comprehensive surveillance for TB disease. Also, the mechanisms and services for treatment adherence under RNTCP will be extended to all health care providers to ensure effective TB management by all sectors to ensure effective TB Control. RNTCP is committed to strengthen partnership with all private health care providers for TB control in India.

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

Notification of TB cases*

TB continues to be a major public health problem accounting for substantial morbidity and mortality in the country. Early diagnosis and complete treatment of TB is the corner-stone of TB prevention and control strategy. Inappropriate diagnosis and irregular/incomplete treatment with anti-TB drugs may contribute to complications, disease spread and emergence of Drug Resistant TB.

In order to ensure proper TB diagnosis and case management, reduce TB transmission and address the problems of emergence and spread of Drug Resistant-TB, it is essential to have complete information of all TB cases. Therefore, the healthcare providers shall notify every TB case to local authorities i.e. District Health Officer / Chief Medical Officer of a district and Municipal health Officer of a Municipal Corporation / Municipality every month in a given format (attached).

For the purpose of case notification, a TB case is defined as follows:

- A patient diagnosed with at least one sputum specimen positive for acid fast bacilli, or Culture-positive for Mycobacterium tuberculosis, or RNTCP endorsed Rapid Diagnostic molecular test positive for tuberculosis

OR

- A patient diagnosed clinically as a case of tuberculosis, without microbiologic confirmation, and initiated on anti-TB drugs.

For the purpose of this notification, healthcare providers will include clinical establishments run or managed by the Government (including local authorities), private or NGO sectors and/or individual practitioners.

For more detailed information, the concerned State TB Officers / District TB Officers, whose details are available on www.tbcindia.nic.in, may be contacted.

*Issued by Ministry of Health and Family Welfare on 7th May, 2012.

Monthly Report

TB Notification

Period of reporting: From ...../...../..... To ...../...../.....

Name of the health facility / practitioner / Laboratory :.....
Registration Number:..... Telephone (with STD):..... Mobile number:.....
Complete Address: .....

Sr No	Name of TB Patient / ID of patient	Age (yrs)	Sex (M/F/O)	GoI issued identification number (Aadhaar, etc), if available	Complete residential address	Patient Phone number	Date of TB Diagnosis	Date of TB treatment initiation

Signature:..... Date: ...../...../.....

FORUM

STEM CELLS AND TUBERCULOSIS

This is in response to the editorial article published in your esteemed journal titled, "Stem cells and its importance in respiratory medicine with special reference to Tuberculosis".[1] At the outset, I would like to thank the author in seeking out-of-the-box solutions to the ever-increasing rise in diseases like Tuberculosis, Multi-Drug Resistance TB, and XDR-TB. Late Prof.KJR Murthy and our group believed from the outset that the immune- modulatory properties of mesenchymal stem cells could be exploited in dose dependent manner to evince the protective immune responses of the host, and balancing the inteleukin 10 releases.[2-4] We approached funding bodies to further the proof-of-concept in the animal model. The concept though was appreciated including international researchers; our reviewers did not gauge the potential application of the stem cells in Tuberculosis. The recent publication by the British group demonstrating some evidence of therapeutic benefit in treating AIDS by way of using stem cells, demonstrates an impending need to understand the underlying mechanisms of host resistance and susceptibility to *Mycobacterium tuberculosis*. Our group believes that the compounding problem of drug resistance can be dealt more effectively by way of cell therapy, which we presume would be a holistic approach to the new therapeutic intervention, rather than the use of potent drug intervention.

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Indian J Tuberc 2012; 59: 178-181

GUIDELINES FOR CONTRIBUTORS

GENERAL

The *Indian Journal of Tuberculosis (IJT)* is published four times in a year; January, April, July and October. It publishes original articles on tuberculosis, respiratory diseases, case reports, review articles, and abstracts of articles published in other medical journals and book reviews. Every issue contains editorial, sections on contemporary subjects, radiology forum and a forum for readers to express their opinions on published articles and raise questions on subjects appearing in the journal.

SUBMISSION OF ARTICLES

All correspondence relating to the *IJT* should be addressed to: *The Editor, Indian Journal of Tuberculosis,* Tuberculosis Association of India, 3 Red Cross Road, New Delhi - 110 001.

Articles are published on the understanding that every author confirms his participation in the study concerned and approves its content, and an affirmation that the article is original and has not been published/submitted for publication elsewhere and will not be so submitted, if accepted for publication in the *IJT.* A letter to this effect signed by the author should accompany the article.

All received articles are published, if found suitable, after completion of basic formalities. Notification of acceptance or rejection will be sent within three months of receipt. The decision of the Editor is final who reserves the right to make editorial corrections.

PREPARATION OF MANUSCRIPTS

Manuscripts should conform to the Uniform Requirements for Manuscripts submitted to the Biomedical Journals (for further details see Ann Intern Med 1997; 126: 36-47). Articles on clinical research should conform to the standards defined in the Helsinki Declaration.

Manuscripts, including diagrams and photographs, typed on one side of the page with double spacing and wide margins, should be submitted by email at tbassnindia@yahoo.co.in. The preferred package is MS Word 2003 version. The author should mention e-mail address, telephone and fax numbers apart from complete postal address with PIN code.

All submitted manuscripts should have a definite format comprising the following sections: Title page, Summary, Introduction, Material and Methods, Results, Discussion, Acknowledgements and References.

Title page

This should contain: (1) A concise informative title; (2) The name of the principa1 author followed by names of other authors without giving qualification or position held, except numera1 on top of last letter of name; (3) A running title usually not exceeding five words; (4) A word count of the text, excluding references, tables and figures; (5) In the case of original articles, a few key words for indexing purposes, using where possible, terms of medical subjects headings list from index medicus. The position held by each author in any institution should be indicated at the bottom of the title page along with the name and address of the author to whom correspondence regarding the manuscript has to be sent. Fax and telephone numbers (both landline and mobile) and e-mail ID should also be given.

Summary

An informative summary of not more than 250 words should be provided that can be understood without reference to the text (see Ann Intern Med 1990; 113: 69-76). The summary should be as per Vancouver format as follows: Background, Aims, Methods, Results and Conclusions. Unstructured

summaries may be submitted for review articles, case reports and short communications (100 words).

Text

Heading should conform to the text of the article. Normally only two categories of heading are used. Major headings shou1d be in capital letters and minor in upper lower case letters at the left-hand margin. The sub-titles should not be numbered in figures or alphabetically

The text should be written as lucidly as possible.

Numerals should be spelt out from one to nine (except measurement) and when beginning a sentence.

l. Research and experimental manuscripts should follow the usual conventions, as fo11ows:

Introduction: Setting forth clearly the aim of the study or the main hypothesis, with reference to previous studies and indicating the method used.
Material and Methods: used in the study.
Results: Presented in logical sequence in the text, with tables and illustrations. All the results of the tables should not be repeated in the text; only important results should be emphasized.
Discussion should be related to the aims, objects and results of the study.

Care should be taken that language is grammatically correct and fluent, that all relevant information is included, irrelevant details omitted and repetitions, especially from section to section, avoided.

In case reports, the sections on *"Material and Methods"* and *"Results"* are replaced by the section *"Clinical Record',* and all other sections are appropriately shortened.

2. Other papers can be sub-divided, as the authors desire: the use of headings enhances readability.

References

References cited in the text and given at the end of the manuscript should conform to the Vancouver style. The authenticity of the references is the responsibility of the author. They must be numbered in the order in which they are cited in the text, and should be numbered in Arabic numerals in superscript. References that are cited more than once should retain the same number for each citation. The truly scientifically acceptable references are those of publications that can be consulted. Permission from the source(s) of information for citing their work must be obtained beforehand. All the numbered references in the text should be typed out in detail at the end of the manuscript, in the same numerical order as they appear in the text.

Journal: References to an article in a periodical should include the authors' names (list all authors when six or fewer, when there are more, list only the first three authors and add "et al"), the full title of the article, the name of the cited journal in its usual abbreviated form according to the *Index Medicus,* year of publication, tome or volume number, first and last page numbers in full:

e.g. Gauri Wankhade, Anindita Majumdar, Pranita D. Kamble, Sajal De and B.C. Harinath. Multi-antigen and antibody assays (Seva TB Elisa) for the diagnosis of tuberculous pleural effusion. *Indian J Tuberc* 2012; **59(2)**: 78-82.

Book References to a piece of work (book or monograph) should include the authors' names, the title of the piece of work, the place and year of publication:

e.g. Crofton, J. and Douglas, A. *Respiratory Diseases,* 1st Edition. Edinburgh: Blackwell Scientific Publications Ltd, 1969.

Chapter in a book: Reference to a chapter in a book should include the authors' names, the title of the chapter with the word "In" preceding the reference of the work:

e.g. Fraser RS, Muller NL, Colman N, Pare PD. Upper airway obstruction. *In:* Fraser RS, Muller NL, Colman N, Pare PD, Bralow L, ed Fraser and Pare's *Diagnosis of Diseases of Chest;* 4th Ed; Vol III. Philadephia: W.B. Saunders Co, 1999: pp 2021-2048.

Reference to electronic material: If references are made to electronically published material, as much of the information as for other reference sources should be provided, the html address and the date last accessed.

Personal communication: References to personal communications should be given in the text with the name of the individual cited and with his/her consent.

Acknowledgements

Acknowledgements should be brief (not more than six lines). Acknowledge only those persons who made substantial contribution to the study and all sources of support in the form of grants.

Tables

Tables should be referred to consecutively in the text, placed after the list of references on separate sheets of paper, and should be numbered in Arabic numerals which are used for reference in the text. A short descriptive title should appear above the table, each column should have a short or abbreviated title. All abbreviations and necessary explanatory notes should be given below the table. The number of tables should be kept to a basic minimum to explain the most significant results.

Figures

Figures should be referred to consecutively in the text, placed after the list of references on separate sheets of paper, and should be numbered in Arabic numerals which are used for reference in the text. A short descriptive title should appear above the figure. Figures can be inserted into the word document for submission or uploaded separately as image files (.jpg, .gif, or .tif). If this is not possible, good quality (camera ready) prints of the figures must be provided.

Line drawings (curves, diagrams, histograms) should be provided in black and white. For optimal clarity, avoid shading.

Half-tone figures should be clear and highly contrasted in black and white. Photo- micrographs should have internal scale where appropriate. X-ray films should be careful1y made to bring out the details to be illustrated with an overlay indicating the area of importance.

Illustration: Legends for photographs should be typed separately with appropriate indication regarding the photograph to which a legend pertains. Photographs (black and white prints) should be clear, glossy and unmounted. Facilities for printing photographs in four colours as illustrations in case reports are available. Contributors are requested to preferably send colour photographs of their clinical material. Each photograph should carry, on its reverse, the title of the paper, and an arrow indicating the top edge of the photograph in pencil. It should be put in an envelope and properly labelled on the outside and attached to the article.

Patient confidentiality: Where illustrations show recognisable individuals, consent must be obtained for publication. If not essential to the illustration, authors should indicate where it can be cropped, or mask the eyes.

Permission to reproduce illustrations or *tables* should be obtained from the original publishers and authors, and submitted with the article by email or fax. They should be acknowledged in the legends as follows:

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Abbreviations and units

Avoid abbreviations in the title or summary. All abbreviations or acronyms used in the text must

be defined at the first mention, and should be kept to a minimum. Symbols and units of measure must conform to recognized scientific use i.e. SI units.

LENGTH OF TEXT

Editorial text can be approximately 500 words with five references

Review articles are from those especially requested persons, who have acknowledged competence in given subjects. Text can be up to 4500 words, a structured or unstructured summary of maximum 250 words, 10 tables/figures and 50 references. **Leading articles** are by those who have expertise in selected aspect of a subject.

Original articles deal with planned studies that have been duly completed and convey definite conclusions from the data presented in the text. Text can be up to 2500 words, a structured summary of maximum 250 words, seven tables/figures and 35 references. Preliminary communications from research still in progress could be submitted exceptionally, if the topic is important and the interim results could be of interest.

Short communications can be of a text up to 1000 words, a summary of 100 words, two tables/figures and 10 references.

Case reports present problems of unusual clinical interest which have been systematically and fully investigated and where a firm diagnosis has been established with reasonable certainty, or the result of therapeutic management is of significance. Text can be up to 1000 words, a summary of 100 words, two tables/figures and 10 references.

Workers in the field of Tuberculosis and Respiratory Diseases are invited to contribute to the **Radiology Forum** by submitting brief reports of patients with interesting clinical and radiological features for publication. These will be published, provided that:

(a) the condition is of clinical and radiological interest;
(b) photographs (10 cm x 8 cm) are of suitable quality for printing;
(c) the diagnosis in each case has been confirmed;
(d) the chest radiograph is accompanied by brief clinical account, not exceeding 500 words, and five references

Forum, in the form of letters to the Editor, provides a platform to readers for expressing their opinions and is a channel of communication with the journal and its readers. It could be used for making suggestions, scientific critique on published articles or for reaching independent conclusions, for asking questions on subjects covered by the journal and for providing supplementary information, either confirming or contradicting the conclusions reached in the article. Such letters can be up to a text of 1000 words with two tables/figures and 10 references. Only the most important agreements, disagreements/suggestions may be chosen for commenting. It is usual to send a copy of such letters to the authors for obtaining a response, if any, after editorial changes. The response, similarly, has to be brief and relevant.

Correspondence can be up to 500 words without tables or figures and five references.

IJT has been indexed in MEDLINE of National Library of Medicine, USA

The journal is available online on our website www.tbassnindia.org.

ABSTRACTS

Population-based surveillance of extensively drug-resistant tuberculosis in Shandong Province, China

X. Li, H.Wang, H.Jing, Y.Wang, C.Yu, J.Wang, Z. Liu, E. A. Graviss and X. Ma. *The International Journal of Tuberculosis and Lung Disease* 2012; **16(5)**: 612-4(3).

To investigate the prevalence of extensively drug-resistant tuberculosis (XDR-TB) in Chinese populations, we analyzed the drug resistance profiles of 1787 *Mycobacterium tuberculosis* isolates through a population-based surveillance project in Shandong Province, China. We found 330 (18.5%; 95% CI 16.1-20.3) isolates resistant to ≥ first-line drug and 65 (3.6%; 95% CI 2.9-4.6) multidrug-resistant (MDR) isolates, of which 13 (20.0%; 95% CI 11.9-31.4) were XDR; 47/65 MDR-TB isolates (70.8%; 95% CI 58.2-81.4) were resistant to fluoroquinolones. Our results indicate that inadequate application of second-line anti-tuberculosis drugs has caused increased prevalence of XDR-TB in certain Chinese populations.

Survival of HIV-infected patients after starting tuberculosis treatment: a prospective cohort study

M. Maruza, M. F. P. M. Albuquerque, M. C. Braga, M. T. S. Barbosa, R. Byington, I. Coimbra, L. V. Moura, J. D. L. Batista, G. T. N. Diniz, D. B. Miranda-Filho, H. R. Lacerda, L. C. Rodrigues and R. A. A. Ximenes. *The International Journal of Tuberculosis and Lung Disease* 2012; **16(5)**: 618-24(7).

To estimate the probability of survival and to evaluate risk factors for death in a cohort of persons living with human immunodeficiency virus (PLHIV) who had started tuberculosis (TB) treatment, a prospective cohort study was conducted between June 2007 and December 2009 with HIV-infected patients who had started anti-tuberculosis treatment in the State of Pernambuco, Brazil. Survival data were analysed using the Kaplan-Meier estimator, the log-rank test and the Cox model. Hazard ratios and their respective 95% CIs were estimated. Of a cohort of 2310 HIV-positive individuals, 333 patients who had commenced treatment for TB were analysed. The mortality rate was 5.25 per 10,000 person-years (95% CI 4.15-6.63). The probability of survival at 30 months was 74%. Risk factors for death in the study population were being female, age ≥ 30 years, having anaemia, not using highly active antiretroviral therapy (HAART) during treatment for TB and disseminated TB. Protective factors for death were a CD4 lymphocyte count >200 cells/mm^3 and treatment for TB having started in an out-patient clinic. The use of HAART can prevent deaths among HIV-TB patients, corroborating the efficacy of starting HAART early in individuals with TB.

Interleukin-10 and interferon-gamma patterns during tuberculosis treatment: possible association with recurrence

P. M. Lago, N. Boéchat, D. P. Migueis, A. S. Almeida, L. C. Lazzarini, M. M. Saldanha, A. L. Kritski, J. L. Ho and J. R. Lapa e Silva. *The International Journal of Tuberculosis and Lung Disease* 2012; **16(5)**: 656-9(4).

Interleukin (IL) 10 and interferon-gamma (IFN-) levels in induced sputum supernatants of 21 tuberculosis (TB) patients at diagnosis and during chemotherapy were correlated to recurrence rates. IL-10 decreased until day 60 of treatment (T60), and between T60 and T180 it increased again in seven cases (Pattern 1) and further decreased in 14 cases (Pattern 2). Follow-up of 69 months was performed in 20/21 cases; six had recurrence of

TB, of which 5/7 (71%) had Pattern 1 and 1/13 (7.7%) Pattern 2 (OR 30.0, 95% CI 2.19411.3, *P* 0.0072). This was not observed for IFN-. High IL-10 levels at the end of treatment may function as a risk factor for TB recurrence.

FEV_3, FEV_6 and their derivatives for detecting airflow obstruction in adult Chinese

D. C. L. Lam, D. Y. T. Fong, W. C. Yu, F. W. S. Ko, A. C. W. Lau, J. W. M. Chan, K. L. Choo, T. Y. W. Mok, C. Y. Tam, M. S. M. Ip and M. M. W. Chan-Yeung. *The International Journal of Tuberculosis and Lung Disease* 2012; **16(5)**: 681-6(6).

Forced expiratory volume in three seconds (FEV_3) and six seconds (FEV_6) could complement FEV_1 and forced vital capacity (FVC) for detecting airflow obstruction. The objective was to compare FEV_1/ FEV_6 and FEV_3/FVC with FEV_1/FVC in the detection of airflow obstruction. Previous lung function data were re-analysed to establish reference values for FEV_3 and FEV_6. Data from a separate cohort of male smokers were used as test set. FEV_1, FEV_3, FEV_6, FVC, FEV_1/FVC, FEV_1/ FEV_6 and FEV_3/FVC were regressed against age, standing height, weight and body mass index, and the mean and 95% confidence intervals for the lower limit of normal (LLN) values for these parameters were determined. The percentage of smokers with airflow obstruction in the test population using FEV_1/FVC < LLN was 15.0%, while using FEV_1/ FEV_6 < LLN and FEV_3/FVC < LLN they were respectively 18.5% and 18.1%. Using FEV_1/FVC < LLN as reference, the sensitivity and specificity of FEV_1/ FEV_6 < LLN in identifying airflow obstruction were 82.3% and 92.8%, while those for FEV_3/FVC < LLN were 78.5% and 92.6%; the positive and negative predictive values were 67% and 96.7% for FEV_1/ FEV_6 < LLN and 65.3% and 96% for FEV_3/FVC < LLN. FEV_3/FVC < LLN and FEV_1/ FEV_6 < LLN are comparable to FEV_1/FVC < LLN for detecting airflow obstruction. FEV_3/FVC < LLN could be useful in screening for airflow obstruction, while FEV_1/ FEV_6 < LLN is useful in detecting airflow limitation in the elderly or in subjects with severe airflow obstruction.

High rate of hypothyroidism among patients treated for multidrug-resistant tuberculosis in Lesotho

H. Satti, A. Mafukidze, P. L. Jooste, M. M. McLaughlin, P. E. Farmer and K. J. Seung. *The International Journal of Tuberculosis and Lung Disease* 2012; **16(4)**: 468-72(5).

Hypothyroidism is a known side effect of treatment for multidrug-resistant tuberculosis (MDR-TB), but it is considered to be rare. Hypothyroidism has vague and non-specific symptoms, and can be easily missed by clinicians. The objective was to report the high rate of hypothyroidism in a cohort of MDR-TB patients in Lesotho and to describe our approach to diagnosis and management. A retrospective study of 212 patients who initiated treatment for MDR-TB in Lesotho between 27 July 2007 and 24 March 2009 was performed. Among 186 patients screened, 129 (69%) had hypothyroidism, defined as at least one documented thyroid-stimulating hormone (TSH) result > 10.0 mIU/l; 100 (54%) patients had a maximum TSH > 20.0 mIU/l. At 93 days after starting MDR-TB treatment, half of the patients had developed hypothyroidism. Hypothyroidism may be more common during MDR-TB treatment than previously recognized. Screening all patients, even those without symptoms, for hypothyroidism within two-three months of starting MDR-TB treatment should be considered until prospective studies can inform screening guidelines.

Reasons for non-participation in an international multicenter trial of a new drug for tuberculosis treatment

D. Lamunu, K. N. Chapman, P. Nsubuga, G. Muzanyi, Y. Mulumba, M. A. Mugerwa, S. Goldberg, L. Bozeman, M. Engle, J. Saukkonen, S. Mastranunzio, H. Mayanja-Kizza and J. L. Johnson.

The International Journal of Tuberculosis and Lung Disease 2012; **16(4)**: 480-5(6).

Clinical trials can provide a high standard of patient care and contribute to scientific knowledge; however, only a fraction of the patients screened participate and receive treatment as part of a trial. The objective was to explore reasons why patients were not enrolled in an international tuberculosis (TB) treatment trial and to compare experiences among study sites. An analysis of reasons why patients were not enrolled was conducted among patients screened for a TB clinical trial at 26 sites in North and South America, Africa, and Europe. Staff at study sites screened 1119 potential candidates for the trial: 61% (n = 686) were not enrolled due to 1) failure to meet eligibility criteria (n = 405, 59%), 2) site's decision (n = 168, 24%), or 3) candidate's choice (n = 113, 16%). Study staff recorded a total of 144 reasons for why they believed patients chose not to participate, including concerns over research (28%), conflicts with work or school (21%), and lifestyle and family issues (20%). Socio-demographic and geographic factors also influenced participation. Increased evaluation of screening outcomes and of specific interventions, such as improved education and communication about trial procedures, may increase the efficiency of screening and enrollment in clinical trials.

Laboratory diagnosis of tuberculosis in a large pediatric hospital in Cambodia

K. Schopfer, H. L. Rieder, T. Bodmer, J. F. Steinlin-Schopfer, Y. Chantana, T. Somathea, P. Studer, D. Laurent and B. Richner. *The International Journal of Tuberculosis and Lung Disease* 2012; **16(4)**: 503-9(7).

Tuberculosis laboratory in the Jayavarman VII Children's Hospital in Siem Reap, part of the Kantha Bopha Hospitals, is the largest pediatric hospital complex in Cambodia. The objective was to determine the efficiency of on-site microscopy and rRNA amplification in children with a clinical diagnosis of tuberculosis (TB) and specimen sampling for culture. From 1 July 2005 to 31 March 2006, 52,400 children were admitted to the hospital. Among these, 405 children had tuberculosis, including 91 (22.5%) laboratory-confirmed cases, or respectively 7.7 and 1.7 per 1000 admissions. Among cases confirmed by microscopy or rRNA assay, rRNA identified 91.2%. Among all culture-confirmed cases, rRNA identified 90.5%. Culture alone contributed 7.1% to all laboratory confirmed cases. The yield of culture from preserved specimens was not affected by shipment delay. For 97.4% of the children, the maximum turnaround time for the on-site laboratory result was 48 h. Implementation of a mycobacteriology service in a referral hospital is feasible, as the molecular technique is highly efficient. Storage of specimen aliquots allows subsequent culture without loss of viability due to shipment delay.

Association of streptomycin resistance mutations with level of drug resistance and *Mycobacterium tuberculosis* genotypes

N. T. Q. Nhu, N. T. N. Lan, N. T. N. Phuong, N. van V. Chau, J. Farrar and M. Caws. *The International Journal of Tuberculosis and Lung Disease* 2012; **16(4)**: 527-31(5).

The objective of the study was to determine 1) the relationship between specific streptomycin (SM) resistance mutations and the minimum inhibitory concentration (MIC), and 2) whether these mutations are preferentially associated with the Beijing genotype in Viet Nam. A total of 131 consecutive *Mycobacterium tuberculosis* isolates resistant to either isoniazid (INH) or rifampicin (RMP), collected previously, were tested for SM resistance, spoligotyped and sequenced in the *rps*L, *rrs* and *gid*B genes. The MIC for 50 mutants was also determined. Overall, 116/131 isolates were SM-resistant. The three most frequently occurring mutation sites in *rps*L and *rrs* were at codon 43 of *rps*L (72/116, 62.1%), *rps*L88 (22/116, 18.9%) and *rrs*514 (8/116, 6.9%). Mutations in the *rrs*910 region were found in two isolates (1.7%), and three isolates had mutations in both *rps*L and *rrs* (2.6%). *gid*B mutations were found in both resistant and susceptible strains. Among SM-resistant isolates resistant to INH/RMP, the Beijing genotype was

strongly associated with *rps*L43 mutation (aOR 23.6, 95% CI 2.9-193.4, $P = 0.002$). The median MIC for each mutation was as follows: *rps*L43 = 256 g/ml,*rps*L88 = 16 g/ml, 515 loop = 4 g/ml, 910 region = 8 g/ml, and double mutation = 256 g/ml. We found a strong association between *rps*L43 and high drug resistance levels, with all *rps*L43 mutants having an MIC >256 g/ml ($P < 0.001$).

Obstructive lung disease does not increase lung cancer mortality among female never-smokers in Hong Kong

C. C. Leung, T. H. Lam, W-W. Yew, W. S. Law, C. M. Tam, K. C. Chang, S. McGhee, S. Y. Tam and K. F. Chan. *The International Journal of Tuberculosis and Lung Disease* 2012; **16(4)**: 546-52(7).

High lung cancer mortality is observed among female never-smokers in Hong Kong. The objective was to examine the relationship between obstructive lung disease (chronic obstructive pulmonary disease and/or asthma) and lung cancer mortality by sex and smoking status. A cohort of elderly clients (aged ≥ 65 years) in a health maintenance programme were followed prospectively through linkage with the territory-wide death registry for causes of death, using identity card number as the unique identifier. After 516 055 person-years of follow-up, respectively 1297, 872 and 1908 deaths were caused by lung cancer, other tobacco-related malignancies and non-tobacco-related malignancies. In the overall analysis, obstructive lung disease was independently associated with mortality due to lung cancer (aHR 1.86, $P < 0.001$) after adjustment for potential confounders. However, no association was detected among female never-smokers (HR 0.97, $P = 0.909$), in sharp contrast with female ever-smokers, male never-smokers and male ever-smokers (HR 1.98, 2.34 and 2.09, respectively, P from 0.047 to <0.001). Consistent results were observed after exclusion of all deaths in the initial three years. Obstructive lung disease exerted differential effects on lung cancer mortality across different sex and smoking subgroups in this Asian population, with a conspicuous absence of effect among female never-smokers.

Incidence and prevalence of chronic bronchitis: impact of smoking and welding. The RHINE study

M. Holm, J-L. Kim, L. Lillienberg, T. Storaas, R. Jogi, C. Svanes, V. Schlünssen, B. Forsberg, T. Gíslason, C. Janson and K. Toren. *The International Journal of Tuberculosis and Lung Disease* 2012; **16(4)**: 553-7(5).

The objective was to investigate the prevalence and incidence rate of chronic bronchitis (CB) in relation to smoking habits and exposure to welding fumes in a general population sample. Subjects from Northern Europe born between 1945 and 1971 who participated in Stage 1 (1989-1994) of the European Community Respiratory Health Survey were mailed a respiratory questionnaire in 1999-2001 (the RHINE study); 15909 answered the questionnaire and gave complete data on smoking. CB was defined as chronic productive cough of at least three months a year for two consecutive years. The questionnaire comprised an item about age when CB started and items about exposure to welding fumes. The incidence of CB was retrospectively assessed for the observation period 1980-2001. CB had a prevalence of 5.4%, and was associated with current smoking and welding exposure. The incidence rate of CB was 1.9 per 1000 person-years, and was increased in relation to welding exposure (low exposure HR 1.4, 95% CI 1.1-1.8; high exposure HR 2.0, 95% CI 1.6-2.7) and in relation to smoking (HR 2.1, 95% CI 1.8-2.5). Smoking and occupational exposure to welding fumes are both associated with an increased risk of CB.

Influence of Lineage-Specific Amino Acid Dimorphisms in GyrA on *Mycobacterium tuberculosis* Resistance to Fluoroquinolones

Hyun Kim, Chie Nakajima, Youn Uck Kim, Kazumasa Yokoyama and Yasuhiko Susukhi. *Jpn J Infect Dis* 2012; **65**: 72-4.

We conducted *in vitro* DNA supercoiling assys, utilizing recombinant DNA gyrases, to elucidate the influence of the lineage-specific serine or threonine

residue at position 95 of GyrA on fluoroquinolone resistance in *Mycobacterium tuberculosis*. There was little effect of the GyrA-Ala74Ser amino acid substitution on activity of the GyrA-Ser95 gyrase, while activity of the GyrA-Asp94Gly-Ser95 gyrase was reduced. These findings were in striking contrast to previous resports analyzing GyrA with Thr95 and suggest an important impact of the amino acid in the development of fluoroquinolone resistance.

Errata

On page 83 under Summary of the article "Morbidity and Mortality at five years after initiating Category I treatment among patients with new sputum smear positive pulmonary tuberculosis" published in the April, 2012 issue, an inadvertent printing error has occurred as follows:

Printed as

***Background*:** Evaluation of disease outcome is central to the assessment of tuberculosis control programmes. Most of the follow up studies in RNTCP are short term.

evaluate the outcome of Category I treatment in smear positive tuberculosis, five years after treatment in terms of relapse, sequelae and death and to know the associated factors.

Read as

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

The error is regretted.

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