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Editorial

STEM CELLS AND ITS IMPORTANCE IN RESPIRATORY MEDICINE WITH SPECIAL REFERENCE TO TUBERCULOSIS

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Background

Regenerative medicine in curing respiratory diseases has led us to a great deal of interest in stem cells, as they are likely to play an important role in the repair of diverse lung injuries, pathologies & tumours. However, very low rates of cellular proliferation *in vivo* in the normal steady state, cellular and architectural complexity of the respiratory tract, lung stem cells remain poorly understood compared to those in other major organ systems.

Stem cells are divided into embryonic and adult stem cells.[1]Embryonic stem cells are derived from the blastocyst of a developing embryo and are able to produce progeny of all cell lineages; however their use has generated moral and political objections.[2] Adult stem cells are located in many tissues throughout the body and play an essential role in their growth, maintenance and repair[1]. Adult bone marrow-derived stem cells may have more plasticity and are able to differentiate into bronchial, alveolar and vascular endothelium, interstitial cell types, making them prime candidates for repair. A potential advantage of using adult stem cells, expanded in culture and reintroduced into the same patient, is the avoidance of immunologic rejection, as this process constitutes an autologous transplantation. Once a cell is differentiated, their phenotypes are stable. However, a number of reports have shown that tissue stem cells, which are thought to be lineage-committed multipotent cells, possess the capacity to differentiate into cell types outside their lineage restrictions (called *transdifferentiation*). This feature may provide a means to use tissue stem cells derived directly from a patient for therapeutic purposes, thereby eliminating the need to use embryonic stem cells or elaborate procedures such as nuclear reprogramming a patient's somatic cells.

In this editorial, we explore the framework of stem cell biology and recent advances pertaining to the lungs, and tubercular disease in which manipulation of stem cells may be quite helpful.

Air-exposed lungs are a subject to an array of potentially damaging agents, including various toxins,oxidants and proteolytic enzymes. Presumably, daily oxidant and protease wear and tear on structural components such as elastin and collagen contributes to inevitable age-related decline in pulmonary function in normal individuals. [3,4.] Acute and chronic lung disease, or its treatment with oxygen and positive pressure ventilation, may further damage lung tissue in excess of the capacity for orderly repair, resulting in characteristic pathologic changes including tissue destruction or fibrotic scarring [5-7].

Stem cells of the respiratory tract have not been fully elucidated. Studies[8] of epithelial damage in animal models suggest different local stem populations situated throughout the tract, basal epithelial cells in the trachea and larger airways, and Clara cell secretory protein-expressing cells the likely candidates in the smaller airways. Type 2 pneumocytes are thought to act as the main local endogenous stem cells of the lung parenchyma. Whatever the dominant population, this endogenous repair is insufficient to prevent the many progressive respiratory diseases[9].

Stem Cells and TB

Tuberculosis (TB) is a leading public health problem. About half of the 10% of *Mycobacterium tuberculosis*-infected individuals develop overt clinical disease, however many patients still report quite late in our country and have marked destruction of lung parenchyma. Reported incidence of Tuberculosis in India, as per WHO, is 168/100000 [10]and there are about 24 deaths/lac population in India.[11] The role of innate and adaptive immune responses in the control of mycobacterial growth and the regulation of inflammation is an area of great interest. T.B. heals by fibrosis and therefore morbidity continues even after the disease has been cured. This is more often true in MDR and chronic cases. Since 90% of the subjects do not develop the disease even after they are infected, therefore it is important to understand the genetic role controlling the immunity in patients who develop the disease either primary or secondary in nature. Targeted and genome-wide explorations are being employed in large familial samples to identify novel susceptibility genes involved in the immune control of primary *M. tuberculosis* infection and secondary pulmonary TB. The outcome for the design is novel vaccination, immunotherapeutic strategies and better diagnostic biomarkers.

The gene, named Toll-like receptor 8 (TLR8), has a probable role in human susceptibility to *Mycobacterium tuberculosis* infections. The study also found that males are more susceptible than females. Analysis of the results from cohort studies in Indonesia and Russia suggested that susceptibility was attributed to genetic variants of TLR8, which is located at the X chromosome. Males carrying only one copy of the gene could have a higher chance of suffering from the disease. The identification of a role for TLR8 in TB infection has the potential to open up new areas of exploration in TB host/pathogen interactions and provide researchers and clinician scientists with novel targets for therapeutic intervention. This is extremely important given the emergence of multi-drug resistant strains of *M. tuberculosis* that are refractive to current treatment regimes.

Challenges

The realistic prospects for beneficial stem cell therapy of the lung are still not understood. We must identify lung diseases/cases/timing in which cell and tissue damage occurs in excess of the capacity for timely endogenous repair. Establishing standardized sources of relevant stem/progenitor cells and methods for their delivery to the appropriate lung sub-compartment is yet to be found. It remains unknown whether bone marrow cells must transit through an intermediate compartment prior to lung colonization or whether circulating stem cells can be mobilized from sources other than bone marrow. Major lung diseases potentially addressable by stem cell therapy may pose unique challenges. Ongoing research is looking at the manipulation of stem cells and specifically the use of extrinsic stem cells to augment lung repair to improve the response of the lung parenchyma to injury and disease

Future

Several studies have demonstrated that cell fusion occurs both *in vitro* and *in vivo*, which likely explains why some of the cells contain both donor and lung cell markers. Alternatively, cells may reprogramme in the lung environment- transdifferentiation. There is hope that exogenous and/or mobilized endogenous stem cells may be harnessed to prevent or treat acute and chronic lung diseases and even regenerate abnormally developed or lost tissue. Our understanding of lung stem cells and the regulation of lung morphogenesis is still rudimentary, and the complex, integrated function of multiple cell types underlying normal lung structure and function poses unique challenges. Thus, the therapeutic prospects for stem cell therapy in lungs appear more distant than in some other organs. This realization should stimulate meaningful new studies from the lung research community. The first trial of embryonic stem cells in humans has

already been declared and begun in patients after getting the green light from regulators.[12] Whether stem cells can play an important role in regeneration of lung parenchyma destroyed by the disease is an exciting area of interest in the field of T.B., COPD & bullous disease, cystic fibrosis and interstitial lung diseases .

Further there is a scope for treatment options, wheras technologies derived from stem cell research to treat a wider variety of diseases of lungs can be realistic exciting option of future.

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Original Article

EFFECT OF HIV PROTEASE INHIBITORS AND ORLISTAT ON MYCOBACTERIAL ES-31 SERINE PROTEASE, A POTENTIAL DRUG TARGET IN *MYCOBACTERIUM TUBERCULOSIS**

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Summary

Background: Mycobacterial excretory secretory-31 (SEVA TB ES-31) antigen is shown to possess protease and lipase activities.

Aim: To study the effect of commonly used HIV-protease inhibitors and lipase inhibitor Orlistat if any on mycobacterial ES-31 serine protease *in vitro* enzyme activity and on the growth of *M.tb* H_{37}Ra bacilli in axenic culture.

***Methods*:** Effect of HIV-protease inhibitors namely Ritonavir, Lopinavir and Indinavir and Orlistat on protease activity of ES-31 was assessed using azocasein assay and on bacillary growth in axenic culture of *Mycobacterium tuberculosis* H_{37}Ra. The concentration of ES-31 antigen in culture filtrate was determined by sandwich peroxidase ELISA using anti ES-31 antibody and the growth of bacilli by CFU count.

***Results*:** HIV-protease inhibitors such as Ritonavir, Lopinavir and Indinavir and lipase inhibitor Orlistat inhibited serine protease activity by 41.3 - 69.7% *in vitro.* These inhibitors also showed decreased bacterial growth in axenic culture and further confirmed by decreased concentration of ES-31 serine protease secretion in the culture fluid. Ritonavir showed maximum inhibition of 77% on the growth of the bacilli in axenic culture while anti obesity drug Orlistat showed 61% inhibition.

***Conclusion*:** SEVA TB ES-31 with serine protease and lipase activities may be a potential drug target in tuberculosis management. **[*Indian J Tuberc 2011; 58:* 4-10]**

Key words: *Mycobacterium tuberculosis*, HIV, Lopinavir, Ritonavir, Orlistat, Mycobacterial ES-31 serine protease.

INTRODUCTION

Mycobacterium tuberculosis (*M. tb*), a causative micro-organism of tuberculosis (TB) is an intracellular pathogen. Following infection, *M. tb* uses macrophages as reservoir for continuous bacterial multiplication and as a vehicle for bacillary dissemination. Microbial pathogens frequently utilize extracellular proteases as virulence factors, which often contribute significantly to pathology.[1] These proteases participate in tissue destruction, inactivation of host defense molecules, activation of key regulatory proteins or peptides, nutrient acquisition and the processing of secreted signaling molecules which regulate gene expression. Little is known about the biology and role of these enzymes in *M. tb*, hence it is hoped that characterization and potential of these proteases as drug target may provide new strategies in understanding the biology and function of these enzymes in the mycobacterial cell and in host-pathogen interaction.

Brown *et al*[2,3] (2000) identified five *M. tb* genes (myc P 1-5) that encode a family of serine proteases (mycosins-1 to 5) and Mycosin-1 was found to be cell wall associated and expressed during infection of macrophages. Earlier studies from our laboratory reported that ES-31, a glycoprotein antigen with metalloserine protease activity was promising in the diagnosis of pulmonary TB, some

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forms of extrapulmonary TB like TB Lymphadenopathy, TB meningitis and in human immunodeficiency virus (HIV)-TB co-infection.[4-5]In spite of development of multidrug (MDR) and extensive drug resistance(XDR) in tuberculosis strains, the progress has been slow in detection of new drug targets and development of more effective and safe antitubercular drugs. Mycobacterial ES-31 antigen has been shown to have serine protease activity as well as lipase activity similar to chymotrypsin family.[6-7] Inhibition of mycobacterial ES-31 serine protease secretion and decreased bacterial growth in axenic culture and macrophages by isoniazid, serine protease inhibitors and anti-serine protease antibody showed drug target potential of ES-31 serine protease.[6] Further serine protease was shown to be inhibited by Lipase inhibitor-Orlistat[7]. Increasing incidence of HIV-TB coinfection has been reported.[9]Inhibitors of HIV-encoded aspartic protease such as Lopinavir, Ritonavir, Indinavir etc. inhibits HIV-protease and interrupts the life cycle of HIV cleaved before nascent viral particles (virions) can mature.[10-11] It will be of interest to study the effect of HIV Protease and lipase inhibitors if any on mycobacterial serine protease and growth of bacilli in axenic culture. In this communication we report the inhibitory effect of HIV-protease inhibitors and Orlistat (anti obesity, lipase inhibitor) on mycobacterial ES-31 serine protease activity *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 at 37 ^{0}C on an orbital shaker for 10 days as described earlier.[12] ES-31 serine protease was isolated from culture filtrate protein by affinity chromatography using anti ES-31 serine protease antibody. 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). Analysis of ES- 31 serine protease using gelatin substrate gel electrophoresis and studies with protease inhibitors, phenyl methyl sulphonyl fluoride (PMSF) and 1, 10 phenanthroline showed that it is a zinc containing serine protease.[13]

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.[13] 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 aminoethyl-cellulose ion exchange column chromatography as described earlier.[13] Anti ES-31 serine protease antibody was isolated from anti-DSS IgG by affinity chromatography using ES-31 serine protease coupled Sepharose-4B column.[15]

Study of HIV-PIs and Orlistat on protease activity of mycobacterial ES-31 in vitro:

The effect of HIV-protease inhibitors such as Ritonavir, Lopinavir and Indinavir on the proteolytic activity of ES-31 was studied using azocasein as protease substrate as described earlier.[8] In brief, 5 ml of azocasein assay incubation mixture consists of the mycobacterial ES-31 antigen (100 µg) with 25 mg azocasein in 0.5M Sodium Bicarbonate buffer (ph 8.3). The azocasein assay mixture was incubated at 37^{0}C for 6 hours. Further, 1ml aliquot solution was removed and 4ml of trichloroacetic acid (5%) was added to the solution. 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. Absorbance of the

liberated dye at 440 nm was measured 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 protease inhibitors of different concentrations (50% of minimum effective concentration to 300% of MEC; MEC of Ritonavir, Lopinavir and Indinavir are 100ng/ml, 1000ng/ml and 2700ng/ml respectively, 0.5 – 1.5 M Orlistat) at 37 ^{0}C for 1 hour followed by azocasein assay of protease activity.[8] Azocasein assay without protease inhibitor incubation served as control.

Study of HIV-PIs and Orlistat on M. tuberculosis bacilli in axenic culture:

The effect of protease inhibitors such as Ritonavir, Lopinavir and Indinavir, lipase inhibitor namely Orlistat was studied on secretion of ES-31 serine protease by *M. tuberculosis* H_{37}Ra bacilli 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 on an orbital shaker for 10 days. Rapidly growing cells were harvested by centrifugation at 4000 g at 4 ^{0}C for 20 minutes. The inhibitors ritonavir, lopinavir and indinavir and lipase inhibitor namely Orlistat were added separately to the Sauton medium (100 ml) with inoculum, 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.[16] Using the standard graph with purified ES-31 serine protease, the concentration of mycobacterial ES-31 serine protease (ng) in culture filtrate was determined.[16] 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.

Peroxidase Sandwich ELISA for Quantitation of secretory ES-31 antigen in culture filtrate:

To study the effect of serine and metalloprotease inhibitors 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.[16] 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^{0}C followed by blocking with BSA (1%) for 2 hours at 37^{0}C. The plate was washed twice with PBS containing 0.05% Tween 20 (PBS/T) followed by

Table 1: Effect of HIV-protease inhibitors and Orlistat on protease activity of Mycobacterial ES-31 serine protease *in vitro*

Inhibitor	Concentration	ES-31 protease activity (% inhibition)
Control*	100 µg ES-31	17.40
Lopinavir	500 ng/ml	10.66 (38.7)
	1000 ng/ml (**MEC**)	5.32 (69.4)
	2000 ng/ml	5.27 (**69.7**)
	3000 ng/ml	5.29 (69.6)
Ritonavir	500 ng/ml	12.58 (27.7)
	1000 ng/ml (**MEC**)	6.21 (**64.3**)
	2000 ng/ml	6.29 (63.9)
	3000 ng/ml	6.23 (64.2)
Indinavir	1350 ng/ml	14.74 (15.3)
	2700 ng/ml (**MEC**)	12.29 (29.4)
	5400 ng/ml	10.22 (**41.3**)
	10800 ng/ml	10.27 (41.0)
Orlistat	0.5 mM	5.27 (69.7)
	1 mM	1.84 (**89.4**)
	1.5 mM	1.88 (89.2)

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

Table 2: Effect of protease inhibitors on the growth of *M. tb.* bacilli in axenic culture

Inhibitor	**Concentration**	**Concentration of ES-31 protease (ng/ ml) in culture filtrate**[#] (% inhibition of secretion)	**CFU count**[$] (% growth inhibition)
Control	-	66 ng	15.6×10^5
Lopinavir	500 ng/ml	56 ng (15.2)	9.8×10^5 (37.2)
	1000 ng/ml **(MEC)**	52 ng (11.2)	8.6×10^5 (44.9)
	2000 ng/ml	24 ng (**63.6**)	7.4×10^5 (**52.6**)
	3000 ng/ml	26 ng (60.6)	9.2×10^5 (41.0)
Ritonavir	500 ng/ml	40 ng (39.4)	9.0×10^5 (42.3)
	1000 ng/ml **(MEC)**	14 ng (**78.8**)	3.6×10^5 (**76.9**)
	2000 ng/ml	16 ng (75.8)	4.2×10^5 (73.1)
	3000 ng/ml	18 ng (72.7)	4.6×10^5 (70.5)
Indinavir	1350 ng/ml	48 ng (27.3)	11.4×10^5 (26.9)
	2700 ng/ml **(MEC)**	36 ng (**45.5**)	8.4×10^5 (**46.6**)
	5400 ng/ml	36 ng (**45.5**)	8.8×10^5 (43.6)
	10800 ng/ml	40 ng (39.4)	9.4×10^5 (39.7)
Orlistat	Orlistat 0.5 mM	32 ng (51.5)	7.6×10^5 (51.3)
	Orlistat 1 mM	26 ng (**60.6**)	6.0×10^5 (**61.5**)
	Orlistat 1.5 mM	34 ng (48.5)	8.2×10^5 (47.4)

MEC: Minimum effective concentration

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

[$] : CFU/ml of culture = $\frac{\text{Number of colonies} \times \text{dilution factor}}{\text{Volume of culture suspension}}$

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

addition of 100 µl of culture filtrate and incubated for 1 hour at 37^0C, 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 37^0C. The wells were washed five times with PBS/T with 1 minute interval. The color was developed using TMB substrate and reaction stopped using 50 µL 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.

RESULTS

HIV-protease inhibitors i.e., Lopinavir, Ritonavir and Indinavir showed maximum inhibition on ES-31serine protease activity *in vitro* at the concentration of 2000ng/ml, 1000 ng/ml and 5400 ng/ml respectively. The inhibition observed by Lopinavir, Ritonavir, Indinavir was 69.7%, 64.3% and 41.3% respectively (Table 1).

HIV-protease inhibitors were also observed to be inhibiting secretion of mycobacterial ES-31 serine protease by *M. tb*. in axenic culture. Maximum inhibition of secretion of ES-31antigen by HIV-protease inhibitors namely Lopinavir, Ritonavir and Indinavir was found at the concentration of 2000ng/ml, 1000 ng/ml and 2700 ng/ml with 63.6%, 78.8%, and 45.5% inhibition respectively (Table 2). HIV-protease inhibitors simultaneously inhibited growth of *M.tb*. bacilli in culture medium. Inhibition of bacillary growth by Lopinavir, Ritonavir and Indinavir was observed to be 52.6%, 76.9%, and 46.6% respectively as assessed by CFU count (Table 2). Similarly Orlistat also inhibited secretion of ES-31antigen at the concentration of 0.5mM, 1mM, 1.5mM with 51.3%, 61.4% and 47.4% respectively with similar inhibitory effect on growth of mycobacteria in axenic culture.

DISCUSSION

Discovery of novel drug targets is important due to the development of MDR and XDR TB. Culture filtrate proteins such as the 45 kDa, MPT 32, the 38 kDa PstS homolog and an 88 kDa MPT 32 have been shown to exert strong humoral immune response and have shown potential for serodiagnosis.[17-18] In our laboratory, presence of 31 kDa antigen protein has shown diagnostic potential for detecting active TB infection.[19] Biochemical characterization of SEVA TB ES-31 antigen showed that it is a zinc containing serine protease.[12] Mycosin-1, a membrane and cell wall associated mycobacterial serine protease shed into the culture supernatant was identified in *M. tuberculosis* culture filtrates.[3] The proteolytic activity in the culture filtrate was inhibited by mixed serine / cysteine protease inhibitors, a typical feature of the subtilisins. Harth et al reported that inhibition of secretory glutamine synthetase released by pathogenic mycobacterial species, selectively blocks bacterial multiplication in axenic culture.[20]Further the inhibitory effect of serine and metalloprotease inhibitors, isoniazid and anti ES-31 antibody on multiplication of tubercle bacilli in macrophage cultures has been reported earlier[15]. Anti ES-31 antibody inhibited the protease activity of ES-31by 96% *in vitro* as well as addition of anti ES-31antibody in axenic culture showed 89% inhibition in colony count[15].

In the present study, it was found that protease activity of ES-31 was inhibited by HIV-protease inhibitors and Orlistat, an anti obesity drug *in vitro*. Secretion of ES-31 and mycobacterial growth was also inhibited in axenic culture. HIV-Protease inhibitors in particular Ritonavir, Lopinavir do significantly inhibit mycobacterial ES-31 serine protease *in vitro* and *in vivo* decreasing bacterial cell growth in axenic culture.

Orlistat binds covalently to the active site of pancreatic lipase and causes acylation of a hydroxyl group on serine residue making the enzyme inactive.[21] HIV-protease inhibitors bind to the active site of HIV-protease homodimer and inhibit the protease activity.[10] These drugs are also showing affinity for ES-31 serine protease, may be binding at active site of the enzyme and blocking its action. Orlistat has been shown to inhibit serine protease acting at the active site[7]. In axenic culture, inhibition of ES-31 enzyme activity by drug reduced secretion of ES-31antigen followed by decrease in growth of bacilli. **Thus protease activity of ES-31 is possibly**

needed for mycobacterial multiplication. Interestingly Ritonavir is more effective than Lopinavir *in vivo*. Possible role of HIV-protease inhibitors in preventing TB in HIV infected cases under treatment needs further extensive study and may be helpful in understanding HIV-TB coinfection. Similar to anti ES-31 antibody, HIV Protease inhibitors and lipase inhibitor Orlistat inhibited serine protease activity of mycobacterial ES-31 *in vitro*. These inhibitors also decreased bacterial growth in axenic culture with decreased secretion of ES-31. Orlistat, a lipase inhibitor used in obesity treatment significantly inhibited mycobacterial ES-31 serine protease and bacterial cell growth, thus confirming potential of ES-31 as a new drug target for developing anti tubercular drugs. Orlistat and anti-ES-31 serine protease as therapeutic antibody may be useful in antitubercular treatment, in particular drug resistant cases, which need further evaluation.

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The Editor-in-Chief and the members of the Editorial Board of the *Indian Journal of Tuberculosis* wish all its readers a Very Happy and Prosperous New Year 2011.

D. BEHERA
EDITOR

Original Article

REASONS FOR INTERRUPTION OF ANTI-TUBERCULAR TREATMENT AS REPORTED BY PATIENTS WITH TUBERCULOSIS ADMITTED IN A TERTIARY CARE INSTITUTE

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Summary

***Setting*:** Department of Tuberculosis and Chest Diseases, Tertiary Level tuberculosis (TB) institute in Delhi, India.

***Objective*:** To study the reasons for interruption of Anti-Tubercular Treatment (ATT) as reported by tuberculosis patients admitted at LRSI.

***Design*:** Retrospective cohort-based analysis.

Results: A total of 201 patients were enrolled (179 of pulmonary tuberculosis, eight of extra-pulmonary tuberculosis and fourteen of both pulmonary as well as extra-pulmonary tuberculosis); who had interrupted treatment 327 times. Maximum interruptions (72.17%) were found to occur by third month of ATT. More than one reason was often reported for discontinuation of treatment. In all, 366 responses were obtained from 201 patients, in response to reasons for treatment interruption. The rate of treatment interruption was higher in the private health sector (56.27%), as compared to DOTS (34.25%) and other sources of treatment (9.48%). Early improvement (30.05%) and high cost of treatment (16.39%) were found to be the two most common reasons, leading to treatment interruption.

Conclusion: Early improvement and high cost of treatment were found to be the two most common reasons, leading to treatment interruption. Continuous health education should be provided to all tubercular patients emphasizing the need to continue treatment despite early improvement in symptoms. ***[Indian J Tuberc 2011; 58: 11-17]***

***Key words*:** Tuberculosis, Treatment interruption, Anti-Tubercular Treatment (ATT).

INTRODUCTION

Tuberculosis affects one third of the world's population[1]. It remains a major public health problem in the world with approximately 9.27 million new cases reported in 2007 and around 1.7 million deaths occurring each year[2]. India alone accounts for one-fifth of the world's new TB cases[1].

Under India's previous National Tuberculosis Programme (NTP), treatment completion rate of only 30 per cent could be achieved[3]. Since the programme was not DOT based, adherence to treatment remained a serious problem[3]. Chatterjee *et al*[3] estimated that 70 to 90 per cent of patients failed to take their drugs regularly. Revised National Tuberculosis Control Programme (RNTCP) was designed in 1992 to overcome the drawbacks of NTP. However, interruption in tuberculosis treatment still remains the major barrier to its control and is the most important challenge for control of TB. Inability to complete the prescribed regimen, is an important cause of treatment failure, relapse, acquired drug resistance and continuous transmission of infection.

Adherence to the long course of TB treatment is a complex, dynamic phenomenon with a wide range of factors impacting on treatment-taking behaviour. Many studies have been conducted across the world to study the reasons for default from ATT[4-6], and some are also reported from India (mostly done under RNTCP setting)[23-26]. The objective of this study was to study the reasons for discontinuation of Anti-Tubercular Treatment (ATT) among patients admitted in an Institute catering to a heterogeneous population.

METHODS

The present study was conducted over a six month period from May to October 2007, and

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consisted of an analysis of the data of pulmonary as well as extra-pulmonary TB patients admitted at LRS Institute of Tuberculosis and Respiratory Diseases, New Delhi during the said period. Ethical approval was taken and patients were interviewed using a semi-structured questionnaire after obtaining consent. Information recorded in the questionnaire included personal data, socio-demographic data, past and present history of ATT, sputum and X-ray results, and reasons for discontinuation of ATT. Data were analysed using Microsoft Excel and percentages were calculated and applied.

All TB patients admitted to the Institute during this period were interviewed regarding the past history of ATT and whether they had ever interrupted their treatment for two months or more anytime. For the purpose of this study, any patient suffering from TB (PTB/EPTB) at the time of interview, and also with a past history of treatment default was said to have interrupted treatment, which was defined as ATT intake of more than a month, with a gap of more than two months between two courses of ATT.

Patients who gave a history of treatment interruption as defined above were enrolled for the study. All these patients were then interviewed in detail using a pre-tested semi-structured questionnaire. The questionnaire was initially pre-tested in fifteen patients. In addition to the personal and socio-demographic data, treatment history was recorded in detail.

The recall period (from time of first treatment interruption to the time of interview) in this study, was lengthy in some patients, especially for those who had taken ATT more than twice in the past.

RESULTS

A total of 2505 patients were admitted to LRS Institute during the study period. Among them, 1488 patients suffered from Tuberculosis. Among the 1488 TB patients, 201 (13.51%) were found to have a past history of ATT interruption and were included in the study. 179 (89.05%) suffered from Pulmonary Tuberculosis, eight were cases of extra-pulmonary Tuberculosis (EPTB); while 14 had both pulmonary as well as extra-pulmonary tuberculosis.

Among the 201 patients interviewed, 156 (77.61%) were males and 45 were females. The highest number of treatment interrupters were in the age group 25 to 44 years (n=116), constituting nearly 57% of all the patients studied, while only 1.49% were below 15 years of age.

Of the 201 patients interviewed, 107 belonged to Delhi, while remaining 94 had come to LRS Institute from outside Delhi. 62 (30.85%) of the patients studied had been ill for only six months, while other patients had been ill for a longer duration, with nine (4.48%) patients having been ill for more than five years. 57.71% (n=116) patients were smokers among which 56.03% (n=65) had a smoking index of < 300, 12.93% (n=15) had a smoking index between 300 – 400 and 36 patients (31.03%) had a smoking index of >400. Almost half [49.25% (n=99)] of the patients interviewed had a history of alcohol intake.

179 of the patients interviewed had no co-morbidities. Among the remaining, nine had Diabetes, two had co-existing Hypertension and other co-morbidities were present in 11 patients.

Among the 201 patients enrolled, 42 (20.9%) were unmarried, 145 (72.14%) were married, 12 were widowed, while two were divorced. 58 (28.86%) patients had a family history of TB. 92 (45.77%) patients were residents of urban areas, while the other 109 (54.23%) patients used to reside in rural areas.

Among the 201 patients interviewed, 115 (57.21%) had interrupted treatment only once, while 59 (29.35%) had interrupted treatment twice; and remaining patients had interrupted treatment more than two times. Thus, the 201 patients included in the study had interrupted treatment 327 times.

Among the 327 treatment interruption episodes, 184 (56.27%) occurred when the prescribing source was a private practitioner, 112

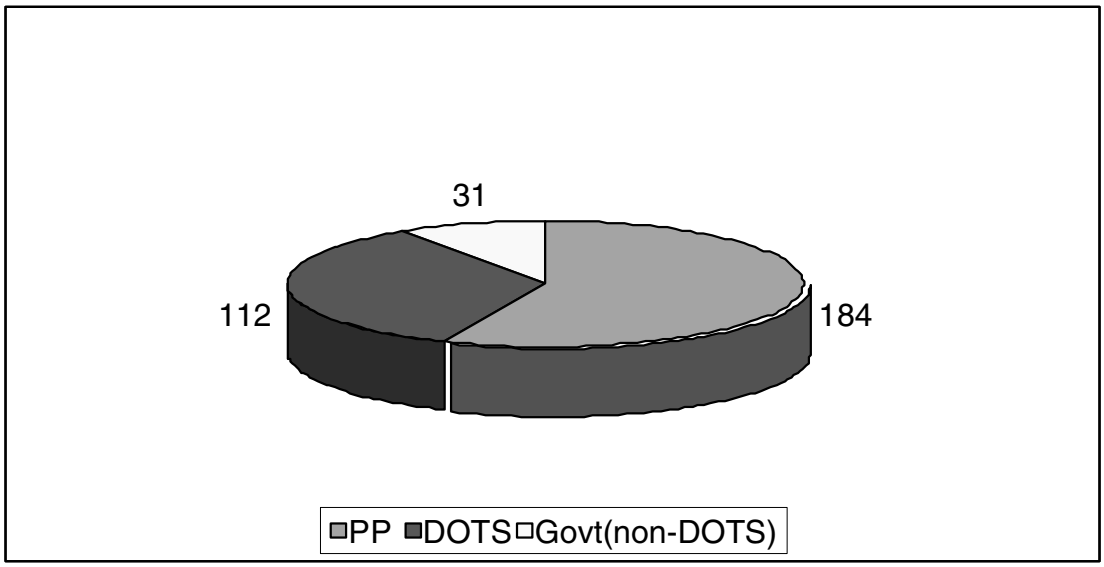

Fig. 1: Source of ATT among patients who interrupted treatment (n = 327)

(34.25%) took place while on treatment under DOTS, and remaining 31 (9.48%) interruptions took place while on non-DOTS treatment from a Government source (Figure 1).

Seventy-two percent of patients had stopped treatment by the end of third month of treatment. Maximum interruptions were found to occur between second and third months (Figure 2).

Among the 201 patients interviewed, 81 (40.3%) stated only one reason for interrupting their treatment. An equal number of patients stated two reasons, while 33 (16.42%) and six (2.99%) gave

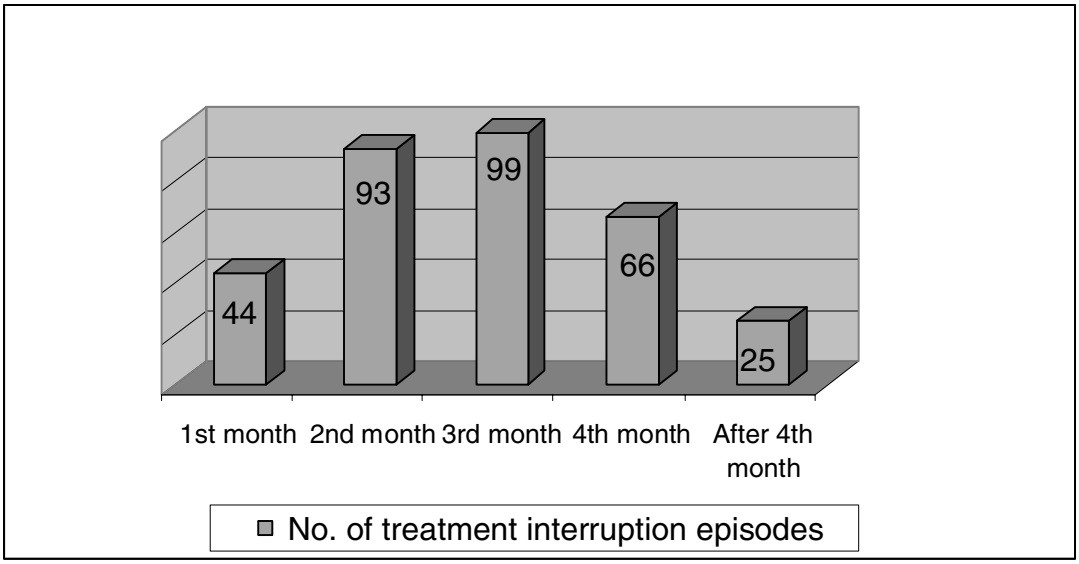

Fig. 2: Time of treatment interruption (n=327)

Table 1: Reasons for treatment interruption (n=366)

S. No.	Reasons for treatment interruption	No. of Patients who interrupted treatment	%age (n=366)
1.	Early improvement	110	30.05
2.	High cost of treatment	60	16.39
3.	ATT-induced side effects	47	12.84
4.	Unaware about long duration of treatment	33	9.02
5.	No improvement/ deterioration	26	7.10
6.	Alcoholism	16	4.37
7.	Advised to stop treatment by Physician	15	4.10
8.	Others	59	16.12
	TOTAL	366	

Table 2: Other reasons for treatment interruption (n=59)

S. No.	Other Reasons		No. of Patients	%age (n=59)
1.	Lack of Faith in treatment		10	16.95
2.	DOTS related	Long Distance travel to Centre	14	23.73
3.		Missing of work	4	6.78
4.		Shortage of medicines at Centre	6	10.17
5.		Refused treatment by health worker	3	5.08
6.	Personal/ family reasons	Family problems	5	8.47
7.		Went to village	13	22.03
8.		Non-compliant attitude	2	3.39
9.		Change of address	2	3.39
	Total		59	

three and four reasons respectively. Thus, 366 responses for treatment interruption were obtained from 201 patients. The most common reason stated was a feeling of early improvement (30.05%), followed by high cost of treatment (16.39%) and ATT-induced side effects (12.84%). (Table 1)

Among the various ATT-induced side effects (n=47), the most commonly reported side effect was nausea and vomiting (53.19%), followed by restlessness (14.89%), next being ATT-induced skin rash (n=6), drug-induced hepatitis (n=5); hearing loss (n=2), nephrotoxicity (n=1) and seizures (n=1).

Fifty-nine (16.12%) patients cited other reasons to be responsible for their treatment interruption. Other reasons being lack of faith in treatment (n=10); DOTS – related (n=27); and personal or family reasons (n=22). (Table 2)

DISCUSSION

This was an interview based analysis of the reasons for ATT interruption as reported by tuberculosis patients admitted to LRS Institute between May 2007 and October 2007. Among the 1488 TB patients admitted during the study period, 201 (13.51%) had a history of treatment interruption, of which 89.05% patients had pulmonary tuberculosis.

In this study, out of 327 treatment interruptions, 184(56.27%) interruptions occurred on private treatment, while 34.25% interruptions took place on DOTS; and remaining 9.48% treatment interruptions occurred on non-DOTS Government treatment. This emphasizes the need to provide DOTS to all as it is the only way to minimize treatment interruption.

In our study, 72% patients had interrupted treatment by the end of third month; and maximum (30.28%) interruptions were found to occur between second and third months. Other studies have also reported that maximum number of patients interrupted their treatment by the end of second or third month. Kaona *et al*[7] reported up to 39% patients stopped taking their medication within the first two months of commencing treatment; while Chan-Yeung *et al*[8] found that 45% of those who defaulted did so in the first two months of treatment. Oliviera *et al*[9] from Brazil have found that 43.3% of the defaulters, stop treatment in the first two months of treatment. On the contrary, some investigators have reported higher default rate after third month of ATT. Dodor *et al*[10] have determined the mean defaulting moment to be 3.4 months. Tekle *et al*[11] found defaulting to be the highest (81%) during the continuation phase of treatment. In a case control study by Demissie *et al*[12], most of the defaults occurred in the third and fourth months of treatment. Chee *et al*[13] have stated that 70% patients defaulted during the continuation phase of treatment.

When trying to assess the reasons for treatment interruption, 366 responses were obtained. The most common reason was a feeling of early improvement as stated by 110 patients (30.05%). Kaona *et al* [7] also found that 29.8% of TB patients failed to comply with ATT once they started feeling better. Social problems and feeling of improvement were the top two reasons for patients to default in study by Demissie *et al*[12]. In another survey by Tissera[14] at Colombo Chest Clinic, relief from symptoms (13%) emerged as the most common reason for treatment interruption. However, in a study by Jaggarajamma *et al*[15], relief from symptoms was found to be the third commonest reason for discontinuation of treatment (20%).

As interruption frequently occurs at the third month of treatment, which coincides with clinical improvement and early improvement is the commonest reason, it is imperative to provide repeated health education to patients emphasizing the need to continue treatment despite improvement.

The next most common reason was high cost of treatment cited by 60 (16.39%) patients in our study. This was exclusively reported by patients who had interrupted ATT from non-DOTS sources as they had to purchase their medicines from the market. It is thus necessary to incorporate all TB patients for treatment under DOTS, so as to reduce the number of interruptions occurring due to high

cost of treatment. To achieve this, public-private mix is a must.

ATT-induced side effects leading to treatment interruption ranked as the third commonest reason, in the present study, stated by 47 (12.84%) patients. In a study from Tiruvallur district, South India, Jaggarajamma *et al* [15] have found drug related problems to be the leading cause of treatment interruption in 42% patients. Similarly, Wares *et al*[16] found the most common reason for stopping treatment being the adverse effects of ATT.

Thirty-three (9.02%) patients said that they interrupted treatment as they were unaware about the long duration of treatment. In a study by Bam *et al* [17] from Kathmandu, 61% non-adherent patients claimed insufficient knowledge about the need to continue treatment, especially after they felt better.

Twenty-six (7.1%) patients stopped taking their drugs as they were unable to appreciate any improvement or felt they were deteriorating. Sixteen (4.37%) patients blamed alcoholism as the reason for their treatment interruption. In a study from the Russian Federation, Jakubowiak *et al* (2007)[18] have found alcohol use to be the second commonest reason (30%) for treatment default.

Fifteen patients in our study said that their treating physicians had advised them to stop their treatment. Fifty-nine (16.12%) patients cited other reasons to be responsible for their treatment interruption. Among DOTS related other reasons, 14 patients had interrupted treatment due to long distance of travel to their DOTS centre. In a study by Chatterjee *et al*[3], the most common reason for treatment interruption was distance from the treatment centre. Many studies have demonstrated the indirect costs of treatment to be responsible for treatment interruption. In the pre-Rifampicin era, Pathak[19] reported that many patients defaulted if visit to the clinic involved loss of wages. Similarly, Mishra *et al*[20] reported that the risk of non-adherence to treatment was significantly associated with cost of travel to the TB treatment facility. In a study by O'Boyle *et al*[21], cost of transport was the reason most frequently given for non-attendance at DOTS centre. Hill *et al*[22] have reported a higher default rate among those who incurred significant time or money costs travelling to receive treatment.

CONCLUSIONS

Maximum interruptions were found to occur by third month of ATT. Multiple treatment interruptions were common. More than one reason was often reported for discontinuation of treatment. The rate of treatment interruption was higher in the private health sector, as compared to DOTS and other sources of treatment. Early improvement and high cost of treatment were found to be the two most common reasons, leading to treatment interruption.

The need for direct supervision of treatment and continuous health education emphasizing the need to continue treatment despite early improvement cannot be over-emphasized. Default occurring due to early improvement also calls for the introduction of drugs that would further shorten the duration of chemotherapy. Also the number of DOTS centres should be increased so that it comes within reach of everyone.

LIMITATIONS

1. This is a hospital based study and not a field study; therefore the results may not be reflective of reasons for treatment interruption in the community.
2. The study is interview-based and documents were not always available, so there could be error in reporting of events from patient's side. Also, for some patients, the recall period was six to seven years.
3. Due to lack of control group in the study, statistical tests could not be applied.
4. As the study was conducted at a tertiary referral hospital, where patients usually present with advanced disease or complications, the results may not be applicable to the general population suffering from T.B.

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Original Article

SUSTAINING PPM-DOTS: THE CASE OF PIMPRI CHINCHWAD, MAHARASHTRA, INDIA

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Summary

Background: Globally, Public-Private Mix (PPM) models of service delivery are recommended as a strategy for improving tuberculosis (TB) control. Several models of PPM-DOTS have been initiated under the Revised National TB Control Programme (RNTCP) in India, but scaling up and sustaining successful projects has remained a challenge.

Aim: This paper examines factors accounting for the sustainability of a PPM-DOTS initiated in 1998 in Pimpri Chinchwad (PC), a city in Maharashtra, India.

Methods: A two-year intervention research project documented the workings of the PPM–DOTS programme. This paper draws on in-depth interviews with programme officers and staff, and semi-structured interviews with private practitioners (PP) practising in the study area.

Results: PPM-DOTS was originally introduced in PC, in order to increase access to DOTS. Over the years it has become an integral part of the RNTCP. Multiple approaches were employed to involve and sustain private providers' participation in PPM-DOTS. Systems were developed for supervision and monitoring DOTS in the private sector. Systematic use of operations research and successful mobilisation of available local resources helped set future direction for expanding and strengthening the PPM. The private sector's contribution to case detection and treatment success has increased, however ensuring referrals of TB suspects from all private providers continues to present a challenge.

Conclusion: PPM-DOTS in PC is one of the few Indian models implemented as envisaged by global and national policy makers. Its successful operation for over a decade reiterates the importance of public sector initiative and leadership and makes it an interesting case for study and replication. **[*Indian J Tuberc 2011; 58: 18-28*]**

Key words: PPM-DOTS, Sustainability, India

INTRODUCTION

Public-Private Mix (PPM), a strategy for optimising the strengths of both public and private sectors in health care delivery, has been implemented for tuberculosis (TB) control. It is regarded as a model that could be adapted to other disease control programmes[1,2,3]. However, the contribution of PPM to TB control, its scaling up and sustainability as a health systems strategy is debatable[4]. Specifically, integration of PPM-DOTS into the public health system has proven challenging[5].

We present the case of PPM-DOTS in Pimpri Chinchwad (PC) a city in Western Maharashtra, India. PPM was initiated in PC in 1998, and has been sustained for over a decade exclusively with resources from the Revised National TB Control Programme (RNTCP). We discuss its sustainability using six evaluation criteria that have been proposed in the literature on PPM-DOTS[3,6] - (1) Initiation of PPM; (2) Clarity about roles and responsibilities of partners; (3) Supervision and monitoring; (4) Efforts at ensuring sustainability; (5) Evaluation based on case detection and cure rates and; (6) Challenges in expansion and sustainability.

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STUDY SETTING

With a population of 1.01 million (Census 2001), PC is the seventh largest city in Maharashtra, covering an area of 171 sq km. Over the past decade, the city has seen a population growth of 95%[7].

The health infrastructure in PC is typical of urban India. The public sector with eight municipal hospitals (one super speciality 600-bedded hospital, two multi-diagnostic centres, and five maternity hospitals) and 15 municipal dispensaries co-exists with a vast private sector that serves as the first provider contact for 83% of new sputum positive patients[8]. In 2006, AYUSH (Ayurveda, Yoga and Naturopathy, Unani, Siddha, Homeopathy) practitioners accounted for 88% of private practitioners (PP) in PC[9]. The City TB Centre (CTC) initiated PPM with the introduction of the RNTCP in 1998, and currently PC has over 250 Directly Observed Treatment (DOT) centres in the private sector[10].

METHODS

This paper is based on primary quantitative and qualitative data and secondary data from the RNTCP, gathered in the course of an intervention research project conducted between 2006 and 2008. Specifically, we draw on in-depth interviews with three programme officers [including the City TB Officer (CTO) who initiated the PPM, the CTO who succeeded him and a Medical Officer (MO) of a TB Unit], and eight TB health visitors (TB HV); semi-structured interviews with 497 PPs (164 DOT provider PPs and 333 PPs not providing DOTS) conducted as part of the baseline survey; and semi-structured interviews with 19 PPs (14 DOT provider PPs and five PPs not providing DOTS) who had participated in one of the pilot interventions. The in-depth interviews explored the respondents' perspectives on PPM-DOTS, and the semi-structured interviews with PPs documented their practices and experiences with PPM-DOTS, including communication with the RNTCP. Quantitative data from semi-structured interviews were analysed using SPSS, version 16 (SPSS Inc., Chicago IL). Qualitative data from in-depth interviews were analysed thematically.

RESULTS

Initiation of PPM-DOTS in PC

Involvement of PPs*:* The CTC introduced PPM to enhance patients' access to TB care in response to the RNTCP's mandate to provide DOT within two kilometres of a patient's residence. Initially, programme officers, Senior Treatment Supervisors (STS) and Senior TB Laboratory Supervisors (STLS) approached PPs in peripheral areas of the city that had inadequate public sector health care facilities. The CTC also advertised through local newspapers inviting PPs to undergo training and become DOT providers.

Over the years, the CTC recognised the need for regular liaising with the PPs to ensure their involvement in PPM-DOTS. With the increase in the number of PPs, it assigned TB HVs – a cadre of outreach workers introduced in 2003 by the Central TB Division for urban areas primarily for address verification and defaulter retrieval through home visits – the task of approaching existing DOT provider PPs and requesting other PPs to participate in PPM-DOTS or to refer their TB suspects/ patients to the RNTCP. Subsequently, continuing medical education (CME) sessions on PPM-DOTS were organised for recruited PPs.

Over the years the programme has engaged almost a fifth of the PPs from PC as DOT providers. The proportion of DOT providers among non-allopaths (34%, 150/437) was higher than among allopaths (23%, 14/60) (Chi square, $p=0.090$)[9]. PPs reported multiple motives for their participation as DOT providers. Seventeen percent (28/164) said that their practice would gain from their participation. Of these, almost all (27/28) were trained in AYUSH systems of medicine and about half (12/28) had set up practice in the last five years. More than two-thirds (68%, 111/164) regarded PPM as a way of contributing to society and 56% (92/164) reported their participation would benefit their patients.

Several reasons were attributed to the difference in the participation of allopaths and non-allopaths in PPM-DOTS. The former CTO felt that allopathic PPs were less likely to refer a TB patient to the public sector because they were trained in TB management at the MBBS level. However, for non-allopaths, PPM represented an opportunity to become familiar with 'modern' TB management practices.

> *"*....[PPM training] *was informative especially for non-allopaths. They were inquisitive. Also because of the training, they could give complete TB treatment to their patients....they got to know the protocol."* (MO, TB Unit)

Involvement of other partners: Although the RNTCP in PC has focused on PPs as partners in the PPM, it has also reached out to other sector providers. Over the years, the CTC has involved nine NGOs, two corporate health facilities, five private pathology laboratories, one private medical college with a tertiary hospital and the State Insurance Hospital in PPM-DOTS. Community volunteers, especially members of women's small savings groups and women health workers under the government's Integrated Child Development Scheme (*anganwadi* workers) have also been involved as DOT providers. As with PPs, the main criteria for selection of other partners were location and ability to provide easy access to DOTS. The process gained momentum after the PPM guidelines were issued by the Government of India[11-13].

Incentives: In PC, the programme officers' views and the programme's experiences with incentives are mixed. Involvement of PPs steadily increased in the initial years even in the absence of incentives. According to the programme officers, incentives introduced in 2006 by the RNTCP did not influence motivation for participation in PPM-DOTS too much.

> *".....They* (PPs) *know that there is a* [incentive] *scheme now. Initially there was no scheme so they did not ask, but when the scheme was declared people* (PPs) *started asking... For 30%* (participating PPs)*, it is motivation and for 70% it is not. That is my observation"* (Former CTO)

The former CTO also noted that the small proportion of PPs who find incentives important are *"... the people who have an 'average level' of practice,.... I remember Dr. X from Y. He used to treat so many patients. He refused the money. He said 'I don't need it."*

The CTO felt that although the incentives were useful in motivating few PPs, the programme's approach towards the partners was more important for ensuring partnership. He believed that incentives should be paid as a measure of increasing accountability:

> *"Incentives alone will not work...incentives are for few people but motivation, encouragement and leadership* [by the programme] *are important. You give them the incentives* [and say] *'...utilize this for the phone calls, for mobile calls, for water for the patients. It is not* [intended] *as a profit-making* [mechanism]. *This should be utilized for the poor patients. Certainly this should work*...[If] *we give incentives then we can ask them for more returns."*(CTO)

Clarity about roles and responsibilities

According to the 2002 PPM policy, DOT provider PPs were expected to give DOT, mark treatment cards, inform programme representatives regarding missed doses within a stipulated time period, and refer patients for follow-up sputum examinations[14]. When assessed using these guidelines, a wide variation was seen in PPs' commitment levels as well as the quality of DOT at DOT provider PPs' clinics.

On the one hand, programme staff and MOs reported of DOT provider PPs, who successfully managed confirmatory and retrieval visits and marked all cards independently. On the other hand, there were PPs whose commitment to

the programme, as the CTO put it, did not translate "*into taking on more responsibility towards the patients*" including the administrative work involved in DOTS.

> *"Practitioners do not want to have that much paper work. Tick marking on cards does not take much time but the attitude of PPs should be that treatment cards are essential as it ensures that patients are taking regular treatment…"* (CTO)

The programme officers' observation was reflected in the survey findings which showed that at seven percent (12/162) PP DOT centres their clinic staff supervised DOT; and in 12% (20/162) treatment cards were maintained by these staff or the RNTCP staff. Twenty-eight percent (46/162) and 36% (58/162) of the DOT provider PPs reported handing over more than one blister pack to a patient in a single visit in the intensive and continuation phases respectively. One-tenth (10%, 15/157) of the DOT provider PPs further reported not taking any action in case a patient missed doses (Table 1).

Table 1: Quality of DOT in private sector

Indicators	**DOT provider PPs Number (%)**
Observation of DOT	***n=162****
PP	129 (79)
PP or PP's Assistant or PP's Staff	19 (12)
PP's Staff only	12 (7)
Not observed	1 (1)
Not asked	1 (1)
Maintenance of Treatment cards	***n=162****
PP	137 (84)
Staff at PPs' clinic	13 (8)
PP / PP's Staff / PP's Assistant	5 (3)
RNTCP staff (always / sometimes)	7 (4)
Number of blister packs given to the patient at a time – Intensive Phase	***n=162****
One (either observed being taken at the clinic, or allowed to take at home)	114 (70)
More than one (often / sometimes / rarely)	46 (28)
Can't say / No response	2 (2)
Number of blister packs given to the patient at a time – Continuation Phase	***n=162****
One	101 (62)
More than one (often / sometimes / rarely)	58 (36)
Can't say / No response	3 (2)
***Action taken if patient misses a dose**[#]*	***n=157**^*
PP visits patient	17 (11)
PP tries to contact patient through an acquaintance or over telephone	69 (44)
No action taken	15 (10)
PP informs RNTCP[β]	102 (65)
No patient has missed a dose	17 (11)

*2 of 164 participating DOT provider PPs interviewed for the survey had not provided DOT after they received training and were excluded from the present analysis
[#]Multiple response
^Valid responses only
[β] Duration within which PP informs RNTCP not available

The former CTO attributed complacency among DOT provider PPs to their confidence that TB HVs would take care of the records and patients. The PPs' inability to comprehend the gravity of the assigned tasks and their submission to patient pressure sometimes had serious consequences:

> *"…He* [DOT provider PP] *used to give patients tablets to take home. .....I saw a lot of difference* [between the cards and the treatment boxes]. *Then twice – thrice I requested him, 'do not do this'. But he was so used to it, that he would give the tablets* [to take home]. *Then I went to the patient's home.... I enquired* [and found that] *the patient had left the city with the medicines. The patient did not come for about a month and a half. He had taken the tablets that the doctor had given him. But he delayed his return.... because of that once again he became* [sputum] *positive and had to go on Cat II* [re-treatment regimen]*"* (TB HV)

Supervision and monitoring

Programme officers felt that the only way to ensure that PPs take their responsibilities seriously and reduce default was continuous monitoring of the PP DOT centres. The TB HVs, primarily responsible for regular monitoring, were given explicit directives for their weekly visits:

> *"I tell the HVs that you have to see that they are filling the cards, doing the follow up and* treatment [DOTS] *should be under observation. I keep telling them that at each visit they have to remind* [PPs] *about these three things."* (MO TB Unit)

Among the assigned tasks, retrieval action for defaulter patients was prioritised.

> *"TB HVs have to visit the PP DOT centres weekly. ...But* [if] *he is unable to go there on the assigned days then there is a gap. I have told TB HVs not to have that gap. If there is any interruption of weekly visits, even though it is not part of his advance tour programme, priority should be given* [to retrieval action]". (CTO)

Of the 155 DOT provider PPs who felt that the visits by TB HVs were useful, 28 (18%) specifically mentioned their importance in retrieving patients who interrupted or defaulted treatment,

> *"Patients don't take medicines. Sometimes there are 'social problems'. There is still a lot of 'stigma'. We don't have that much time, but they* [TB HVs] *can counsel* [these patients] *well."* (DOT provider PP interviewed post-intervention)

> *"They go and give information to the patient properly. Patients do not pay attention to their illness.... The family also needs to be told. They have to be 'convinced' about taking their medicines properly. He* [TB HV] *does that."* (DOT provider PP interviewed post-intervention)

The TB HVs' work in turn was supervised by STSs and programme officers through daily review and visits to DOT provider PPs' clinics.

> *"Once, the MO-TU visited a DOT provider in my area and checked the boxes. Two boxes matched with the cards but there was an error in the third one. She explained to me that even if one has good rapport with the doctor, it is important to cross check* [the card with the box]. *Actually the patient had not taken medicines for 15 days but the card had been marked."* (TB HV)

These visits by the programme officer to PP DOT centres were also useful in monitoring quality of DOT at these centres, identifying weaknesses in the monitoring system and taking measures to strengthen them.

Efforts at ensuring sustainability

Senior programme officers attributed the sustained functioning of PPM to its initiation as a felt need and its implementation as an integral part

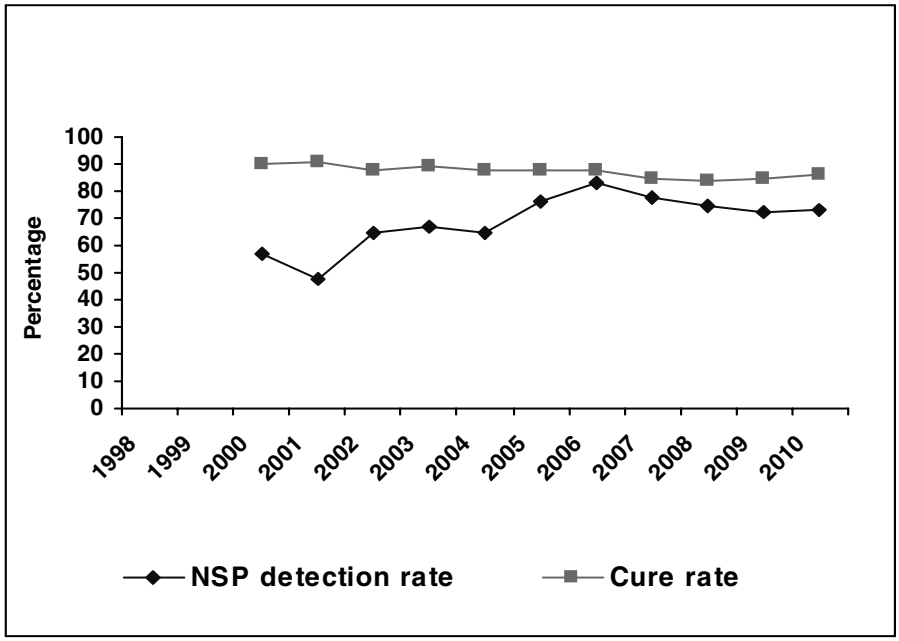

Figure 1: Performance of RNTCP in PC

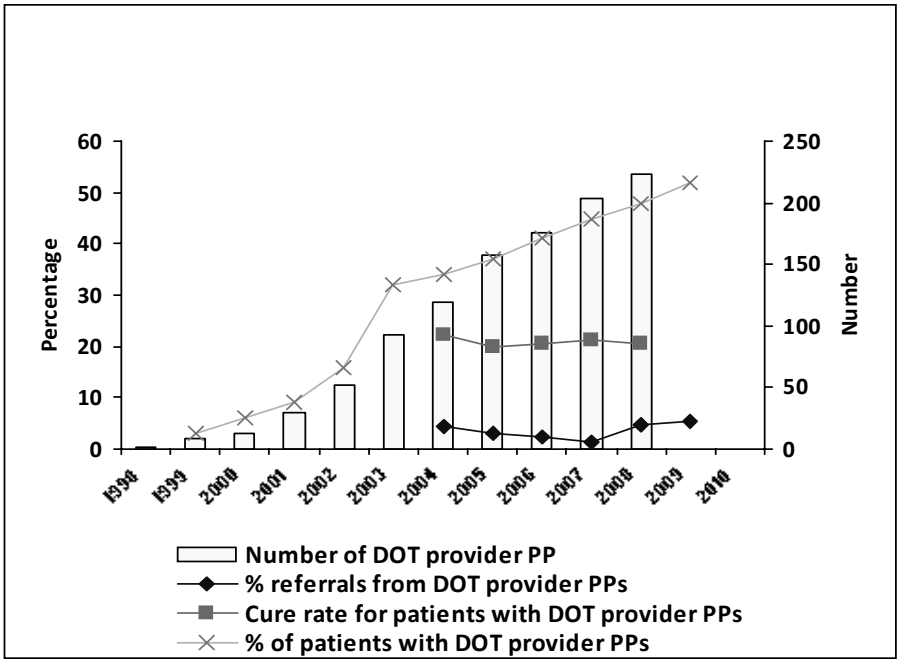

Figure 2: Performance of RNTCP in PC

Table 2: Treatment outcome for new sputum positive cases

Year	Sector	Cure	Default	Died	Failure	Transferred out	Total	Chi square probability (Cure / Default)	Chi square probability (Favourable / Unfavourable outcome)
		(Favourable outcome)	(Unfavourable outcome)						
2004	Public	358	26	21	13	5	423	0.007*	0.002342*
	Private	210	4	9	2	1	226		
2005	Public	376	20	25	14	0	435	0.394	0.266385
	Private	242	17	19	9	3	290		
2006[#]	Public	298	22	17	17	5	359	0.158	0.541541
	Private	272	12	25	8	4	321		
2007	Public	243	24	19	8	6	300	0.025*	0.007071*
	Private	316	15	12	9	5	357		
2008	Public	247	11	14	13	6	291	0.389	0.580931
	Private	299	9	20	11	7	346		

*Significant at 5% level
[#]Data taken from a presentation[39]

of the RNTCP. The CTO remarked that PPM in PC was successful due to a strong underlying health care delivery system.

> "*...the public health care delivery is very strong and that helps us* [programme officers] *to have a direct partnership with the PPs without involvement of any interface or NGOs. We are members of PCDA* [doctors' association], *we participate in various doctors' programmes and it helps us to get familiar with all the doctors there – allopaths, non-allopaths. So it helped us in bridging the gap and there is a direct relationship, direct partnership*". (CTO)

All levels of programme staff – from TB HVs to senior programme officers – have maintained contact with PPs. Senior programme officers' visits provided opportunities to hold clinical discussions with PPs. Efforts to ensure sustainability have also included providing written or verbal feedback about referred patients, organizing periodic trainings for PPs and piloting an intervention to strengthen the referral – feedback system[9].

Over the years, PPM-DOTS has moved from being an activity to meet the policy mandate to an established mechanism for quality assurance, evident in the resource mobilisation, including involvement of TB HVs for liaising with DOT provider PPs, and in the systematic use of operations research for periodic evaluation of the programme and for setting future directions. Two studies looking at the treatment delays in sputum positive patients were conducted in 2000 and 2004 respectively. While the first study hinted towards the benefits of PPM[41], the second study showed evidence regarding the benefits of PPM-DOTS for patients as well as the programme by reducing the provider delays and costs incurred by patients before reaching the RNTCP[8]. In 2006, the CTC initiated the present intervention research study to explore the enablers and barriers for expansion and sustainability of PPM-DOTS that identified the need for increased communication between the PPs and the programme and better behaviour towards patients by the public health care staff. Accordingly interventions were piloted to respond to these needs- workshops on communication for RNTCP staff as well as zonal meetings between representative of RNTCP and PPs and an intervention to strengthen the existing referral and feedback system under the RNTCP.

Evaluation based on conventional indicators

The RNTCP in PC is successful with detection rates close to the national target and cure rates above the national target for most years since its introduction in 1998[15-24] (Figure 1). The private sector's contribution to case detection and treatment success has also increased over the past decade (Figure 2). For most years, the cure rate for patients on DOTS in the private and public sectors was comparable (Table 2).

Challenges faced

Ensuring partners' ownership of DOTS and adherence by the DOT provider PPs to the RNTCP guidelines were the main challenges in sustaining quality PPM in PC. Among the surveyed PPs, 22 (13%) of the 164 DOT provider PPs and 64 (19%) of the 333 PPs not providing DOTS prescribed non-RNTCP regimens to some of their patients. The most commonly reported reasons were that patients, especially when more educated and socio-economically better-off, chose private non-RNTCP treatment primarily because of stigma or prior poor experiences in the public sector. A small number of PPs perceived the RNTCP's insistence on re-diagnosing patients they had referred after diagnosis as challenging their clinical skills. Others felt that being a DOT provider limited their role as clinicians.

Despite repeated efforts by the RNTCP, many DOT provider PPs failed to inform the programme regarding treatment interruption by patients within the stipulated time period. Periodic CME sessions organised by the CTC to re-affirm the importance of treatment adherence were poorly attended as DOT provider PPs found them repetitive and unnecessary.

The CTO felt that one of the weaknesses of PPM-DOTS in PC was the inability of the MOs at peripheral health institutions (PHI) to be more actively involved in PPM-DOTS. Rigorous monitoring of public and private DOT centres by the MOs of PHIs and the MO-TU being solely dedicated to the RNTCP work were necessary to address this problem.

Ensuring referral of all TB suspects to the RNTCP was another challenge. Some PPs, especially allopaths, consultant physicians and PPs, who had started practicing before the RNTCP, did not refer all TB suspects to the RNTCP or referred only after a delay.

> *"Allopathic doctors hesitate to get involved because either they are attached to some physician or they have "relations"* [relationship] *with some hospital. If they come across a patient with Koch's* [TB], *generally they send him to a physician. Initially they get x-ray, and other investigations done from outside* [private sector], *and then if the patient is unable to afford, only then they refer to us.* (TB HV)

Discussion

PPM-DOTS in PC has traits common to many successful projects documented elsewhere[25-34] yet, it has components not commonly seen in most other models[3, 6].

First, PPM-DOTS in PC is an example of an initiative led by a strong public sector TB programme. Programme officers in PC have ensured that PPM-DOTS is sustained without external support or additional resources. In most other documented projects, an NGO or a professional body played a key role in establishing linkages between the public and private sectors[3]. Public-private partnerships that are formed because of policy mandates often fail to sustain beyond demonstration phase[35]. In contrast, PPM-DOTS in PC was initiated and sustained by integrating it within the local RNTCP implementation machinery in order to expand services.

Second, PPM-DOTS in PC is one of the few Indian models implemented in close accordance with the concept of PPM originally envisaged by global and national policy makers by involving various types of private sector providers. There are PPM-DOTS projects in India that have focused on particular types of private care provider like PPs, pathology and radiology clinics, private specialists,

small nursing homes and TB sanatoria[25-29,32,33]. Published literature shows very few Indian models have involved a range of private, NGO, corporate and non-RNTCP implementing public sector providers as partners[26,27,31] using a multipronged approach[29,31,36] in the way PC has.

Third, despite mixed evidence regarding the effective contribution of non-allopaths in PPM-DOTS[27,29,33], the RNTCP in PC has involved them as DOT providers. In a context where non-allopaths form 88% of practising PPs, their involvement is crucial as they may be the most accessible and affordable providers for many vulnerable patients[37].

Fourth, while the monitoring and supervision mechanisms in PC are comparable to those in other PPM projects[27,29,33], they stand out in terms of optimal use of available human resources for the entire range of PPM activities. While other Indian models of PPM-DOTS that used similar processes have acknowledged the benefits of the approach, they have reported difficulties in scaling-up the programme and expressed concerns regarding its sustainability[33]. The programme in PC on the other hand, has ensured that the entire city is covered by TB HVs for over a period of five years.

The contribution of PPM-DOTS to case detection in PC, is comparable to projects in Mumbai, Kerala, and Delhi[24,26,31,34]. As with other projects, treatment outcomes for patients with DOT provider PPs is satisfactory[38] and comparable[27,33] to those with public sector DOT centres[39]. PPM in PC is also comparable to other PPM projects in terms of reduction in costs incurred by patients in accessing DOTS[8,27,40,41]; additionally, it also reduced system delays[8].

Efforts invested by the CTC in PC to establish and maintain communication with partners, mobilise resources, conduct operations research for periodic assessment of the progress and sensitise PPs and patients regarding the benefits of PPM-DOTS have contributed to its success. Through these efforts, PC distinguishes itself from other projects that used similar strategies but were unable to expand due to increased burden of supervision for staff[33]; or could not be sustained due to lack of communication between the partners, weak leadership, or lacking infrastructure and commitment from the public sector[32]. Most importantly, unlike other projects, PPM-DOTS in PC could be sustained because it is well integrated within the local RNTCP[32].

CONCLUSION

The case of PPM-DOTS in PC reiterates the importance of initiative and leadership by the programme for orienting and training the potential partners; and ensuring adequate supervision and monitoring while having a continued dialogue with them. It also demonstrates the importance of the programme's commitment towards a systematic and organised identification of problems and challenges and addressing them through optimal use of local resources to help scale up and sustain PPM-DOTS.

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Case Report

TUBERCULOSIS OF PATELLA IN AN IMMUNOCOMPETENT PATIENT - A CASE REPORT

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***Summary*:** Isolated involvement of patella as extra-pulmonary tuberculosis is very uncommon. It is the clinical presentation of the patellar osteomyelitis that makes it prone to delay in diagnosis as reported in literature. Imaging studies, especially MRI, are of great interest for the diagnosis and to assess the extent of tuberculosis. A tissue or bacterial diagnosis is essential. Early institution of anti-tuberculous drugs minimizes the spread of the diseases, decreases the patient's morbidity and gives a better knee functions. One rare case of patellar tuberculosis is discussed here with its clinical and radiological features.
[Indian J Tuberc 2011; 58:29-31]

Key words: Patella, Osteomyelitis.

INTRODUCTION

Skeletal involvement of extra-pulmonary tuberculosis is common and knee joint is third most common joint involved after spine and hip joint[1]. Isolated involvement of patella is very uncommon with reported incidence of 0.09 to 0.15% in literature[2]. One rare case of patellar tuberculosis is discussed here with its clinical and radiological features.

CASE

An apparently healthy 34-year-old female presented with complaints of throbbing pain in right knee for the last one month duration. Later on, she developed swelling of the right knee and noticed limping while walking and restriction of movements of right knee. There was no history of locking, instability, morning stiffness and involvement of other joints. She had no constitutional symptoms and refused for any history suggestive of tuberculosis or contact with tuberculosis patients. Examination of the right knee revealed swelling, warmth, and tenderness over knee cap, mild effusion and loss of terminal 20^0 - 30^0 of flexion. ROM was full, no crepitations, quadriceps were good and distal pulses were normal.

Laboratory investigations showed Hb of 11.2 gm/dl, TLC of 5900/ mm^3 with polymorph of 70% and ESR of 30 mm in first hour. RFT, LFT, RA Factor, connective tissue markers, urine routine examination, USG abdomen and X-ray chest were non-contributory. Quantitative CRP showed positivity with value of 10 mg/l (>10 mg/l significant) and Mantoux test produced 16 mm of induration at the end of 72 hours. AP and Lateral views of right knee X-ray were looking normal. Skyline view of the knee revealed osteolytic lesions of patella (Fig.1). MRI of the right knee revealed

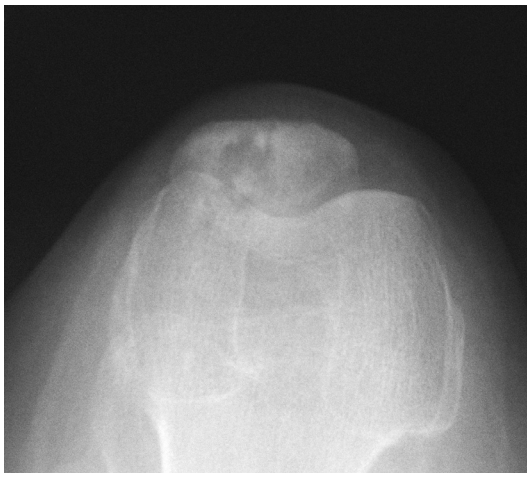

Fig.1: Skyline view of the knee revealed osteolytic lesions of patella.

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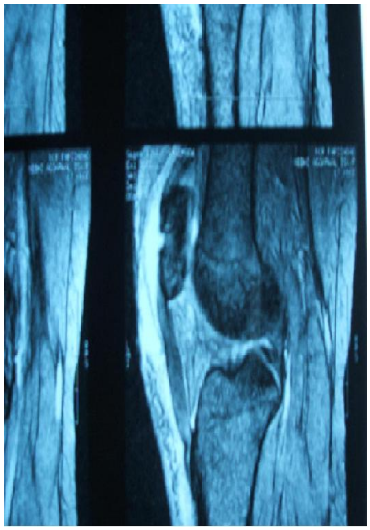

Fig. 2: MRI of the right knee revealed focal area of destruction with patchy confluent areas altered marrow signal intensity involving the patella, predominantly anterior aspect with anterior cortical destruction. There is associated soft tissue collection at anterior aspect of the patella beneath the subcutaneous fat plane.

focal area of destruction with patchy confluent areas altered marrow signal intensity involving the patella, predominantly anterior aspect with anterior cortical destruction. There was associated soft tissue collection at anterior aspect of the patella beneath the subcutaneous fat plane as well as moderate soft tissue thickening around the knee joint involving the adjacent soft tissue including myofascial plane and subcutaneous tissue in anterior, medial and lateral aspect of knee joint with likely inflammatory/ infective aetiology (Fig. 2).

She was subjected to incision and drainage of collection (diagnostic and therapeutic). Drainage consisted of necrotic material with multiple soft tissue pieces. Histopathological examination revealed areas of haemorrhage with dense collection of lymphocytes, plasma cells, histiocytes and multiple epithelial cell granulomas along with Langhan's giant cells. Stain for AFB was positive. She was started on appropriate anti-tuberculosis drugs (2 HERZ + 4 HR). After a follow up of six months, she improved clinically and radiologically with full knee movement without pain or swelling.

DISCUSSION

Although knee joint is the third most common joint involved in tuberculosis in the body, the patella, however, as a primary site, is most uncommonly involved. Earliest reported cases in literature are by D. McCrae Aitken in May 1933[3], LrNscniErD & anid Dahlin in 1966[4] and George Hartofilakidis-Garofalidis, in 1969[5]. Tuli SM in 1991 reviewed 1074 cases of osteoarticular tuberculous lesions and reported an incidence of 90 cases (8.3%) involving the knee, out of which only one (0.09%) was localized in the patella[1]. Martini and Boudjema have mentioned only one case of tuberculous osteomyelitis of the patella in 652 cases (0.15%)[6]. Most of the cases reported are in the age group of 5 to 15 years. Before the age of five years, the patella is more or less cartilaginous in nature and hence osteomyelitis of the patella usually does not manifest at that age[7]. Three modes of acquiring the infection are clear: direct invasion following injury, haematogenous, and local from prepatellar bursitis[8-10].

It is the clinical presentation of the patellar osteomyelitis that makes it prone to delay in diagnosis as reported in literature. The lack of clinical suspicion, due to rarity of the entity, could be another cause for the delay. A high index of suspicion should thus be maintained in cases of patellar pain with or without signs of infection. Richter *et al*[11] gave an account of seven cases of tuberculous osteomyelitis, all of which presented late and the lesion in all was situated within the corpus of the patella. Differential diagnosis of lesions in patella includes tumours (e.g. chondroblastoma, osteoblastoma, infected aneurismal bone cyst to metastatic lesions), tumour like conditions (e.g. Brown tumour) and inflammatory lesions (e.g. gout, pyogenic and osteomyelitis)[12]. Imaging studies, especially MRI, are of great interest for the diagnosis and to assess the extent of tuberculosis. An osteolytic lesion with a sequestrum is usually suggestive of infective pathology and absence of

sclerosis and location in a para-articular region suggests tuberculosis[12, 13]. A review of the literature has revealed only one common denominator in radiographical findings; an osteolytic lesion in the patella. A tissue or bacterial diagnosis is essential, and some form of biopsy, either excisional or incisional, is usually required. Open biopsy may be necessary in some doubtful cases. From a treatment point of view, the basic treatment in the form of early institution of adequate anti-tuberculous therapy and guarded function cannot be over-emphasized. In this case, early institution of anti-tuberculous drugs may have minimized the spread of the disease inside the joint and may have perhaps decreased the patient's morbidity and given her a better knee functions.

We advocate that osteolytic lesions of the patella should be evaluated early to diagnose rare cases of tuberculosis, as these can be cure with early institution of therapy.

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Case Report

PRIMARY CONJUNCTIVAL TUBERCULOSIS IN A 14 YEAR OLD GIRL

Jeyanth Suresh Rose[1], Anupriya Arthur[1], Renu Raju[2] and Meera Thomas[3]

(Received on 9.2.2010; Accepted after revision on 2.12.2010)

Summary: Tuberculosis is a common disease in India. However, tuberculosis primarily affecting the conjuctiva is a rare entity. We report a 14-year-old girl who presented with unilateral eye discharge, watering, redness and itching for two weeks. Giant papillae were present on the upper tarsal conjunctiva. A provisional diagnosis of allergic conjunctivitis was made. Topical therapy with 1% Prednisolone acetate and 2% Sodium cromoglycate was commenced. The patient returned six months later with no improvement in the symptoms.The tarsal conjunctiva had a polypoidal, velvety appearance with giant papillae. A fibrinous membrane was seen over the tarsal conjunctiva and a preauricular node was found. Excision biopsy and histopathologic examination revealed necrotizing granulomatous inflammation suggestive of tuberculosis. Systemic examination and investigations were normal. She was started on anti-tuberculous therapy. In two months she showed complete resolution of symptoms and marked reduction in papillae and conjunctival thickening.

Symptoms and signs of unilateral conjunctivitis may masquerade as primary conjunctival tuberculosis. In an endemic country like India, laterality, chronicity and non-resolution of symptoms with steroids are indications for pursuing a biopsy earlier than later. In our patient, the histopathology clinched the diagnosis of conjunctival tuberculosis resulting in a faster and complete resolution of the disease condition. ***[Indian J Tuberc 2011; 58: 32-34]***

***Key words*:** Conjunctival. Tuberculosis, Histopathology.

INTRODUCTION

Tuberculosis (TB) is a major cause of illness world-wide. Globally 9.2 million new cases and 1.7 million deaths occurred because of tuberculosis worldwide.[1] TB is a systemic disease that commonly affects the lung. Tuberculous involvement in the eye has been reported.[2-7] Most patients with ocular TB have no systemic manifestations of the disease[2,3] We report a girl who presented with a chronic unilateral conjunctivitis, who was treated empirically for allergic conjunctivitis. Due to non-resolution of signs and symptoms, histopathology was pursued. The biopsy report and the therapeutic response to Anti Tuberculous Therapy confirmed the diagnosis of conjunctival tuberculosis in this patient. In areas endemic for tuberculosis, considering TB in the diagnostic algorithm of non-resolving unilateral conjunctivitis would be worthwhile.

CASE REPORT

A 14-year-old school girl presented to a tertiary multispecialty teaching hospital in October 2005 with discharge, watering, redness and itching of the left eye for two months. She had no prior medical illness or eye disease and had never used contact lenses. She had no past or family history of tuberculosis. On examination, her left eyelid was thickened. There was minimal discharge with a mechanical ptosis and giant papillae on the upper tarsal conjunctiva. There were no limbal papillae or Horner Trantas spots. The cornea was clear, the anterior chamber had no cells or flare. The intraocular pressure was 16 mm of Hg. The posterior segment was normal. The fellow eye examination was normal. Besides the eye findings in the left eye, the remaining physical examination was normal. A provisional diagnosis of allergic conjunctivitis was made. Topical therapy with 1% Prednisolone acetate

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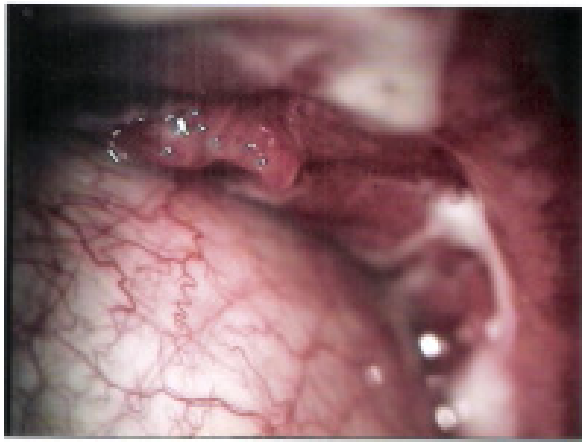


Fig.1: Giant papillae extending upto the lid margin of the upper lid.

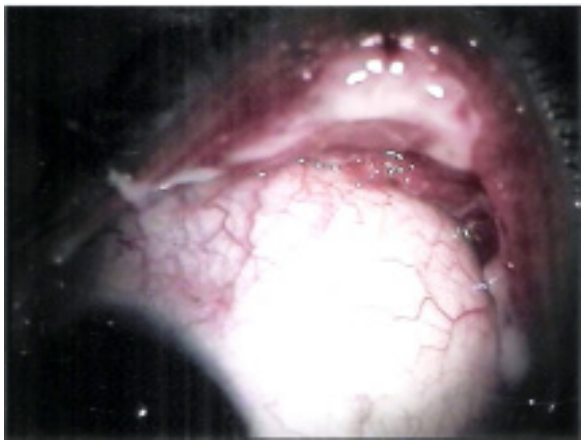


Fig. 2: Giant papillae with fibrinous membrane over the upper tarsal conjunctiva.

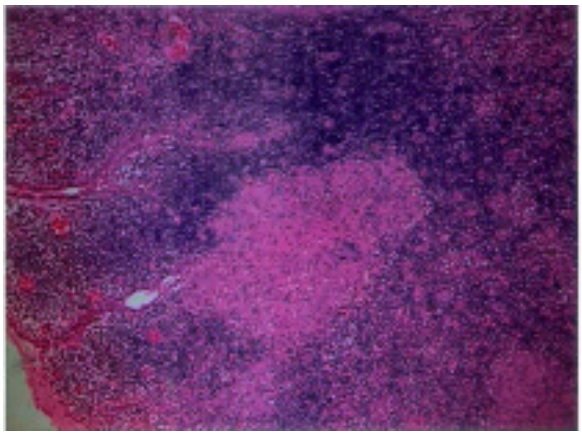
and 2% Sodium cromoglycate was commenced. The patient returned six months later with no improvement in the symptoms. The tarsal conjunctiva had a polypoidal velvety appearance and papillae were seen extending up to the upper lid margin (Fig. 1). A fibrinous membrane was seen over the conjunctiva (Fig. 2). There was one enlarged pre-auricular node. BCG scar was seen on the left arm. Histopathology examination showed necrotizing granulomatous inflammation suggestive of tuberculosis (Fig. 3). Following this, a conjunctival scraping was sent for Acid Fast Bacilli culture and fungal culture which were negative. Chest X-ray and ESR were normal, Mantoux was 12x10mm. Her HIV test was negative.

TREATMENT

The patient was started on Anti-tuberculous therapy according to current guidelines for the treatment of tuberculosis by the DOTS (Directly Observed Therapy Shortcourse).

Isoniazid, Rifampicin, Ethambutol, Pyraziamide were prescribed for two months followed by Isoniazid and Rifampicin for four months. There was no evidence of ocular toxicity with the above medication.

In two months, the patient showed complete resolution of symptoms and marked reduction in papillae and conjunctival thickening (Fig. 4).



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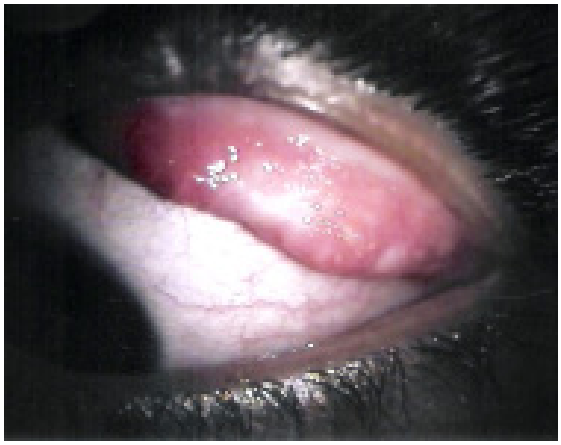

Fig. 4: Upper tarsal conjunctival after two months of ATT.

DISCUSSION

In current clinical practice, the incidence of active tuberculous lesions of the conjunctiva are so rare that the physician's index of suspicion is very low. Variations in the clinical picture depend on the immunoallergic state of the patient.[2] The morphological characteristics of conjunctival lesions fall into 4 groups[3]:

1. Ulcerative- Localized ulceration associated with lymphadenopathy.
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4. Pedunculated- Pedunculated mass without lymphadenopathy.

This patient had features suggestive of hypertrophic granulomatous type. The chronic nature of the symptoms and its refractory nature to therapy suggested the possibility of malignancy or chronic infection. A conjunctival squamous cell carcinoma was a remote clinical consideration but the patient's age group made it less likely (peak prevalence at 60 years).[8,9]

Though this case is not culture proven, the histopathological correlation and the therapeutic response to anti-tuberculous therapy clearly establish the diagnosis which could have been established earlier had there been a higher index of suspicion.

A red eye with itching and irritation is a common presentation of varied aetiologies. Detailed clinical assessment is imperative and often guides treatment in clinical practice. In the event of non-resolution of symptoms, close follow up and a tissue diagnosis are important and must be sought. In our patient, the histopathology clinched the diagnosis of conjunctival tuberculosis. Appropriate treatment was initiated and resulted in complete cure. **In areas endemic for infectious disease (e.g.TB), the threshold for pursuing histopathology should be low. This approach could be "eye saving" as definitive treatments are available.**

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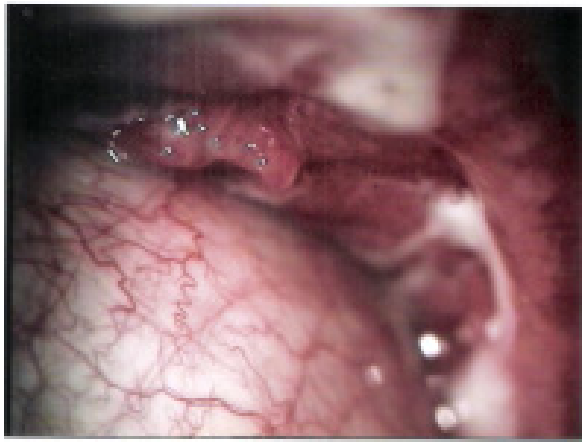

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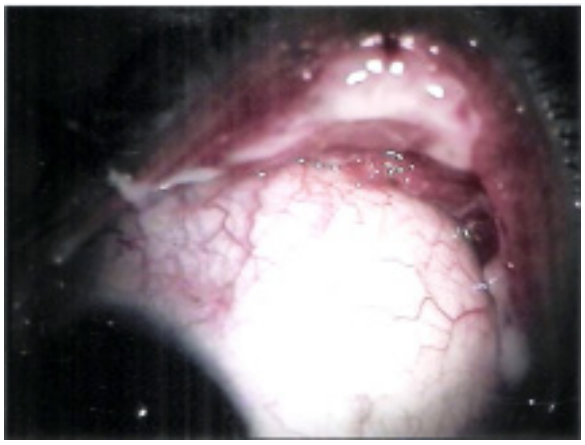

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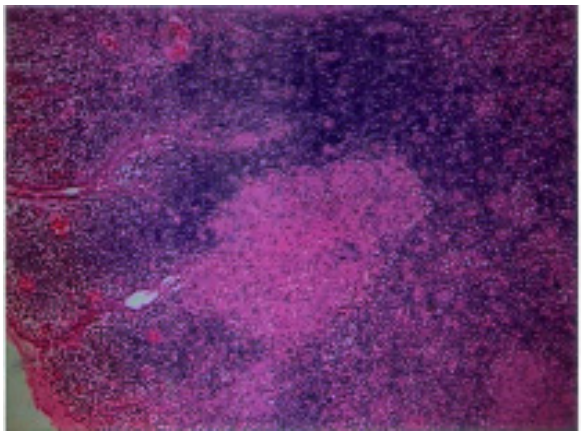

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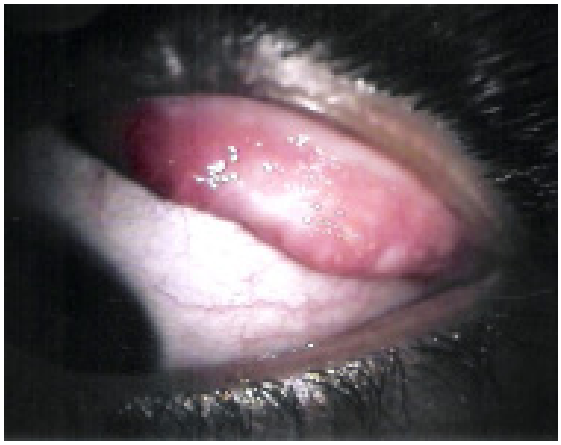

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Case Report

UNUSUAL COMPLICATION OF CERVICAL TUBERCULOUS LYMPHADENOPATHY

K. Gowrinath[1*], Mohammed Ismail Nizami[2*], B. E. Phanil Kumar[3], P. Suneetha[4**] and V. H. Kishor[1**]**

(Received on 17.8.2010; Accepted after revision on 21.12.2010)

Summary: Lymphadenopathy is the most common form of extrapulmonary tuberculosis; cervical region being the most frequent site. Yet, tuberculous cervical lymphadenopathy is rarely associated with Internal Jugular Vein (IJV) thrombosis. We report right IJV thrombosis with isolated cervical tuberculous lymphadenopathy in a 22-year-old woman. Anti-tuberculous treatment resulted in complete regression of lymphadenopathy but anticoagulation treatment failed to restore the caliber of thrombosed IJV to normal. Thrombosis of adjacent IJV is a potential complication of delay in diagnosis and treatment of cervical lymphnode tuberculosis. ***[Indian J Tuberc 2011; 58: 35-37]***

Key words : Tuberculosis, Lymphnode, Internal jugular vein, Thrombosis

INTRODUCTION

IJV thrombosis is unusual and the commonest risk factor is injury to venous wall[1]. Inflammation accompanied by thrombosis of IJV may occur due to spread of oropharyngeal infections to cervical region[2]. Lymph node is the most common extrapulmonary site of tuberculosis and cervical lymph nodes are affected most frequently[3]. Yet, tuberculous cervical lymphadenopathy leading to IJV thrombosis is very rare. We report an unusual case of IJV thrombosis as a complication of isolated tuberculous cervical lymphadenopathy in a young female.

CASE HISTORY

A 22-year-old married woman presented with right - sided neck swelling of six week's duration.Onset of neck swelling was gradual and became painful for the last ten days. There was no cough or fever. There was no significant medical or family history. She received seven day course of an antibiotic (Amoxycillin-clavulanic acid) at local place without relief. On physical examination, she was well built and nourished. There was a tender right supra-clavicular swelling measuring 4×3cms. Clinical examination of all systems were normal. Blood examination revealed haemoglobin of 13.1g%, total leucocyte count of 7,600/cu.mm (51 per cent polymorphonuclear leucocytes, 35 per cent lymphocytes and 11 per cent eosinophils) and platelet count of 2,40,000/cu.mm. Erythrocyte sedimentation rate was 46 mm in the first hour. Mantoux test result was 22x20mm. A chest X-ray film was normal. Blood chemistry was normal. HIV serology was negative. Ultrasound examination of neck showed a well-defined 42x30mm size right supra-clavicular lymphnode as a mixed echogenic Space Occupying Lesion (SOL) posterolateral to IJV breaching its wall and an intraluminal thrombus (Fig.1). Fine needle aspiration of the right supra-clavicular swelling was done cautiously and aspirated material revealed necrotizing granulomatous inflammation on cytological examination. Excision biopsy of the enlarged right supra-clavicular lymphnode was not done to avoid dislodgement of IJV thrombus. Plain and contrast enhanced computed tomography (CT) of chest was normal. The coagulation studies like prothrombin time, bleeding and clotting time were normal. Antiphospholipid antibodies were negative. Other

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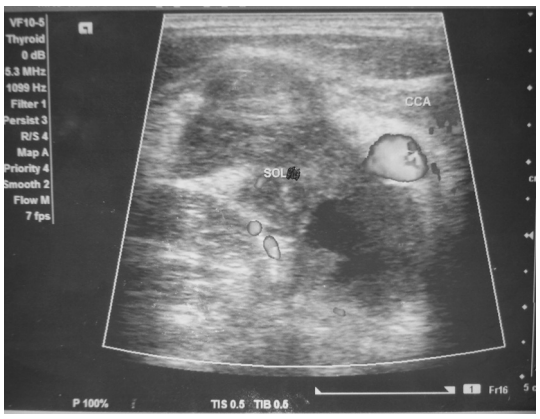

Fig 1: Ultrasound of neck showing SOL (Lymphnode) breaching the wall of IJV and intra- luminal thrombus

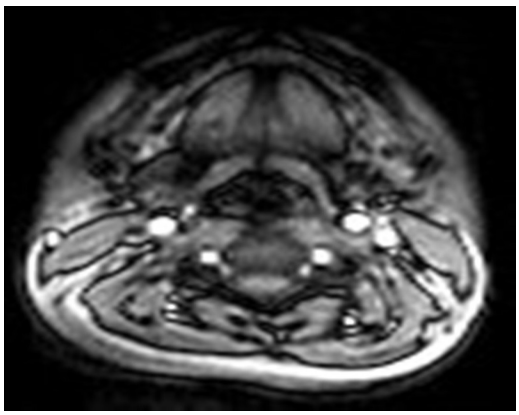

Fig. 2: Axial 2D TOF MRI neck showing loss of flow related enhancement of right IJV.

thrombophilic workup was not possible. Magnetic Resonance Imaging (MRI) 2D Time Of Flight (TOF) of neck showed loss of flow related enhancement of right IJV (Fig.2). Anti-tuberculous treatment with daily isoniazid, rifampicin, pyrazinamide and ethambutol along with anticoagulation therapy (Subcutaneous enoxaparin and oral warfarin) was started. After seven days, oral warfarin was continued. The neck pain subsided and the size of neck swelling reduced significantly within two weeks of treatment. Repeat ultrasound examination of neck after three months of treatment showed complete regression of enlarged right supra-clavicular lymphnode, recanalization of the IJV thrombus with thickening of venous wall and development of collateral circulation. TOF MRI of neck revealed irregular narrowing of right IJV with flow related enhancement along with a collateral blood vessel (Fig. 3). At the end of six months of anti-tuberculous and anticoagulation treatment, the colour doppler study of neck still showed collateral

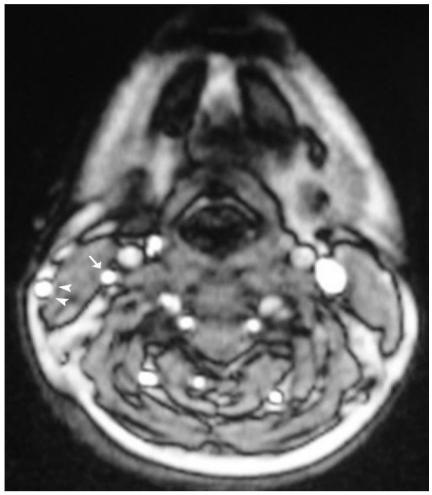

Fig. 3: Axial 2D TOF MRI of neck showing irregular narrowing of right IJV with flow related enhancement (White arrow) with development of collateral circulation.(Arrow heads)

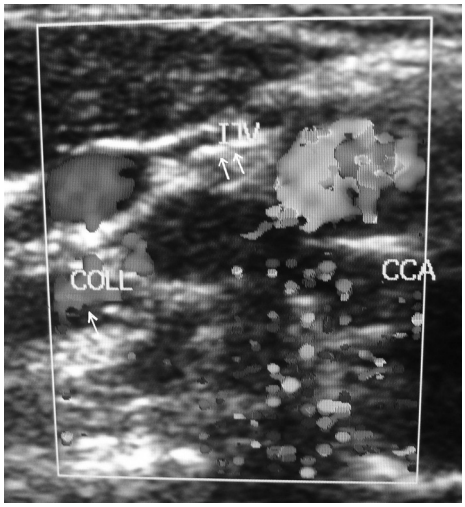

Fig. 4: Colour doppler of neck showing collateral blood flow (Single arrow) and residual thickening of IJV wall (Two arrows).

blood flow (Single arrow) and IJV wall thickening (Two arrows) (Fig 4). Patient remained well and is currently under follow up.

DISCUSSION

The clinical diagnosis of non-catheter related IJV thrombosis is usually not suspected and mistaken for a necrotic lymphnode or abscess. In our case, the tender neck swelling was initially mistaken as cold abscess. Painful neck swelling and fever are presenting features in majority cases of IJV thrombosis[4]. Tuberculosis can involve any group of cervical lymph node and the enlarged lymph node can compress and invade IJV anywhere in the neck before it joins the ipsilateral subclavian vein. Thrombosis of IJV due to oropharyngeal infections in young individuals (Lemierre's syndrome) was frequent in pre-antibiotic era and is not rare even today[5]. IJV thrombosis is rarely complicated by tuberculosis which is common in most parts of the world. Multiple right cervical tuberculous lymphadenopathy with abscess formation and adjacent IJV thrombosis was earlier reported in a case of HIV infection[6]. In our case, isolated cervical tuberculous lymphadenopathy in the absence of early diagnosis and treatment caused adjacent IJV thrombosis. Diagnosis of tuberculous aetiology in our case was based upon clinical suspicion, fine needle aspiration cytology report which suggested tuberculous aetiology and excellent response to antituberculous treatment. Ultrasound and CT studies are accurate and reliable diagnostic tools of IJV thrombosis[7]. Ultrasound examination of neck is also useful in assessing the surrounding soft tissues such as enlarged lymph nodes and serial ultrasound scanning is safe and inexpensive for monitoring response to treatment after initial assessment. In our case, initial ultrasound examination revealed right supra-clavicular lymphadenopathy invading the adjacent IJV and repeat ultrasound examination showed its regression with treatment. But ultrasound study cannot display the portion of IJV behind the mandible or clavicle. We did MRI as this technique provides better view of surrounding soft tissue and has higher sensitivity to blood flow rates compared to CT-scan without exposure to contrast material or radiation[8]. IJV thrombosis should be treated as upto 5% cases of upper extremity deep vein thrombosis were reported to be complicated by pulmonary thromboembolism[9]. The optimum duration of anticoagulation in IJV thrombosis is yet to be standardized. Cohen *et al* suggested one week of heparin and three month course of warfarin to treat IJV thrombosis[10]. We used enoxaparin for one week and warfarin for six months. In our case, invasion of IJV by tuberculous lymphadenopathy might have occurred early causing extensive vessel wall damage and residual venous wall thickening resulted despite optimum anti-tuberculous and anti-coagulation treatment. **In conclusion, IJV thrombosis may occur as a rare complication of tuberculous cervical lymphadenopathy, if there is delay in diagnosis and treatment. Fine needle aspiration biopsy of cervical lymphadenopathy should be attempted with caution to avoid possible dislodgement of thrombus during the procedure.**

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Indian J Tuberc 2011; 58:38-40

STATUS REPORT ON RNTCP*

The Revised National TB Control Programme has maintained NSP case detection rate of > 70% and treatment success rate of > 85% at the national level during the third quarter, 2010 (Figure 1). The programme, while consolidating and sustaining its past achievements, is progressing well towards achieving the TB related Millennium Development Goals.

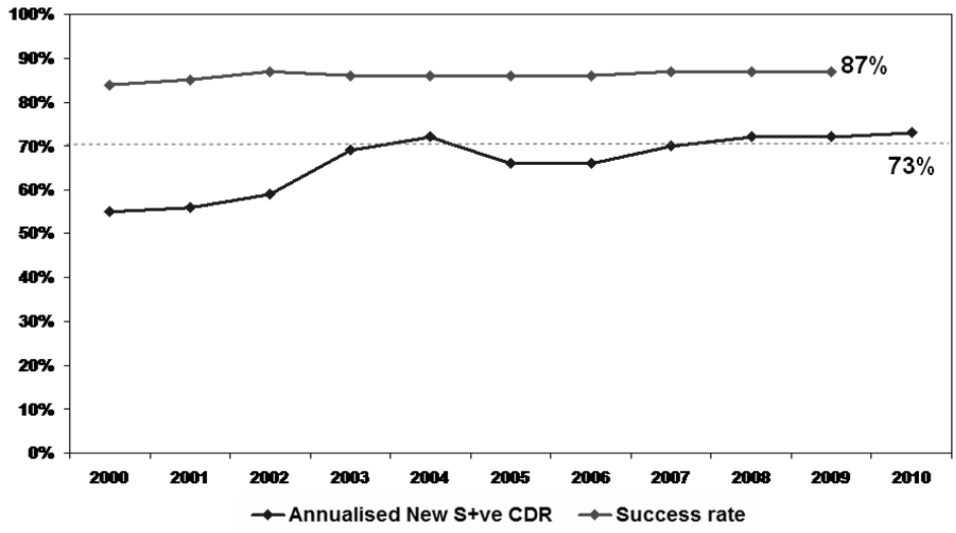
RNTCP performance in third quarter 2010

During the quarter, over 1.94 million TB suspects were examined, 237,206 sputum positive cases were diagnosed, and 383,599 TB cases were registered for treatment. The annualized total case detection rate is 130 cases per 100,000 population. With a total of 157,360 new smear positive cases, being registered for treatment, the new smear positive TB case detection rate (annualized) for the third quarter 2010 is 71%. In addition to this, 99,582 new smear negative cases, 58,126 new extra-pulmonary cases, 52,198 smear positive re-treatment cases and 22,972 re-treatment others cases were also registered for treatment during the quarter. The treatment success rate amongst the new smear positive PTB cases, registered in the third quarter 2009, is 87% and the sputum conversion rate of patients registered during second quarter, 2010 is 90%. The default rates among NSP (5.8%), NSN (6.7%) and re-treatment cases (14.1%) continue to show the declining trend over the past several quarters.

Progress in accreditation of Intermediate Reference Laboratories (IRL)

Ten IRLs across the country have already been accredited. In addition, state C&DST Laboratories of Haryana, UP and Uttarakhand are in the advanced stages of accreditation and the laboratories of other states are under various stages of the accreditation

Fig 1: New Smear-Positive Case Detection Rate and Treatment Success Rate in DOTS Areas, India, 2000-2010 (Jan-Sept)



•Population projected from 2001 census
•Estimated no. of NSP cases - 75/100,000 population per year (based on recent ARTI report)

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Table: Performance of RNTCP Case Detection (2010, third quarter), Smear Conversion (2010, second quarter), and Treatment Outcomes (2009, third quarter)

State	Population (in lakh) covered by RNTCP[1]	Suspects examined per lakh population	No of Smear positive patients diagnosed[2]	Total patients registered for treatment[3]	Annualized total case detection rate	New smear positive patients registered for treatment	Annualized new smear positive case detection rate (%)		No of new smear negative cases registered for treatment	No of new EP cases registered for treament	No. of retreatment cases registered for treatment	3 month conversion rate of new smear positive patients	Success rate of new smear positive patients
Andaman & Nicobar	5	246	108	200	167	75	63	83%	50	55	20	84%	90%
Andhra Pradesh	840	181	19927	29111	139	12547	60	80%	7147	3278	6097	92%	89%
Arunachal Pradesh	12	224	278	644	210	177	58	77%	165	130	168	93%	88%
Assam	302	127	6130	10435	138	4462	59	79%	2714	1447	1794	87%	83%
Bihar	964	89	10618	18892	78	7738	32	43%	6076	1344	3686	87%	88%
Chandigarh	14	293	636	768	225	285	83	88%	95	235	153	90%	85%
Chhattisgarh	239	121	3240	7184	120	2608	44	54%	2749	1049	770	89%	86%
D & N Haveli	3	162	77	94	112	38	45	56%	20	11	25	89%	78%
Daman & Diu	3	235	60	65	100	20	31	39%	13	11	21	74%	82%
Delhi	179	203	5766	12121	270	3291	73	77%	2018	3972	2819	88%	85%
Goa	17	231	359	569	133	214	50	62%	106	127	122	91%	92%
Gujarat	582	209	15497	19311	133	9040	62	78%	2069	2382	5802	92%	89%
Haryana	250	151	6124	9300	149	3382	54	57%	1717	1723	2478	89%	85%
Himachal Pradesh	67	268	2054	3523	209	1252	74	78%	640	856	763	93%	89%
Jammu & Kashmir	116	154	1812	3009	104	1338	46	49%	419	717	532	94%	90%
Jharkhand	310	134	5917	10505	135	4601	59	79%	3290	815	1787	90%	90%
Karnataka	588	222	10975	17241	117	6917	47	63%	3633	3172	3512	87%	82%
Kerala	343	248	3781	6411	75	2702	31	63%	1446	1474	789	84%	85%
Lakshadweep	1	153	3	4	21	4	21	28%	0	0	0	0%	100%
Madhya Pradesh	710	132	13667	23254	131	8946	50	63%	7242	2621	4434	90%	88%
Maharashtra	1111	169	19201	33330	120	13024	47	59%	7423	5782	7095	90%	86%
Manipur	24	146	385	1098	181	291	48	64%	383	223	201	88%	86%
Meghalaya	26	233	719	1421	219	436	67	90%	317	343	314	82%	82%
Mizoram	10	240	150	584	235	102	41	55%	163	201	118	90%	90%
Nagaland	22	187	525	1010	182	373	67	89%	227	227	183	93%	93%
Orissa	404	142	7531	12539	124	5468	54	64%	3070	2229	1761	87%	87%
Puducherry	13	420	664	322	97	136	41	54%	60	74	52	88%	88%
Punjab	274	153	6276	10312	151	4198	61	65%	1758	2206	2143	90%	88%
Rajasthan	668	155	18915	29006	174	10980	66	82%	7487	3953	6586	92%	90%
Sikkim	6	360	188	430	284	125	83	110%	89	121	95	90%	86%
Tamil Nadu	670	242	11305	20860	125	8139	49	65%	5594	4104	3022	90%	87%
Tripura	36	154	497	745	83	409	46	61%	138	117	79	89%	91%
Uttar Pradesh	1973	149	44461	70204	142	30685	62	65%	18839	8436	12125	91%	89%
Uttarakhand	98	181	2682	3566	146	1362	56	59%	720	594	885	89%	84%
West Bengal	887	167	16678	25531	115	11995	54	72%	4705	4097	4733	88%	86%
Grand Total	**11767**	**165**	**237206**	**383599**	**130**	**157360**	**53**	**71%**	**92582**	**58126**	**75164**	**90%**	**87%**

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

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

3 Total patients registered for treatment, include new sputum smear positive cases, new smear negative cases, new extra-pulmonary cases, new others ,relapse,failure,TAD and retreatment others

process. To supplement and support the state laboratory network, the programme is also involving mycobacteriology laboratories of Government Medical Colleges as well as laboratories, in the NGO and Private Sector. Till date, five laboratories in other sectors (CM Vellore, PD Hinduja Hospital Mumbai, BPRC-Hyderabad, RMRCT Jabalpur and SMS Jaipur) have been accredited. Another five laboratories (Quest Diagnostics Gurgaon, SRL Religare Gurgaon, SRL Religare Mumbai, Chaitram Hospital, Indore and Bhopal Memorial Hospital, Bhopal) are nearing accreditation, and several other laboratries have applied for accreditation. Apart from these, Government medical college laboratories (AIIMS New Delhi, JJ Hospital, Mumbai, and PGI, Chandigarh) are also in the accreditation process.

Progress in the DOTS- Plus services for MDR TB cases

DOTS Plus services for management of MDR-TB are now available in 130 districts covering a population of 260 million in 10 states. Till date, a total of around 2985 MDR-TB patients are on treatment in these states. Other states are in various stages of preparatory activities for rolling out DOTS-Plus services.

Progress in TB-HIV Collaborative Activities

Intensified Package of TB-HIV collaborative activities have been rolled out in total 29 states including 11 states in 2010. In third quarter, 2010, TOT on Intensified Package of TB-HIV Collaborative Activities has been completed in states, namely Arunachal Pradesh, Chhattisgarh, Haryana, Himachal Pradesh, Madhya Pradesh, Meghalaya, Sikkim, Tripura and Uttarakhand. We would like to congratulate the states which are performing well like Chandigarh, Goa, Karnataka, Puducherry and Tamil Nadu, where more than 80% of TB patients registered in third quarter, 2010 know their HIV status and urge other states to accelerate progress in this regard. The proportion of HIV-positive TB patients put on CPT has improved to 93%, but linkage to ART, though improved (50%), still remains the biggest challenge.

Progress in Partnerships

The Confederation of Indian Industry (CII) conducted Regional Conferences on "TB Management at Workplace and Beyond" on 6th July at Ranchi, 9th July at Pune and 22nd July at Mysore for the Eastern, Western and Southern states respectively. It was attended by representatives from the Industry including medical advisers, NGOs as well as RNTCP programme managers of the states. The consultations provided an opportunity to discuss the workplace interventions under RNTCP.

The Partnership for TB Care and Control organized the Regional Consultative meetings for Partners at Dehradun on 15th -16th July and Shillong on 23rd-24th September to develop a common understanding and agreement among the key stakeholders for involving partners in TB care and control at state and regional level. This provides the partnership a more geographic focus as well as a platform to share experiences and views among partners.

The Global Fund Round 9 India TB project was initiated by the two civil society Principal Recipients (The Union and World Vision-India). The civil society component of this project aims to improve the reach, visibility and effectiveness of RNTCP through civil society support and to effectively engage communities and community-based care providers in 374 districts across 23 states by 2015 to improve TB care and control, especially for marginalized and vulnerable populations including TB-HIV patients.

Progress in Operations Research

A one year TB Operational Research (OR) Training project for professionals associated with the RNTCP was initiated during this quarter. This project is a collaborative effort between 'The Union (IUATLD) South-East Asia (USEA), Regional Office, New Delhi', World Health Organization, India and The US Centres for Disease Control, Atlanta in coordination with Central TB Division, MoHFW, and National TB Institute (NTI), Bangalore. The first protocol development workshop was held in Sept, 2010 at NTI. Thirty three participants worked and refined 17 RNTCP priority Operations research topic concept notes into operations research protocols. They will be supported to implement these OR studies. These studies when completed will provide further evidence to stream line the policy and programmatic processes to benefit the TB patients.

Indian J Tuberc 2011; 58; 41-42

SIXTY FIFTH NATIONAL CONFERENCE ON TUBERCULOSIS AND CHEST DISEASES – BENGALURU – JANUARY, 2011 : A BRIEF REVIEW

R.C. Jain*

The 65th National Conference on Tuberculosis and Chest Diseases (NATCON 2010) was organized by the Karnataka State Tuberculosis Association (KSTBA) under the auspices of the Tuberculosis Association of India (TAI) from 10th to 12th January, 2011. The venue of the Conference was NIMHANS Convention Centre, Bangalore. Dr.Shashidhar Buggi, Honorary Secretary, KSTBA and his team worked hard to make the conference a grand success. The team worked under the overall advice and guidance of Dr. R.C. Jain, Vice-Chairman,Tuberculosis Association of India.

Over 450 delegates attended the Conference.

The conference was inaugurated by the Hon'ble Health Minister, Government of Karnataka, Shri B. Sreeramulu. Shri Ramadas, Hon'ble Minister for Medical Education, Government of Karnataka, also graced the occasion and released the souvenir brought out on the occasion.

Dr. Prahlad Kumar, President of the conference, delivered the presidential address. In his address, he highlighted the journey of TB control from pre-chemotherapy era till RNTCP expansion. He said "The goal of a 'TB-Free India' should be in the heart and mind of every citizen of this country in order to prevent the tentacles of TB bacteria from further dissemination in the community. In order to achieve the ultimate objective of taking out the scourge of TB, each has a role to play in translating the vision of planners of RNTCP into reality. Every TB patient in the country should have universal access for TB care."

Speaking on the occasion, Dr. R.K. Srivastava, Director General of Health Services Government of India and Chairman, TAI said that India is the largest contributor of global tuberculosis load. Though RNTCP has achieved a treatment success rate of 85% at the national level, still there are many areas where we are facing challenges like areas outside the pulmonary system. Sustainability of the programme, cost of treatment, emerging threats of XDR/MDR TB, co-existence of HIV-TB, etc. are major challenges. He hoped that deliberations of this conference would give a direction to this country.

He congratulated the Tuberculosis Association of India (TAI) on organizing this biggest event and highlighted its role, as an NGO, in supplementing and complementing the efforts of the government.

Dr. S.P. Agarwal, President of Tuberculosis Association of India, in his key-note address focused on challenges of tuberculosis in urban settings. He said "the burden of suffering and economic loss caused by TB is an affront to our conscience. TB is a curable and preventable disease. TB continues to be a public health problem in the world despite the availability of highly effective treatment regimens. HIV and TB form a lethal combination, each speeding the other's progress. TB-HIV co-infection and drug resistant tuberculosis aggravate the TB situation globally. TB is a leading cause of death in HIV infected persons and HIV infection is the most potent risk factor for developing active TB disease from a latent TB infection.

Dr. R.C. Jain, Vice-Chairman, TAI read out the citations for various awards of TAI which were presented to the recipients by the Karnataka Health Minister. The inaugural function ended with a vote of thanks by Dr. Shashidhari Buggi, Organising Secretary of the Conference.

The Scientific Programme Committee of the Conference had chalked out a very useful programme. On the first day the conference (10th

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January, 2011) a CME programme was held for the post graduate students and young delegates. Three sessions of lectures on various aspects of tuberculosis, respiratory diseases and RNTCP were conducted during the CME.

During the main conference, besides three prestigious orations, two guest lectures, six symposia and two panel discussions, there were 50 oral paper presentations and 60 poster presentations. Dr. P.K. Sen TAI Gold Medal Oration was delivered by Dr. N. Selvakumar of TRC, Chennai on the subject of "Laboratory support to RNTCP". TAI Oration was delivered by Dr. Kandala Venu on the subject of "Mycobacterial Menace – a medical challenge". Lupin-TAI Oration was delivered by Dr. A.K. Janmeja on the subject of "MDR-TB". The OA Sarma guest lecture was delivered by Dr. Rupak Singla on the subject 'XDR-TB'. The meeting of the State Secretaries was held on 11th January 2011 in which next venue of the conference was discussed in addition to other discussions. The meeting of the Standing Technical Committee was held on 12th January 2011.

A colourful exhibition was the highlight of the conference. Over 15 medical companies, including TAI, FIND and IUATLD, had put up informative stalls giving useful information on TB and chest diseases through charts and working models. A number of informative literature, brochures and books were distributed among visitors.

NATCON 2010 held in Bangalore was an excellent package of academic, social and cultural feast. The hospitality offered by the Organizing Committee was superb and the delicious food picked from the various areas of Bangalore were the highlights of the various meals wherein different menu was served on different days.

In the Business and Concluding Session, the President of the Conference, Dr.Prahlad Kumar gave a brief resume of the Conference activities from the start till the conclusion of the Conference wherein it was highlighted that all the sessions were well attended and the younger workers were participating in large numbers.

Under Rule 3 (xiii) of the Rules and Regulations of TAI, Drs. A.K. Janmeja, R.P. Vashist, S.M. Govil and S.S. Revadi were elected as representatives of the National Conference to serve on the Central Committee.

Dr. Rohit Sarin proposed a vote of thanks on behalf of the delegates and Dr. Shashidhar Buggi on behalf of the Organising Committee.

Indian J Tuberc 2011; 58:43-44

FORUM

CORNEA HARVESTING FROM RESPIRATORY/TB INSTITUTIONS

Cataract is the dominant cause of blindness in India as it accounts for nearly two-third of blind population and hence the thrust of the National Programme for Control of Blindness (NPCB) in increasing the availability and accessibility of high quality cataract surgery services. Eye care services are witnessing a dramatic improvement in India, that is comparable to best in the developed nations. With the approval and implementation of eleventh five year plan, a greater impetus is being given under the programme to blinding conditions other than cataract including refractive errors, glaucoma, diabetic retinopathy, strabismus, squint, retinopathy of prematurity, childhood and corneal blindness etc.[1]

It is estimated that there are 190,000 persons with bilateral corneal disease in India and every year 20,000 join the list.[2] Various conditions that may damage cornea include injury, infection, inflammatory, iatrogenic condition or nutritional disorders. The negative impact of corneal blindness is far greater as these patients are usually of younger age group in comparison to cataract or glaucoma. The prevention strategies are always considered cost-effective, however sight lost due to corneal disease can only be restored by corneal transplantation, a procedure with high success rate and outcome among all organ donation interventions. Cornea harvesting is the surgical removal from a deceased person of either the whole eye [enucleation] or the cornea [*in situ* corneal excision].

Cornea is an avascular tissue that can be removed preferably by six-eight or up to 24 hours after death. Any person between the ages 3-70 years, healthy or otherwise suffering from diabetes, hypertension, asthma, using spectacles or who underwent cataract operation can donate eyes/ corneas after death, though ideal candidates for eye donation would be 'young' patients dying due to road traffic accident. The known absolute contraindications for eye donations are rabies, HIV, hepatitis & other viral diseases, progressive neurological ailments like Creutzfeldt-Jakob disease, death due to unknown cause, septicemia, ocular malignancy, lympho-proliferative disorders and corneal pathologies.[3]

It is not a necessary pre-requisite to pledge eyes during life for donation but what is vital is the informed written consent of kith/kin at the time of death for eye removal and collection of blood sample for serological testing. The entire process of eye removal takes nearly 15-20 minutes at no cost to donor family and the recipient of corneal transplantation will always remain anonymous.

Inspite of high reported institutional & home deaths and favourable regulatory environment, eye donation movement is still considered in infancy. There is an estimated annual requirement of more than one lac corneas in the country. However, the programme could collect ever maximum of 42,000 corneas in the year 2009, of which utilization rate i.e. corneal transplantation was only up to 40%. Because of this huge lack of natural donors cornea in the country, new patients are piling up for receiving corneal graft. In order to enhance eye donation, it is essential to increase awareness among potential donors and dispel their misconceptions. Medical professional attitude towards eye donation can also be expected to influence donation rates.

NPCB, Government of India and State government along with stakeholders like teaching institutions and NGOs are regularly taking various measures for informing, educating and motivating community for eye donation. There are around 250 eye banks currently existing in country but majority of them have started becoming non-functional entity due to lack of cornea harvesting. However, solution does not lie in opening chains of newer centres but rather close networking and establishment of

functional inter-linkages between health institutions/ hospitals and existing eye banks.

In the present circumstances, existing respiratory/TB institution and chest wards in medical colleges can take up a new leadership role of promoting eye donation/cornea harvesting for reported in-patient deaths. The activity can be initiated after some confidence building measures among ophthalmologists, eye bank staff, chest specialists, microbiologists, etc., at local level and may initially become applicable for in-patient death reported among non-fulminant young cases. The medical and nursing personnel can identify potential donors, initiate a dialogue with attendants, persuade them and solicit corneas at the time of death and inform/coordinate with nearest eye bank for seeking written consent, enucleation, blood sample collection, transportation, tissue processing, evaluation and corneal transplantation. With initial hiccups, this endeavour may herald new era of teaching, training, research and collaborative activities and may facilitate in bridging the gap between the demand and supply of cornea in the country.

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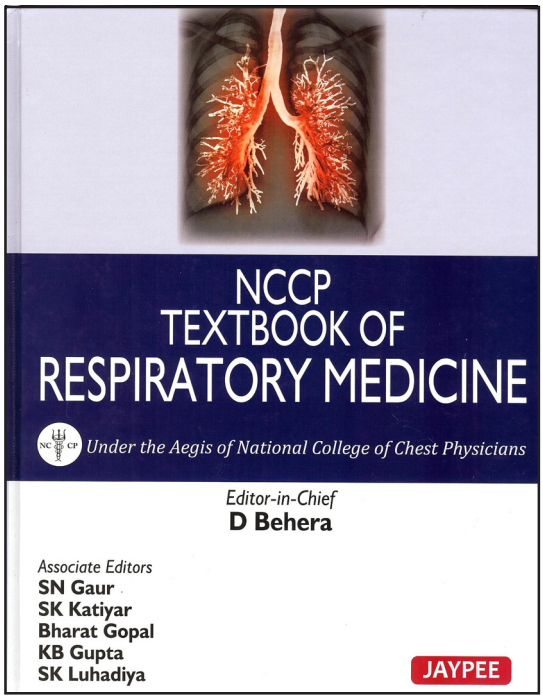
BOOK REVIEW

NCCP (I) Text Book of Respiratory Medicine - First Edition - 2011; (Editor-in-Chief) Dr. D. Behera; Published by Jaypee Brothers Medical Publishers (P) Ltd., B-3, EMCA House, 23/23 B, Ansari Road, Darya Ganj, New Delhi - 110 002; 1012 pages, 41 chapters with 18 colour plates and hardbound cover; ISBN-978-93-5025-212-3; Price Rs.2,495/-.



NCCP (I) has published a text book on Respiratory Medicine. This is a multi-authored textbook on Respiratory Medicine that provides an updated version of various pulmonary diseases covering all major conditions like tuberculosis, bronchial asthma, lung cancer, COPD, ILDs, respiratory failure, smoking and air pollution. Editor-in-Chief Dr. D. Behera is an eminent Chest Physician of the country, apart from heading the prestigious LRS Institute of Respiratory Diseases, New Delhi. He has been bestowed with the B. C. Roy award twice. Along with Dr. D. Behera, there are five Associate Editors involved. There has been a rapid advancement in the diagnosis and management of respiratory diseases in addition to opening up of new avenues for research. This textbook has been designed to keep pace with these developments. It contains 41 chapters contributed by noted and senior authors in the field both from India and abroad. Chapters on Tuberculosis, Bronchial Asthma and Lung Cancer are extensive and a vivid account of the various issues therein, has been given by the experienced authors. Other major diseases such as COPD, Pulmonary Embolism, Respiratory Failure, Cor-Pulmonale, Pneumonias, ARDS, 1LDs, and Pleural Diseases are also discussed appropriately. This textbook highlights the problem of tuberculosis and lung cancer, their pathogenesis, newer diagnostic modalities and the Revised National TB Control Programme. It a1so includes discussions on acute respiratory failure and ARDS by the experts, apart from adopting an approach to physical and clinical examination of the respiratory system, which is still a mainstay in the teaching of clinical medicine by an experienced teacher. This book would prove to be an excellent referral book for the undergraduate, postgraduate and postdoctoral students and a handy reference for the busy practitioners. The book contains 18 colour plates and is priced at Rs.2495/-.

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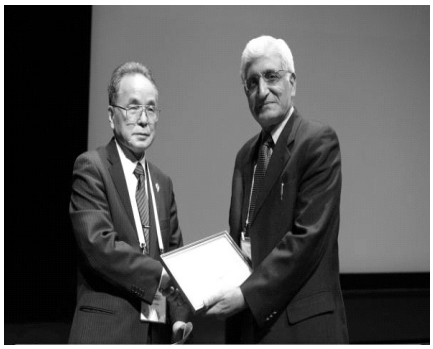
Indian J Tuberc 2011; 58: 46

2010 PRINCESS CHICHIBU GLOBAL MEMORIAL TB AWARD



Dr G.R. Khatri, Senior Public Health Adviser to World Lung Foundation, New York was awarded on 12th November, 2010 the Japan Anti-Tuberculosis Association's 2010 Princess Chichibu Global Memorial TB Award at the World Conference in Berlin.

Dr Khatri began his professional career in 1969 in an urban slum health centre in Delhi and rose to become Director of India's TB Control Programme. For more than four decades, India's TB control efforts had made little progress. Dr. Khatri supported a radically different approach and successfully obtained a World Bank soft loan of US$ 144 million in 1996 to implement it.

He was responsible for persuading the medical fraternity, public sector and other partners, as well as the public, to adopt and accept the DOTS strategy. The result was that India's TB control programme tripled its treatment success rate from 26% to more than 80% and saw a seven-fold decrease in death rate from 29% to 5% in areas covered by DOTS. The population benefitting from DOTS jumped from a meagre 18 million in 1998 to about 500 million in 2002. This DOTS programme became recognised internationally as fastest and best-quality expansion to occur anywhere in the world which till date has treated 11 million TB patients, averting more than two million deaths.

Dr. Khatri replicated this success in TB control through the World Health Organization in Bhutan, DPR Korea, Indonesia, Afghanistan and Myanmar. He has also been a consultant at the WHO SEARO headquarters and served as Chairman of the SAARC TB centre.

From 2003 to 2007, Dr Khatri worked as Global Contracts Director of FIDELIS (The Fund for Innovative DOTS Expansion through Local Initiatives to Stop TB) which funded 53 projects covering more than 600 million people in 19 low-income Asian and African countries. He continues to serve on the faculty of The Union's International Management Development Programme.

In 2005, he established the World Lung Foundation — South Asia (WLF-SA), which he serves as its President. In this capacity he has become involved in tobacco control, notably Delhi University's Smoke Free Initiative

Dr Khatri has received numerous awards, including The Union's Karel Styblo Public Health Prize in 2000 at Florence, Italy.

ABSTRACTS

Antibiotic susceptibility pattern of rapidly growing Mycobacteria

R. Gayathri, K. Lily Therese, P. Deepa, S. Mangai and H.N. Madhavan. *J Postgrad Med* 2010; **50**: 76-8.

The rapidly growing mycobacteria (RGM) causing human infections primarily consist of the *Mycobacterium fortuitum* group, *Mycobacterium abscessus* and *Mycobacterium chelonae.* The antibiotic susceptibility testing is important to determine the appropriate therapy as the antibiotics used to treat RGM are different from those used for treating infections caused by slow growers of mycobacteria. Aim was to determine antibiotic susceptibility of RGM using Kirby Bauer method and following Clinical and Laboratory Standards Institute (CLSI) guidelines. The retrospective styudy was conducted at Larsen and Toubro Microbiology Research Centre, Vision Research Foundation, Sankara Nethralaya, Chennai. The antibiotic susceptibility testing was performed following CLSI method for the drugs Amikacin, Azithromycin, Tobramycin, Ceftazidime, Cephotaxime, Cefuroxime, Cefaperazone, Ceftriaxone, Ciprofloxacin, Ofloxacin, Norfloxacin, Gatifloxacin and Moxifloxacin. Out of the 148 RGM isolates 146 (98%) were susceptible to amikacin, 138 (91%) to gatifloxacin, 132 (87%) to moxifloxacin, 122 (76%) to ciprofloxacin and 116 (74%) to Norfloxacin. Majority of the RGM were resistant to Ceftazidime, Cephotaxime and Cefaperazone. All the M. *abscessus* isolates were resistant to tobramycin. The *in vitro* antibiotic susceptibility testing by disc diffusion method showed that.majority of the RGM were sensitive to Amikacin followed by Gatifloxacin, Moxifloxacin and Ciprofloxacin.

jefA (Rv2459), a drug efflux gene in *Mycobacterium tuberculosis* confers resistance to isoniazid & ethambutol

Anuj Kumar Gupta, Vineel P. Reddy, Mallika Lavania, D.S. Chauhan, K. Venkatesan, V.D. Sharma, A.K. Tyagi and V.M. Katoch. *Indian J Med Res* 2010; **132**: 176-88.

Drug efflux pumps have been contributing factor(s) in the development of multi drug resistance in various clinically relevant bacteria. During efflux pump gene expression studies on mycobacteria, we have found a previously uncharacterized open reading frame (ORF) Rv2459 to be over expressed in drug stressed conditions. The objective of the present study was to investigate the role of this ORF as a drug efflux pump, which might add new information in our understanding about the alternative mechanisms of drug resistance in mycobacteria. The open reading frame Rv2459 of *Mycobacterium tuberculosis* encoding a probable drug efflux protein has been cloned using pSD5 E.coli-Mycobacterium shuttle vector and overexpressed in *M. tuberculosis* H_{37},R_v. This ORF was named as *jefA*. Over expression of this gene in clones has been verified by real-time reverse transcription PCR. Minimum inhibitory concentrations (MICs) of recombinant as well as non-recombinant clones were determined by resazurin microtitre assay plate method (REMA) with and without efflux pump inhibitors carbonyl cyanide m-chlorophenylhydrazone (CCCP) and verapamil. In recombinant strains of *M. tuberculosis,* the over expression of this gene led to an increase in MIC of anti-tubercular drugs isoniazid and ethambutol when tested by REMA. In the presence of CCCP and verapamil, the recombinant strains showed decrease in MIC for these drugs. Bioinformatic analysis has shown a close relation of JefA protein with drug efflux pumps of other clinically relevant bacteria. In

homology derived structure prepared from nearest available model, it was observed that amino acids forming TMH 1, 8 and 11 participated in ethambutol specificity and those forming TMH 2, 7 and 10 participated in isoniazid specificity in JefA. The increased transcription of *jefA* leads to increased resistance to ethambutol and isoniazid in *M. tuberculosis* via efflux pump like mechanism and contributes in the development of resistance to these drugs. JefA amino acid sequence is well conserved among clinically important bacterial genera, which further provides evidence of being a potent drug efflux pump. The involvement in drug resistance and very little homology with any of the human proteins makes JefA important to be included in the list of potential drug targets.

Streptomycin induced protein expression analysis in *Mycobacterium tuberculosis* by two-dimensional gel electrophoresis & mass spectrometry.

Prashant Sharma, Bhavnesh Kumar, Neelja Singhal, Vishwa Mohan Katoch, Krishnamurthy Venkatesan, Devendra Singh Chauhan and Deepa Bisht. *Indian J Med Res* 2010; **132**: 400-08.

The resistance of *Mycobacterium tuberculosis* to streptomycin, a core drug for treatment of category II tuberculosis (TB) has posed a major challenge to the health providers as well as research workers worldwide and has severely compromised the therapeutic options. A significant proportion of streptomycin resistant *M. tuberculosis* isolates failed to show mutations in conventional targets like *rpsL and rrs.* Although efflux, permeability, etc. are also known to contribute, yet a substantial proportion of isolates remains resistant suggesting involvement of other unknown mechanism. A resistant isolate may show altered gene as well as protein expression under drug induced conditions and a whole cell proteome analysis under induced conditions might help in further understanding the mechanisms of drug resistance. The present study was therefore designed with the objective to identify proteins related to streptomycin resistance in *M. tuberculosis* isolate grown in presence and absence of streptomycin (SM). A clinical isolate of *M. tuberculosis* from Mycobacterial Repository Centre at the Institute (NJIL & OMD), Agra was grown in Sauton's medium for 36 h with/without subinhibitory concentration of the drug (2 JigJml) and the cell lysate of isolates was prepared by sonication and centrifugation. Two-dimensional (2D) gel electrophoresis was employed to study the protein profile. The selected proteins were finally identified by MALDI-TOF mass spectrometry. Our study revealed eight inducible proteins (DnaK, fabG4, DNA-binding, hypothetical, two 14 kDa antigen and two 10 kDa chaperonin) that were upregulated in the presence of drug. This preliminary study has thrown light on whether or not and how the resistant isolate responds to streptomycin at its non-toxic but sub-inhibitory concentration. An in-depth study of the upregulated proteins will give an insight into probable sites of drug action other than established primary sites.

Real-time polymerase chain reaction in bronchial aspirate for rapid detection of sputum smear-negative tuberculosis

J-W. Min, H. I. Yoon, K. U. Park, J-H. Song C-T Lee and J. H. Lee. *Int J Tuberc Lung Dis* 2010; **14:** 852-58.

The real-time polymerase chain reaction (RT-PCR) has increasingly been used for the detection of various micro-organisms, including mycobacteria. Aim was to determine the role of RT-PCR in confirming the diagnosis of tuberculosis (TB) when acid-fast bacilli (AFB) smear results in sputum samples were not available (i.e. no sputum or negative smear results). We analysed the data of consecutive patients whose bronchial aspirate (BA) was tested for RT-PCR for the diagnosis of TB from January 2006 to April 2008. Computed tomography (CT), bronchoscopy and tissue biopsies were performed in all patients for confirmatory diagnosis, and BA was collected for microbiological analyses and RT-PCR. Final diagnoses were based on micro-biological or clinicopathological criteria. Final diagnoses were made in 136 patients, and TB was confirmed in 77 (including 65 culture-positive patients). RT-PCR was positive in 51.9% (40/77) of

the confirmed TB patients. More TB patients (20.8%) were detected using RT-PCR than using BA-AFB stain (40 vs. 20, $P < 0.001$). Of the 77 TB patients, 44 (57.1 %) were detected within a few days using a combination of BA-AFB and RT-PCR. Real-time RT-PCR of bronchial aspirate seems to be useful for the rapid diagnosis of TB in suspects with smear-negative TB sputum or no sputum.

Detection of human immunodeficiency virus infection in the sputum of tuberculosis patients in South India

W. Grandin, A. V. Dev, A. Latha, L. Armstrong, D. Mathai, K. R. John and P. Daley. *Int J Tuberc Lung Dis* 2010; **14:** 1288-94.

The study was conducted at a DOTS clinic in an academic tertiary referral hospital in South India. Aim was to evaluate the performance of two rapid enzyme-linked immunoassays (EIAs) for the detection of human immunodeficiency virus (HIV) infection in sputum samples of patients with tuberculosis (TB). We prospectively recruited 522 consecutive out-patients presenting to the DOTS clinic with confirmed TB of any type to undergo HIV testing using reference serum EIA and index-blinded parallel sputum HIV testing with two rapid EIAs designed for oral mucosal transudate. HIV positivity was 14.9% (95%CI 12.1- 18.4). Compared to reference serum EIA, the Oraquick™ assay was 93.1 % sensitive (95% CI 83.8-97.4) and 95.3 % specific (95% CI 92.7-96.9), while the Aware™ assay was 92.3% sensitive (95% CI 83.4-96.8) and 96.6% specific (95% CI 94.4-98.0). The positive predictive values were respectively 77.0% and 82.7%. After freezing of sputum, the sensitivity of both assays declined, but the specificity significantly increased. Higher sputum volume reduced the odds of obtaining a true result with both assays. HIV testing of fresh sputum is not sufficiently accurate for anonymous HIV surveillance among TB patients in a setting of low «10%) HIV prevalence. Freezing sputum samples and limiting sputum volume for HIV testing may improve assay specificity.

Significant variation in presentation of pulmonary tuberculosis across a high resolution of CD4 strata

G. Chamie, A. luetkemeyer, M. Walusimbi-Nanteza, A. Okwera, C.C. Whalen, R.D. Mugerwa, D. V. Havlir and E.D. Charlebois. *Int J Tuberc Lung Dis* 2010; **14:** 1295-1302.

The human immunodeficiency virus (HIV) alters the presentation of pulmonary tuberculosis (PTB), but it remains unclear whether alterations occur at a CD4 cell threshold or throughout HIV infection. Aim was to better understand the relationship between CD4 count and clinical and radiographic presentation of PTB. Initial presentations of culture-confirmed PTB patients evaluated at a Ugandan national TB referral center and an affiliated research unit were compared by HIV status and across 11 CD4 cell count strata: 0-50 to >500 *cells/µl.* A total of 873 HIV-infected PTB cases were identified. Among HIV-infected PTB cases with CD4 < 50, 21% had a normal chest X-ray (CXR) vs. 2% with CD4 > 500, with a continuous trend across CD4 strata (test for trend, $P < 0.001$). All radiographic manifestations of PTB displayed significant trends across CD4 strata. HIV-infected vs. non-HIV-infected patients had no significant difference in CXR findings of miliary patterns or pleural effusion at CD4 > 100, normal CXR or fibrosis at CD4 > 150, adenopathy at CD4 > 250, and cavitation or upper lung disease at CD4 > 300. Twenty- three per cent of co-infected cases with CD4 < 50 and 1% with CD4 > 500 had negative acid-fast bacilli (AFB) smears, with a significant trend between ($P < 0.001$). CONCLUSION: Variations in CXR appearance and AFB smear correlate with CD4 decline in significant, continuous trends.

Patient and family costs associated with tuberculosis, including multidrug-resistant tuberculosis, in Ecuador

V.A. Rouzier, O. Oxlade, T R. Verduga, L. Gresely and D. Menzies. *Int J Tuberc Lung Dis* 2010; **14:** 1316-22.

There is little published information on the costs of multi drug-resistant tuberculosis (MDR-TB) for patients and their families in low-or middle-

income countries. Between February and July 2007, patients with microbiologically confirmed active TB who had received 2 months of treatment completed an interviewer- administered questionnaire on direct out-of-pocket expenditures and indirect costs from lost wages. Clinical data were abstracted from their medical records. Among 104 non-MDR-TB patients, total TB-related patient costs averaged US $960 per patient, compared to an average total cost of US$6880 for 14 participating MDR-TB patients. This represents respectively 31% and 223% of the average Ecuadorian annual income. The high costs associated with MDR-TB were mainly due to the long duration of illness, which averaged 22 months up to the time of the interview. This resulted in very long periods of unemployment. Most patients experienced a significant drop in income, particularly the MDR-TB patients, all of whom were earning less than US$100/month at the time of the interview. Direct and indirect costs borne by patients with active TB and their families are very high in Ecuador, and are highest for patients with MDR- TB. These costs are important barriers to treatment completion.

Errata

October, 2010 Issue

Page 228, Second column, Second paragraph, incomplete second sentence starting with "World Health Organization……..." will be read as follows:

World Health Organization recognizes the importance of tuberculosis-related knowledge, attitude and practice surveys in advocacy, communication and social mobilization strategy planning[11].

Page 229, second column, after reference No.10, please read the following reference No.11:

11. Advocacy, communication and social mobilization for TB control: A guide to developing knowledge, attitude and practice surveys. World Health Organization. 2008

We regret for the inadvertent misprints/omissions.