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BIO-SAFETY PRECAUTIONS IN TUBERCULOSIS LABORATORY

[Tuberculosis (TB) laboratory requires proper handling of Mycobacterium tuberculosis (M.tb) - the causative bacterial agent of tuberculosis. The pathogen possesses a rigid cell wall that can tolerate adverse environmental conditions. Conventionally, mycobacterial infection spreads through air. Though rarely accidental exposure in the form of inhalation or inoculation of the pathogen can lead to infection. In the laboratory, handling of infected material and the cultures is hazardous not only to those who get directly exposed to such as health care workers, but also those in vicinity of the laboratory through air borne spread of M.tb. Therefore, any manipulation of M. tuberculosis culture or samples containing this organism must be done with utmost care to minimize the risk of transmission to the laboratory personnel and the society.

For this, use of laboratory must be kept limited to trained TB laboratory personnel only. As the handling of M. tuberculosis infected samples falls in category II and manipulation of pure cultures of M. tuberculosis in the category III risk group1, these should be handled in accordance with national laws and practices. Arrangements should be made for appropriate health surveillance of TB laboratory workers e.g. before enrolment in the TB laboratory; at regular intervals thereafter, annually or bi-annually; after any biohazard incident; and in case of onset of TB symptoms. It is ideal to get all protocols approved by institutional biosafety committee (IBSC) which consists of a chairman, an outsider who is a scientist of repute, a medical scientist and a nominee of Department of Biotechnology.1 The medical officer of the IBSC will not only help the committee to recommend or reject the protocols but also monitors the functioning of the laboratory and biosafety practices in the laboratory. All laboratory exposures and records of health of the personnel working in the TB laboratory will be supervised by him. The TB laboratory needs to be designed as per standard engineering guidelines2, There is a standard protocol for placement of equipment e.g: the centrifuges (always lid bucketed), microscope and incubators must be arranged towards the air exit points and vortexing of the cultures/sample and sonication of the cultures must always be done inside the biological safety cabinets (BSC).

The personnel are required to be knowledgeable of the procedures in TB laboratory and each laboratory staff shall confirm that they can properly perform the procedure before commencing work. The laboratory managers will make sure that before allowing them to work in the laboratory, personnel have undergone a training about potential risks to health (symptoms of TB disease and transmission); precautions to be taken to minimize aerosol formation and prevent exposure; hygiene requirements; wearing and use of protective equipment and clothing; handling of potentially infectious material; also if possible about laboratory design, including monodirectional airflow; use of biological safety cabinets (BSC) (operation, identification of malfunctions, maintenance); use of autoclaves, incubators (operation, identification of malfunctions, maintenance); prevention of incidents and steps to be taken by workers in the case of incidents (biohazard incidents, chemical, electrical and fire hazards); good laboratory practices and good microbiological techniques; organization of work flow and procedures of waste management; and importance of laboratory results for patient management.2,3 Details of proper use of biological safety cabinets can be found in most of the TB laboratory manuals and protocols published elsewhere.2,3
The laboratory and hospital staff are at higher risk of getting TB infection than general public. They can get infected during the sample collection, transportation and processing of clinical material, through accidental spillage and droplet formation, if plastic ware and centrifuges of inferior quality are used and proper respiratory masks are not used. They can also get exposed through the sample containers and unstained smears of infected material, if handled without gloves. Relative risk of getting infected to the healthcare workers depends on the nature of TB-related work they do. For example, for those who only do smear and microscopy, risk is only 1.4%; for those who process the sample for culture, the risk is 7.8% but for those who undertake culture and DST, the risk is remarkably high (22%). It is because the culture and DST generate maximum amount of aerosols.

There is common perception in laboratory personnel that once the smears prepared from infected clinical material or cultures are heat-fixed, these smears become risk free. Appearing elsewhere in this issue, Kumar et al., highlight that heat fixation of the smears does not kill the mycobacteria and the smeared sample remains infectious until it is stained with Ziehl Neelsen or other acid fast stain. They emphasize that not only samples from suspected TB patients, but clinical samples such as urine and stool from severely immuno-compromised patients can be a source of mycobacterial infections. These patients may excrete a large number of mycobacterial species in their excretory samples, even though unsuspected, hence universal precautions must be practiced in the general laboratory as well.

Laboratory personnel can also get exposed with *M. tuberculosis* through needle pricks, in TB research laboratories. Even though biosafety guidelines clearly forbid homogenization of mycobacterial cultures using syringes and needles, this author, has witnessed at least three cases of needle pricks contaminated with pure *M. tuberculosis* H37Rv. In all three cases, the research staff was homogenizing or inoculating the cultures of pathogenic strain in animals. He developed fever and was extremely scared. An aspirate from the papule grew *M. tuberculosis* and matched genetically with source strain. The second patient had needle prick on his palm with a genetically modified strain of *M. tuberculosis*. He reported on third day when he developed erythema and swelling on the site of inoculation, but the organism could not be culture-isolated. In third case, the research scholar got exposed in her hand, but she reported on the same day. ATT was started on the day of reporting and all three researchers are healthy after follow-up period of 6-18 months.

In clinical microbiology laboratories that handle and culture clinical samples, safety at every step is crucial. As mentioned above, the pure cultures and drug susceptibility tests must be done after following category III risk precautions. This includes proper maintenance of safety cabinets which must meet NSF/ANSI; biosafety cabinets need to be cleaned with 5% phenolic or 1% hypochlorite solution before and after work and HEPA filters are cleaned/changed at least once a year; double pair of gloves should be used at every stage of handling the cultures; to keep disposal bin/vessel with 5% phenolic or 5% hypochlorite disinfect inside the cabinet at right side corner while all un-infected material should be arranged on left side; Do not process more than six specimens at a time, inside the cabinet and take all other universal precautions to minimize the risk of inhaling or direct contact with the culture. Aerosol generating procedures must be done minimally and with extra care.

Despite all precautions, accidents may happen inside the safety cabinets in the TB laboratory. To prevent exposure due to spillage, all workers using the bio-safety cabinets must keep absorbent material (gauge cloth/adsorbent sheet) and 5% phenol within the cabinet. In case of breakage or spillage, all people in the laboratory and immediate vicinity must be informed and the spillage should be covered with absorbent material soaked with 5% phenol and wiped after 15-20 minutes. The spills should be wiped from the edges
Pipetting should be done using disposable droppers, but mouth pipetting must never be done. If unavoidable, only autoclavable micropipettes should be used. Eating, drinking, smoking, applying cosmetics, use of mobile phones, or applying contact lenses in the TB laboratory must be discouraged at all levels and unauthorized personnel must not be allowed to enter the TB laboratory. If personal clothing is contaminated, remove all outer clothing and place in the autoclave or container for autoclaving. Waste management is crucial for all laboratories, but more so for TB laboratory. All disposables must be segregated at the point of generation and disposed of according to standard guidelines, for example and spoiled HEPA filters, gloves and masks must be incinerated. The Gene-Xpert MTB/Rif is being installed in more and more laboratories. It is imperative to dispose of GeneXpert cartridges in proper manner after use, preferably by incineration, though there are no national or international guidelines published on this matter, so far.

Sarman Singh*

REFERENCES


THE 44TH UNION WORLD CONFERENCE ON LUNG HEALTH

The 44th Union World Conference on Lung Health will be held in Paris (France) from 30th October to 3rd November, 2013. The theme of the Conference is “Shared air, Safe air?”.

For more details, please visit the website: www.worldlunghealth.org.

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ELISA PROTOCOL FOR RAPID SCREENING OF POTENTIAL ANTI-TUBERCULAR DRUGS BASED ON ANTIGENIC REACTIVITY OF MYCOBACTERIAL ES-31 SERINE PROTEASE – A DRUG TARGET SUPPORTED BY AXENIC CULTURE OF MYCOBACTERIUM TUBERCULOSIS H₃₇₇ RA STRAIN IN THE PRESENCE OF INHIBITOR*

Vinita Hutke¹, Gauri Wankhade¹, Pranita J Waghmare² and Harinath BC³

(Received on 20.7.2012; Accepted after revision on 8.3.2013)

Summary
Background: Mycobacterial ES-31 serine protease has been reported to be a drug target using protease and lipase inhibitors in axenic and macrophage cultures. Simple screening techniques are needed for rapid testing of anti-tubercular drugs.
Aim: To demonstrate the usefulness of ELISA protocol based on antigenic reactivity of mycobacterial serine protease by indirect ELISA for detecting anti-tubercular activity.
Material and Methods: Indirect ELISA for assessment of antigenic reactivity of mycobacterial ES-31 serine protease was standardized using ES-31Ag and anti-DSS-goat-serum and assessed the inhibition of the antigenic reactivity by isoniazid, an anti-tubercular drug and serine protease inhibitor and orlistat, a lipase inhibitor.
Results: Optimal antigenic reactivity of mycobacterial ES-31 serine protease was observed at 5µg/well of ES-31 antigen and at 1:25 dilution of anti-DSS-goat-serum. Isoniazid showed 42% inhibition of ES-31 serine protease at 0.4µg/well, while orlistat showed inhibition of 60% at 0.5µg/well. Inhibition of Mtb H₃₇₇ Ra bacilli is further confirmed in axenic culture. 35% and 29% inhibition by isoniazid at 0.4µg/well and orlistat at 0.5µg/well were observed respectively on bacterial growth.
Conclusion: Simple ELISA protocol based on assay of antigenic reactivity of mycobacterial ES-31 serine protease, a drug target, has been standardized for rapid screening of potential anti-tubercular drugs. [Indian J Tuberc 2013; 60: 138-141]

Key words: Mycobacterial ES-31 serine protease, ELISA, Isoniazid, Orlistat, Axenic culture.

INTRODUCTION
Emergence of drug resistant tuberculosis has become a serious threat for successful control of this infection. New regulatory standards demanding rigorous characterisation of the effect of a new drug for drug sensitive TB generally involving relapse free cure in studies with several hundreds of patients and long term follow up are becoming a hurdle in the development of new drugs. There is an urgent need for creative approaches to in vitro testing and animal models for screening of compounds with anti-TB potential. Recently in 2012, a rapid high-throughput surrogate model to evaluate the growth and viability of Mycobacterium aurum inside the macrophages at different levels has been developed by Antima Gupta et al. Newer techniques to detect antimicrobial activity in liquid medium are being developed to avoid the slow growth of the organism.

Previously, in our laboratory, SEVATB ES-31, a mycobacterial secretory serine protease, was isolated from culture filtrate of M.tb H₃₇₇ Ra. ES-31 antigen was shown to possess serine protease as well as lipase activity and was reported to be a drug target in axenic and macrophage cultures. This communication reports that the inhibitory effect of isoniazid and orlistat on antigenic reactivity of ES-31 serine protease may be assessed by ELISA and thus can be used as a rapid screening system for potential anti-TB drugs.
MATERIAL AND METHODS

Isolation of mycobacterial ES-31 serine protease

Culture filtrate protein was obtained from *M. tuberculosis* H₃⁷Ra bacilli grown in thyroxine-supplemented Sauton medium for 10 days as described earlier⁵, ¹¹. ES-31 serine protease was isolated by affinity chromatography from culture filtrate protein using anti ES-31 serine protease antibody raised in goat⁶. 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 (0.01M, pH 2.5), neutralized with Tris-HCl buffer (0.01M, pH 8.6), concentrated and stored at -20°C.

Production of anti-DSS-goat-serum

Detergent Soluble Sonicate (DSS) antigen was prepared from *M. tuberculosis* H₃⁷Ra 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 for 24 hours and labelled as DSS antigen⁷. Anti-DSS-goat-anti serum was obtained from goat by immunizing it with 500 µg/well DSS antigen with 1 ml Freund’s incomplete adjuvant on days 0, 20, 33 and 45. After immunization, the immune sera were collected on days 32, 44, 57 and 60.

Indirect ELISA to study the inhibition of ES-31 serine protease

In the experimental study, antigenic reactivity with specific antibody is measured in the presence of known inhibitor to demonstrate its usefulness in assessing anti-TB potential of unknown drug. The wells of ELISA plates (NUNC) were coated with two concentrations of ES-31 (1µg/well and 5µg/well) in 0.06M carbonate bicarbonate buffer (pH 9.6), and incubated overnight at 4°C. The plates were then blocked with BSA (2%) for one hour at 37°C. ELISA assay without inhibitor served as control. Isoniazid (0.2µg/well and 0.4µg/well) or orlistat (0.25µg/well and 0.5µg/well) were added to antigen-coated NUNC plates and incubated overnight at 4°C. NUNC plates were then washed thrice with PBS containing 0.05% Tween 20 (PBS/T). Diluted anti-DSS-goat serum (1:25 and 1:50) was added and incubated for one hour at 37°C. Plates were washed again thrice with PBS/T followed by one hour incubation with rabbit-anti-goat-IgG-peroxidase conjugate (1:15,000). Plates were again washed thrice with PBS/T. Yellow colour was developed using TMB substrate (20X) and the reaction was stopped using 50µl 2N H₂SO₄ solution. Optical density was read at 450 nm with ELISA reader⁸.

Results of isoniazid and orlistat on *M. tuberculosis* bacilli in axenic culture

Two loopful of *M.tb* H₃⁷Ra bacilli (12 x 10⁷ bacilli/ml) were scraped from the LJ medium slant and inoculated in 50 ml glass bottles (Borosil) containing 10 ml of Sauton medium supplemented with thyroxine (0.8µg/well)⁵ with minor modifications. The cultures were further incubated at 37°C for 10 days, with shaking on a shaker for two hours twice a day. Each assay included 10ml Sauton medium with two loopful bacilli and isoniazid (0.2 or 0.4µg/well) or orlistat (0.25 or 0.5µg/well). Assay without inhibitor served as control. Incubation mixture without bacilli and with isoniazid or orlistat served as blank for each respective drug. Each incubation mixture sample was done in triplicate. The optical density (OD₅₄₀) was recorded immediately after preparation of incubation mixture (0 day), after 5th day and 10th day of incubation. O.Ds of respective blanks were set at 0 for each test. Percentage inhibition was calculated from the difference between ODs of tests and control.

% inhibition = Control-Test/control × 100.

Results

Maximum antigenic reactivity was observed at 5µg ES-31 Ag/well and at 1:25 dilution of anti-DSS-goat-serum (Table 1). Isoniazid at maximum concentration of 0.4µg/well showed 42% inhibition.
of mycobacterial ES-31 serine protease while orlistat showed inhibition of 60% at concentration of 0.50µg/well by Indirect ELISA (Table 2). In axenic culture, isoniazid at 0.4µg/well showed 35% inhibition while orlistat showed 29% inhibition at 0.50µg/well (Table 3).

**Table 1:** Antigenic reactivity of mycobacterial ES-31 serine protease by indirect ELISA

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>ES-31 Antigen (µg/well)</th>
<th>Antigenic reactivity (OD450)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Anti-DSS-goat serum 1:25 dilution</td>
</tr>
<tr>
<td>1</td>
<td>1 µg/well</td>
<td>0.898</td>
</tr>
<tr>
<td>2</td>
<td>5 µg/well</td>
<td>1.145</td>
</tr>
</tbody>
</table>

**Table 2:** Inhibition of antigenic reactivity of mycobacterial ES-31 serine protease by isoniazid and orlistat

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>ES-31 Antigen (5 µg/well)</th>
<th>Antigenic reactivity (OD450) (% inhibition)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>1.077</td>
</tr>
<tr>
<td>2</td>
<td>Isoniazid (0.2 µg/well)</td>
<td>1.005 (7%)</td>
</tr>
<tr>
<td>3</td>
<td>Isoniazid (0.4 µg/well)</td>
<td>0.556 (42%)</td>
</tr>
<tr>
<td>4</td>
<td>Orlistat (0.25 µg/well)</td>
<td>0.565 (47%)</td>
</tr>
<tr>
<td>5</td>
<td>Orlistat (0.50 µg/well)</td>
<td>0.422 (60%)</td>
</tr>
</tbody>
</table>

**Table 3:** Effect of isoniazid and orlistat on the growth of M. tb H37/Ra bacilli in axenic culture

<table>
<thead>
<tr>
<th>Sr No.</th>
<th>Tests</th>
<th>Day of incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>O day (OD540)</td>
<td>5th day (OD540) (%) inhibition</td>
</tr>
<tr>
<td>1</td>
<td>Control (without inhibitor)</td>
<td>0.058</td>
</tr>
<tr>
<td>2</td>
<td>Isoniazid (0.2 µg/well)</td>
<td>0.052</td>
</tr>
<tr>
<td>3</td>
<td>Isoniazid (0.4 µg/well)</td>
<td>0.044</td>
</tr>
<tr>
<td>4</td>
<td>Orlistat (0.25 µg/well)</td>
<td>0.050</td>
</tr>
<tr>
<td>5</td>
<td>Orlistat (0.5 µg/well)</td>
<td>0.049</td>
</tr>
</tbody>
</table>
DISCUSSION

Development of new and slow therapeutics has been a challenge for the treatment of tuberculosis though urgent need is necessitated by emergence of multidrug and extensively drug resistant TB. Innovative techniques are needed to screen potential anti-TB drugs in a faster pace. A study by Kathryn et al. reported a screening of antitubercular drug by growth of tubercle bacilli by using alamar blue dye as indicator. Jeanette et al. reported peptide deformylase (PDF) catalysing the hydrolytic removal of N-terminal formyl group for nascent proteins, essential step in bacterial protein synthesis making PDF an attractive drug target for anti-bacterial drug development leading to a novel class of PDF inhibitors active against TB.

Mycobacterial ES-31 serine protease has been reported as a biomarker with potential drug target for screening anti-TB drugs. Isoniazid, anti-tubercular drug and orlistat, an anti-obesity drug and a lipase inhibitor, have been shown to inhibit mycobacterial ES-31 serine protease by azocasein assay based on protease activity. However it requires a large amount of precious antigen.

Based on the observation that ES-31 serine protease is also an antigen, we explored the effect of isoniazid and orlistat on antigenic reactivity of ES-31 Ag in detecting antibody in ELISA format. Isoniazid showed 42% inhibition at 0.4µg/well while orlistat showed inhibition of 60% at 0.5µg/well in a three hour ELISA assay. This inhibition is confirmed in 10 day axenic inhibition of 60% at 0.5µg/well in a three hour ELISA format. Isoniazid showed 42% inhibition at 0.4µg/well while orlistat showed inhibition of 60% at 0.5µg/well in a three hour ELISA format.

Thus simple ELISA assay based on antigenic reactivity of mycobacterial ES-31 serine protease may be used for large scale screening of drugs, namely protease, lipase and metallo enzyme inhibitors for anti TB potential to be further confirmed by cell culture studies.

ACKNOWLEDGEMENTS

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HEAT FIXED BUT UNSTAINED SLIDE SMEARS ARE INFECTIOUS TO LABORATORY STAFF

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(Received on 9.9.2012; Accepted after revision on 21.3.2013)

Summary
Background and Aim: In a clinical microbiology laboratory, heat fixed slide smears are commonly transported from one place to another for staining with different stains and also for onsite proficiency testing of laboratory technicians for accreditation of the laboratories. These smears are frequently handled without gloves by the staff in developing countries. Therefore, this study was conducted to check the survivability of tubercle bacilli on smears after physical and chemical treatments.

Methods: A total of 196 AFB positive smears were analyzed. Of these, 116 were stained with Ziehl Neelsen (ZN), 60 with cold Kinyoun and 10 were unstained but heat fixed and 10 were neither stained nor heat fixed. The last 20 smears served as controls. The ZN and Kinyoun stained smears were 0-1.5-year-old and stored at room temperature in slide boxes, while control smears were freshly prepared. All smears were prepared from sputum samples positive for acid fast bacilli. All four sets were subjected to slide culture to see if mycobacteria could survive and grow in any. For slide culture, a new and safe device was used, which is designed for three in one purpose: cell cultivation, direct observation of the growth under microscope and cell harvesting inside the closed tube. The slide smears were directly dipped into this tube that contained liquid culture medium. The tubes were incubated at 37°C for four weeks. The growth, if any, was confirmed by MPT-64 rapid test and subculture on LJ slants.

Results: No growth was observed in ZN and Kinyoun stained slide smears. However, significant growth was observed in both control sets; the unstained non heat fixed as well as heat fixed slide smears.

Conclusions: The results of our study indicate that tubercle bacilli remain viable even after heat fixation and carry risk of infection by contact. However, stained smears are safe for handling and storage. [Indian J Tuberc 2013; 60: 142-146]

Key words: Smear, Slide culture, Ziehl Neelsen, Kinyoun, M. tuberculosis.

INTRODUCTION

Tuberculosis (TB) is a highly contagious disease with the incidence of 8.8 million new cases every year. Microscopy remains the mainstay of any clinical microbiology laboratory, whether dedicated to the diagnosis of TB or it caters to other infectious diseases.¹ Though sputum remains the main clinical material but some patients are unable to produce sputum, such as children, immuno-compromised patients and patients with neurological impairment.² Also patients with chronic intractable diarrhoea are investigated for opportunistic coccidian parasites and for mycobacterial causes. The immuno-compromised patients, especially, may shed a high number of the opportunistic coccidian parasites as well as Mycobacterium species in their feces. These suspected fecal smears are stained with cold Kinyoun and as well with hot carbol fuchsin stains for the opportunistic coccidian parasites and Mycobacterium species, respectively.²⁴

In resource limited settings such as primary health care centres and designated microscopy centres, these stained slides are usually being handled without gloves by the staff. Also often, the national and regional reference laboratory staff carries with them, during inspections, unstained smears to evaluate the proficiency of the local laboratory technicians. Hence this may be perilous to the laboratory personnel and the staff who carries such slides to the distant RNTCP laboratories. It has been documented that Laboratory Acquired Infections (LAI) of TB are three to nine times higher in laboratory workers than general...
population. Hence, safety of the laboratory workers from mycobacterial infection should be the first consideration in mycobacteriology laboratory.

Some studies have been performed to check the viability of Mycobacterium tuberculosis (M. tb) from heat fixed smears as well as Ziehl Neelsen (ZN) and Auramine Rhodamine (AR) stained smears. But, to the best of our knowledge, no study is published on viability of mycobacteria using cold Kinyoun stain. Hence, in the present study, we evaluated the viability of Mycobacteria in the heat fixed smear with and without staining with hot and cold acid fast stains.

MATERIAL AND METHODS

Slide smears used for culture

The study was conducted at the Division of Clinical Microbiology, Department of Laboratory Medicine, All India Institute of Medical Sciences (AIIMS), New Delhi from February 2011 to February 2012. A total of 196 AFB 3+ sputum slide smears were used in this study. Of these, 70 were 12-18 months’ old stored ZN stained slides and taken out from the slide storage boxes after reviewing the old records. All old slides are stored only after cleaning with xylene. Remaining 126 were freshly prepared slide smears from the sputum of known pulmonary TB patients and confirmed positive for acid fast bacilli. As a standard practice, the slide smears from sputum samples were prepared and allowed to dry in a biological safety cabinet (HR40-IIB2, Haier, China). Slide smears are then routinely heat fixed by passing the slide three times through the flame of gas burner before removing the slides from biosafety cabinet for staining. Of the 126 freshly prepared slide smears, 46 were stained with ZN (total 70+46=116) and 60 with cold Kinyoun methods. The smears were examined microscopically under oil immersion field and results were graded as per the WHO guidelines. For growth control, 10 heat fixed unstained smears and another 10 slide smears were taken which were neither heat fixed nor stained with any stain. Medium without any inoculation was considered as a negative growth control.

Decontamination of slide smears

Slide smears (heat fixed unstained as well as stained) were decontaminated to prevent the growth of other micro-organisms. In the beginning, decontamination of the slides was done by using 4%, 2% and 1% NaOH solution for different time periods of four, three, two and one minute. It was found that 4% NaOH was too harsh for the mycobacteria but 1% NaOH was not able to decontaminate properly hence decontamination with 2% for one minute was considered as optimum, and this protocol was used throughout the study. For this, two sterilized coplin jars were used. One was filled with 2% NaOH and the second with phosphate buffer solution (to neutralize effect of alkali). With the help of sterilized forceps, slides were dipped in 2% NaOH for one minute and after that neutralized in phosphate buffer solution for 30 seconds.

Smear culture by Thin Layer Agar (TLA) method

Initially, we designed our experiment with the use of TLA method to test the viability of M. tuberculosis in ZN stained smears. The smeared side of the decontaminated slides (20) was placed up-side-down on the agar plate containing middle brook 7H10 agar medium with PANTA (Becton, Dickinson and Company, USA). In each experiment, positive and negative controls were also used. The plates were incubated at 37°C upto six weeks. Plates were observed every 96 hours to check for contamination and cord forming micro-colonies using inverted microscope (RTC-7, Radical Instruments, India).

Smear slide culture by tissue culture tube method

Since the thin layer agar plate method was not very successful, we performed slide culture method using a special type of tissue culture tube (Figure) manufactured by Techno Plastic Products (Ref: 91253, TPP , Switzerland). This tube is designed especially for three in one purpose: cell
cultivation, direct observation under microscope and cell harvesting in a single tube. For slide culture, 10ml Middlebrook 7H9 medium with OADC-PANTA supplement was dispensed into these culture tubes. In the culture tube, the slides under study were placed. The slides were placed in such a way as the whole smear was covered by the liquid medium. Tubes were then incubated at 37°C upto four weeks and examined twice a week using the inverted microscope to detect the growth (cord forming) of \textit{M. tuberculosis} (40x).

After incubation of four weeks, the 200µl liquid medium from the tube was taken out and inoculated on LJ medium in order to confirm the further growth/viability of \textit{M. tuberculosis}.

Fifty microliter of culture was used to confirm the growth using commercially available strips (TB Ag MPT-64 Rapid®, SD BIOLINE, India). The strip is based on the principle of immunochromatography, which detects \textit{M. tuberculosis} MPT-64 antigen.\textsuperscript{10}

\textbf{Figure:} Inoculated slide smear into flat bottom tissue culture tube: (A) standing position during incubation, (B) position during microscopy
RESULTS

None of the ZN stained and Kinyoun stained old slides showed growth after four weeks. However, cord formation was observed within 10 days in both the growth control slides cultured under the same culture conditions (Table).

DISCUSSION

*M. tuberculosis* is usually transmitted through respiratory aerosols in shared air environments and TB infection through surface contact in human-to-human transmission is low. Aerosol may be generated at any stage during laboratory processing of TB specimens, any manipulation with *Mycobacterium* cultures and working with infected animals. Therefore, incidence of TB infections in laboratory staff has been reported three to nine times higher than general population. Moreover, *M. tuberculosis* infections by contacts and cutaneous injuries have also been documented.

Even though hot carbol fuchsin stain (ZN stain) is widely perceived that it kills all mycobacteria, yet no reports are available in the literature to demonstrate, if the cold acid fast stain (such as Kinyoun stain) would also carry the same detrimental effect on the mycobacteria. This study was conducted to compare if the detrimental effect of ZN stain on mycobacteria is due to heat or it is a chemical sterilization. To compare this, we compared heat fixed unstained smears with heat fixed but stained with two types of stains: one uses hot carbol fuchsin while the other uses cold carbol fuchsin. The study clearly showed that mycobacteria resist physical sterilization but cannot withstand chemical sterilization.

The conclusion of the present study is that tubercle bacilli do not remain viable after the slide smears are stained whether with hot ZN or with cold Kinyoun method and are safe for handling and storage. However, the unstained slide smears prepared from samples positive for mycobacteria remain infectious and should be handled as potentially infectious.

ACKNOWLEDGEMENTS

Authors would like to thank Mr. Brijesh Sharma, Mr. Virender Kapil and Mr. Vinod Kumar for their technical assistance.
REFERENCES


CLINICAL PROFILE AND DIAGNOSIS OF EXTRAPULMONARY TB IN HIV INFECTED PATIENTS: ROUTINE ABDOMINAL ULTRASONOGRAPHY INCREASES DETECTION OF ABDOMINAL TUBERCULOSIS*

Sonam Spalgaia1**, Anand Jaiswal2**, Mannmohan Puri2**, Rohit Sarin1 and Upasna Agarwal4

(Received on 15.9.2012; Accepted after revision on 29.4.2013)

Summary

Objective: To study the clinical profile and assess the utility of the procedures performed for the diagnosis of extrapulmonary TB (EPTB) in HIV patients.

Design: Prospective observational study of HIV patients suspected to have EPTB.

Results: Two hundred and thirty HIV-infected patients were enrolled over 18 months. Of them, 87 cases had active TB, 60 (69%) of whom were of EPTB. Major presenting symptoms were fever (93.3%), weight loss (80%) and cough (61.6%). The most common site of active EPTB was the abdomen (70%), which could be detected due to routine use of abdominal ultrasonography, followed by CT scans in inconclusive cases. Peripheral lymph node (22%), pleura (15%), CNS involvement (3%) and one case each of psoas abscess and mediastinal lymphadenopathy were the other extra-pulmonary sites seen. Diagnosis of peripheral lymph node and pleural TB was based on cytological and mycobacterial examinations. Direct smear examinations were positive for AFB in 11 of 24 samples and mycobacterial cultures were positive in five of 18 samples. The median CD4 cell count in our HIV-EPTB cases was 126 cells/µL (IQR=79.5-205.75). There was no statistical difference in the baseline CD4 cell counts in patients with PTB vs EPTB (p=0.70), single vs multiple extra-pulmonary site involvement (p=0.57), and AFB positive vs AFB negative EPTB cases (p=0.51).

Conclusions: EPTB is the most common form of TB in HIV patients with low CD4 cell counts. Fever, weight loss and cough are common presenting symptoms of EPTB. Routine abdominal ultrasonography followed by an abdominal CT scan in inconclusive cases can significantly increase the detection of abdominal TB. [Indian J Tuberc 2013; 60: 147-153]

Key words: Tuberculosis, HIV, EPTB, Abdominal TB

INTRODUCTION

India has 2.3 million Human Immunodeficiency Virus (HIV) infected people, more than 50 per cent of whom have tuberculosis (TB) of one organ or the other1. As per RNTCP report 2012, an estimated 75,000 incident TB cases occur in HIV infected individuals2. HIV infection not only increases the risk of progression of latent infection to active TB, it also increases chances of new TB infections, risk of recurrence and case fatality, if not timely treated. TB is known to be the leading cause of death in HIV infected people in India.

Diagnosis of TB on the whole is difficult in HIV infected patients because of masking of constitutional symptoms, sputum negativity, atypical chest radiographs and resemblance to other opportunistic infections. Extra-pulmonary TB (EPTB) accounts for 53 to 62 per cent of cases in HIV-positive individuals3. Due to higher prevalence of EPTB, involvement of inaccessible and uncommon sites and lack of standardized diagnostic algorithms for HIV infected individuals, diagnosis of EPTB becomes especially challenging. The conventional methods of smear and culture applied to sputum, body fluids and other extrapulmonary specimens remain mainstay investigations to reach a diagnosis. Though use of radiologically guided procedures and minimally invasive diagnostic methods must be made to procure material for histopathological and microbiological testing, quite often clinical judgement,
consistent imaging findings and appropriate response to TB treatment are the only diagnostic strategies possible or available.

Further data on extra-pulmonary manifestations of HIV-TB disease and the appropriate diagnostic procedures to be adopted in these cases would be useful in better diagnosis and early treatment thereby contributing to reduced mortality in HIV infected TB cases. The present study was thus undertaken with the objective to study the clinical profile of extra-pulmonary TB and assess the utility of the procedures performed for the diagnosis of EPTB in HIV infected patients.

METHODS

This study was a prospective observational study conducted at the Antiretroviral Therapy (ART) Centre of LRS Institute of TB and Respiratory Diseases, New Delhi, a tertiary care, referral TB hospital.

Patient selection

All consecutive adult HIV infected patients, newly registered at the ART centre between June 2010 and December 2011 were screened for signs and symptoms related to EPTB. Cases suspected to have EPTB and subsequently diagnosed as EPTB were included in the study.

Patient work up

All HIV infected adult patients were specifically asked for following symptoms (of any duration): fever, weight loss, loss of appetite, chest pain, shortness of breath, cough, swelling in the neck or groin, pain abdomen, diarrhoea, distension of abdomen, headache, vomiting, and altered sensorium. Detailed physical examination was done for all cases. Patients with symptoms and signs suggestive of TB of an extra-pulmonary organ(s) were considered as EPTB suspects and investigated.

Investigative work up

All EPTB suspected patients underwent routine blood counts, biochemistry tests, chest radiographs, abdominal ultrasonography (USG) and baseline CD4 cell counts. Sputum for direct Acid Fast Bacilli (AFB) smear and mycobacterial culture examination, cytology and AFB examination of fine needle aspirates in cases of peripheral lymphadenopathy and body fluid examination (pleural fluid, ascitic fluid and CSF) for cytology, biochemistry, AFB smear and culture were done on a case-to-case basis wherever indicated. Computed tomography of chest/abdomen/head were undertaken, if clinically required.

Diagnosis of extra-pulmonary TB

Extra pulmonary TB was diagnosed as per existing Revised National Tuberculosis Control Programme (RNTCP) guidelines4 by identification of typical clinical features, isolation AFB from a clinical specimen wherever possible, radiological findings and decision to treat for TB. TB was classified as EPTB, pulmonary TB (PTB), or both PTB and EPTB.

Treatment of extra pulmonary TB

Antitubercular treatment (ATT) comprised standard short course regimens available under the RNTCP which consist of rifampicin, isoniazid, pyrazinamide and ethambutol (Category I ) with additional streptomycin (Category II) in retreatment cases.

Diagnosis of HIV and antiretroviral therapy

HIV infection was diagnosed using three antigenically different rapid kits as per the national HIV testing policy. ART regimens followed National ART Programme guidelines5 and comprised efavirenz in combination with either zidovudine or stavudine and lamivudine.

This study was approved by the Institutional Review Board.

Data Analysis: Continuous data are presented as median and inter-quartile range (due to extreme values). Mann-Whitney non-parametric statistics was used to compare continuous variables (due to small sample size). All tests were two-tailed and p<0.05 was considered statistically significant.
RESULTS

Two hundred and thirty adult patients with HIV infection were registered at the ART centre at LRS Institute of TB and Respiratory Diseases, New Delhi between June 2010 and December 2011. Out of the 230 cases, 87 patients were diagnosed to have concurrent active TB, of whom 60 (69%) were diagnosed with EPTB. Of these 60 EPTB cases, 47 (78%) were males and the mean age of the HIV-EPTB patients was 35.5 years (range 18 to 65 years).

Type and site(s) of EPTB: Out of the 60 HIV-infected cases diagnosed to have EPTB, 45 (75%) patients had only EPTB, while 15 (25%) patients had EPTB with pulmonary manifestations. Forty-nine (82%) of EPTB cases presented with single site and 11 (18%) presented with multiple sites of extrapulmonary involvement. The sites of EPTB were abdomen in 42 (70%), peripheral lymph node in 13 (22%), pleura in nine (15%), CNS in two (3%), psoas abscess in one (2%) and mediastinal lymphadeopathy in one (2%) case.

Presenting symptoms: The major presenting symptoms of HIV-EPTB in this study were fever (93%), weight loss (80%) and cough (62%). Other presenting symptoms depended on the site of TB like pain abdomen (60%), diarrhea (48%), abdominal distension (40%), and peripheral swelling (22%).

Diagnosis of EPTB: Abdominal TB (n=42) was diagnosed on the basis of symptoms and findings revealed on routine abdominal ultrasonography (USG). The common findings on ultrasonography and contrast enhanced CT scans of the abdomen are given in Table 1. TB disease of peripheral lymph node (n=13) was based on examination of fine needle aspirates. The findings on cytology were epithelioid granuloma with necrotic debris, lymphocytic inflammation and necrotising granuloma. Pleural fluid aspiration and cytology were done in all the nine patients. The cytology findings were lymphocytic exudation in seven (78%) and degenerative necrotic debris in two (22%) patients.

Mycobacterial isolation from an extra pulmonary sample: Direct smear examinations for AFB could be done in 24 extra-pulmonary samples. We had 13 lymph node fine needle aspirates, nine pleural fluid aspirates, one cerebrospinal fluid sample and one pus sample taken from psoas abscess. The smear examinations were positive for AFB in 11 samples (lymph node = 8, pleural fluid = 2 and pus from

| Table 1: Abdominal ultrasonography and contrast enhanced CT scan findings in patients of abdominal TB |

<table>
<thead>
<tr>
<th>Findings</th>
<th>No. of patients(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Abdominal ultrasonography findings (n=42)</strong></td>
<td></td>
</tr>
<tr>
<td>Multiple enlarged abdominal lymph nodes</td>
<td>42 (100%)</td>
</tr>
<tr>
<td>Central caseation in enlarged lymph nodes</td>
<td>30 (71 %)</td>
</tr>
<tr>
<td>Organomegaly</td>
<td>19 (42 %)</td>
</tr>
<tr>
<td>Splenic micro abscesses</td>
<td>13 (31%)</td>
</tr>
<tr>
<td>Thickened bowel loops</td>
<td>6 (18 %)</td>
</tr>
<tr>
<td>Ascitis</td>
<td>3 (7%)</td>
</tr>
<tr>
<td><strong>Abdominal contrast-enhanced CT scan findings (n=12)</strong></td>
<td></td>
</tr>
<tr>
<td>Abdominal lymphadenopathy with central necrosis</td>
<td>12 (100 %)</td>
</tr>
<tr>
<td>Organomegaly</td>
<td>6 (50 %)</td>
</tr>
<tr>
<td>Splenic lesions</td>
<td>5 (42%)</td>
</tr>
</tbody>
</table>
psosas abscess = 1). Mycobacterial cultures could be done in 18 extrapulmonary samples. The culture was positive for *Mycobacterium tuberculosis* in five samples (two lymph node aspirates and three pleural fluid samples). Details of AFB isolation from different extra-pulmonary samples is given in Table 2. In one case of pleural effusion, the pleural aspirate was negative for AFB on direct smear examination, however was positive for *M. tb* on culture examination.

**X-ray chest PA view:** Chest x-ray was done in all 60 EPTB-HIV patients and was found to be abnormal in 34 (56.6%) patients. Parenchymal lesions suggestive of PTB were seen in 15 (25%) cases, two cases had miliary mottling and one case revealed pneumonic infiltrates. Other abnormalities found on chest x-ray were pleural involvement in 11 (18.3%), prominent hilar nodes in three (5%) and enlarged mediastinal lymph node in one case. One case also showed findings suggestive of pulmonary fibrosis.

**CD 4 Cell count:** Baseline (pre-ART) CD4 cell counts were available for all the HIV-EPTB cases. The median CD4 cell count in our cases was as low

**Table 2:** Sites of EPTB and AFB isolation from extra-pulmonary samples

<table>
<thead>
<tr>
<th>Site of EPTB</th>
<th>No. of cases (N=60)</th>
<th>No. of cases where AFB isolated</th>
<th>No. of cases diagnosed on basis of cytology, biochemistry, CT scan /USG findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abdomen</td>
<td>33</td>
<td>-</td>
<td>33</td>
</tr>
<tr>
<td>Peripheral Lymph Node</td>
<td>7</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>Pleura</td>
<td>6</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>CNS</td>
<td>1</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Psoas abscess</td>
<td>1</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Mediastinal lymphadenopathy</td>
<td>1</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Multiple EP sites involved</td>
<td>11</td>
<td>2</td>
<td>9</td>
</tr>
</tbody>
</table>

**Figure:** CD4 cell counts(cells / µl)in HIV-EPTB cases

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as 126 cells/µl \(^1\) (IQR=79.5-205.75) (Fig.). There was no statistical difference in the baseline CD4 cell counts in patients with PTB vs EPTB (p=0.70), single vs multiple extra-pulmonary site involvement (p=0.57), and AFB positive vs AFB negative EPTB cases (p=0.51).

**Treatment of extrapulmonary tuberculosis - ATT:**
Fifty eight (96.7 \%) of the 60 HIV-EPTB patients were started on ATT. One patient died soon after diagnosis, before ATT could be started and one patient defaulted. Forty one (68.3 \%) received Category I ATT containing isoniazid, rifampicin, pyrazinamide and ethambutol and 17 (28.3 \%) patients were started on category II treatment. All patients were given directly observed, intermittent treatment available under the Revised National Tuberculosis Control Programme.

**Treatment of HIV disease - ART:** ART was started in 54 of the 60 HIV-EPTB patients. Of them, all patients were on efavirenz containing regimen. Twenty seven (50 \%) patients were given ART regimen containing stavudine, lamivudine with efavirenz and 27 (50 \%) were on ziduvudine, lamivudine with efavirenz.

**DISCUSSION**

Tuberculosis is the most common opportunistic infection in HIV positive persons, developing at any stage of the disease, EPTB being a frequent form of TB in these cases. In this present study, we found that about 40 \% newly registered HIV infected patients had active TB, of whom nearly 70 \% of TB cases had EPTB. Three-fourth of these HIV-EPTB cases had extra-pulmonary involvement as the sole presentation of TB while remaining one-fourth had both extra pulmonary involvement and PTB. Similar high proportion of EPTB in HIV has been demonstrated in several other Indian and international studies as well\(^6\)\(^-\)\(^10\). Converse to above, Daniele Bendayan MD et al\(^11\) from Israel and Zubert Ahmad et al\(^12\) from India report a lesser proportion of EPTB in HIV infected TB cases. Possibly, EPTB could be under-diagnosed in a few settings due to unusual site (s) involved and atypical presentations of EPTB in HIV infected individuals. The higher proportion of EPTB reported by us is most likely due to the emphasis in our study on early use of appropriate diagnostic modalities for detection of all the TB affected sites in every patient. The observation that a high percentage of HIV-TB cases have extrapulmonary involvement of sites which can be inadvertently missed highlights the need for robust screening and diagnostic algorithms for EPTB.

Cough of two weeks' duration is a symptom used commonly by different national programmes for screening adults for possible PTB\(^4\). Need for a similar reliable symptom screening for EPTB has been recognized, especially in HIV infected individuals in whom EPTB forms a major type of TB. Fever (93 \%) followed by weight loss (80 \%) and cough (62 \%) were the most common presenting symptoms of EPTB in this study. Similar initial symptoms have been reported by other researchers in EPTB patients\(^5\)\(^-\)\(^8\),\(^11\),\(^14\). In a South African study on active TB case finding in HIV infected cases, a screening instrument comprising weight loss, cough, night sweats or fever was used and the authors found 100 \% sensitivity, 88 \% specificity and 44 \% and 100 \% positive and negative predictive values respectively, with this tool\(^15\). The above studies as well as our study provide evidence to support the use of a set of symptoms including fever, weight loss and cough as a screening tool for diagnosis of EPTB in HIV infected patients. Such an evidence-based symptom screening would be effective, rapid, easy to administer, requires no special training and be cost-effective for HIV clinics in high TB prevalence, resource limited settings. In addition to the symptoms discussed above, we also found certain site-specific symptoms in our EPTB-HIV patients namely pain abdomen, diarrhoea, abdominal distension and peripheral swelling, presence of which depended on the extrapulmonary site involved which could be included in more detailed screening tools for studies on specific sites of EPTB.

In the present study, we found that the most common site of extra-pulmonary involvement was the abdomen and 42 (70 per cent) of EPTB cases were of abdominal TB. The diagnosis of abdominal TB was based chiefly on symptoms along with findings of routine abdominal USG. Contrast-enhanced CT scanning of the abdomen revealed central necrosis of the enlarged lymph nodes in cases
where it was not seen on USG. Among abdominal tuberculosis cases in this data, 12 also had PTB and the extra-pulmonary site involvement was recognized due to a routine abdominal ultrasonography. In variance to our results, most of the studies report peripheral lymph node as the most common site of extra-pulmonary TB in HIV infected individuals. We feel that the reason for this difference is the routine use of abdominal USG in all HIV cases in the present study and early usage of abdominal contrast-enhanced CT scanning intervention in patients with abdominal symptoms. Thus, diagnosis of EPTB disease of uncommon sites especially the abdomen requires a high index of suspicion and routine use of simple investigations like ultrasonography. CT scan abdomen can help enhance the diagnostic yield, especially if USG findings are inconclusive of TB disease activity.

The median baseline CD4 cell count in our EPTB cases was 126 cells/µl. The very low CD4 cell count found in our study was consistent with finding of several studies 6,7,9,11,14. In our data, we could not demonstrate any statistical difference in the baseline CD4 cell counts in patients with PTB vs EPTB (p=0.70), single vs multiple extra-pulmonary site involvement (p=0.57) and AFB positive EPTB vs AFB negative EPTB cases (p=0.51). The reason for this may be that the HIV-TB patients in our cohort had overall very low baseline CD4 counts, which is the case for most Indian settings. This again is basically a reflection of late presentation of HIV-TB patients to medical care. The profound immune suppression at the time of presentation is mostly due to the fact that cases are diagnosed with HIV around the time of diagnosis of TB. Due to unawareness of their HIV status, these patients miss opportunity to receive ART during the asymptomatic phase of HIV infection, which could have prevented the deterioration of their immune status and reduced their risk of tuberculosis. This clearly indicates the need for early diagnosis of HIV and its timely treatment.

Though the study has limitations of small sample size and non-availability of plasma viral loads, it is a prospective study with precise analysis of clinical data of HIV-EPTB cases from a tertiary care TB Institute which cares for patients referred from all over north India.

In conclusion, EPTB is the most common form of TB in HIV infected patients with advanced immunosuppression. Fever, weight loss and cough can be effective screening symptoms for EPTB. Abdominal TB, though a common extra-pulmonary site of TB disease in HIV, may be under-diagnosed. A simple abdominal ultrasound examination used routinely in HIV patients suspected of TB, followed by an abdominal CT scan in inconclusive cases, can significantly increase the detection of abdominal TB.

ACKNOWLEDGEMENTS

The authors are grateful to the Departments of Microbiology, Pathology and Radiology at LRS Institute of TB and Respiratory Diseases, New Delhi, for their diagnostic support. We also acknowledge the staff of the ART Centre for the help provided by them for this study.

REFERENCES

INITIAL DRUG RESISTANCE PATTERN AMONG PULMONARY TUBERCULOSIS PATIENTS

Harshita Gupta1, Surya Kant1, Amita Jain2, S.M. Natu3 and Savita Ahluwalia4

(Received on 6.11.2012; Accepted after revision on 21.3.2013)

Summary

Background: Drug resistant tuberculosis (DRTB) is an emerging problem that adversely affects treatment outcomes and public health in the developing world.

Objective: To determine the initial drug resistance pattern among pulmonary tuberculosis patients registered under the Revised National Tuberculosis Control Programme.

Study Design: A cross-sectional study design.

Setting: Two urban Directly Observed Treatment Supervised (DOTS) centres in Lucknow District of Uttar Pradesh.

Methods: The present study consisted of newly diagnosed sputum smear-positive for acid-fast bacilli (AFB) cases at the time of registration under the tuberculosis control programme. All sputum smear positive cases were subjected to culture and drug-susceptibility testing by 1% proportion method on Lowenstein-Jensen (LJ) medium.

Results: A total of 185 newly diagnosed sputum smear positive for AFB in pulmonary tuberculosis patients were subjected to culture and drug sensitivity test. Among 185 isolates, 170 (91.4%) isolates were culture positive. Of these 170 isolates, 169 (99.4%) were M. tuberculosis and one (0.5%) was Mycobacterium other than tuberculosis (MOTT). Out of 99.4% M. tuberculosis positive isolates, 21.3% were resistant to at least one drug. Resistance pattern of 21.3% strains of M. tuberculosis showing resistance to single, double, triple, and quadruple drugs were 5.9%, 10.7%, 2.4% and 2.4% respectively. Multi-drug resistance (MDR) was observed in 4.7% isolates.

Conclusion: The present study highlights the high rate of drug resistance pattern among the new sputum smear positive pulmonary tuberculosis patients and also high MDR tuberculosis. Routine surveillance of drug resistance profile of patients provides useful information for adopting new strategies of effective treatment within National Tuberculosis Control Programmes in order to combat the threat of MDR-TB in the general population.

Key words: Tuberculosis, Initial drug resistance, Multi-drug resistance

INTRODUCTION

Tuberculosis (TB) is a contagious disease caused by the bacillus Mycobacterium (M.) tuberculosis.1 TB is also more common among men than women, and affects mostly adults in the economically productive age groups; around two-thirds of cases are estimated to occur among people aged 15–59 years.2 If TB is not diagnosed and well treated, a person with active TB can infect 5-10 other persons per year. One in three persons in the world is infected with M. tuberculosis.3 Only 5-10% of the people infected will be sick or infectious at any point of time, in the remaining the disease being latent.1 TB is still a global public health problem since the World Health Organization (WHO) declared the disease a global emergency in 1993.1 According to the WHO, 2008 (based on 2006 data), there were 9.2 million TB cases per annum with 41% sputum smear positive (SS+).4 India reported the largest number of incident cases (2.0-2.5 million) which alone accounted for an estimated one quarter (26%) of all TB cases worldwide.3

Drug-resistant TB is now well established throughout the world. Resistance of M. tuberculosis to drugs is a man-made amplification of spontaneous mutations in the genes of the tubercle bacilli.3 Initial drug resistance develops in a patient, who is denied history of previous chemotherapy. In reality, it consists of true primary resistance and an undisclosed acquired resistance.4 Treatment with a single drug due to irregular drug supply, inappropriate prescription, or poor adherence...
to treatment permits the multiplication of drug-resistant strains. Since drug resistance develops because of inadequate use of drugs, anti-tuberculosis drug resistance surveillance is, together with the monitoring of treatment outcome, an essential tool for evaluating the quality of tuberculosis control programmes, lack of laboratory resources and rapid accurate point-of-care tests.5,6 Globally, multi-drug resistance (MDR)-TB is a major challenge to programme managers.7 Surveillance and analysis of local rates of TB drug resistance are helpful in the detection and monitoring of the extent of MDR strains, indicating the quality of TB control in the country. Knowledge of the prevalence of drug resistance in new cases guided the selection of drugs used in initial treatment of tuberculosis.7 Accurate and rapid diagnosis of TB and drug-resistant TB is of paramount importance in establishing appropriate clinical management and infection control measures.

The aim of the study was to evaluate the possible combinations of isoniazid (INH), rifampicin (RMP), streptomycin (SM), and ethambutol (EMB) and four resistant modes, i.e. mono, double, triple and quadruple resistance among new cases of pulmonary tuberculosis patients under DOTS.

MATERIAL AND METHODS

Study Design and Setting

A cross-sectional study was conducted between January 2010 to March 2011 at the two urban Directly Observed Treatment Short-course (DOTS) centres of Lucknow Medical University and a TB Hospital located near Medical University, Lucknow District of Uttar Pradesh.

Study Population

The study consisted of 185 newly diagnosed SS+ for AFB pulmonary tuberculosis patients of both sexes and between the age group of 12 to 65 years at the time of interview and were about to be registered for treatment. Patients were excluded if they fulfilled any of the following exclusion criteria: previous history of Anti-Tuberculosis Treatment (ATT); pregnant and lactating women; subjects known to be HIV positive/ or suffering from any immuno-deficient state; and use of corticosteroids or supplements containing Vitamin A, zinc, iron, etc. during the previous month. All subjects were free from alcoholism.

Ethical Considerations

The study was ethically approved by the institutional ethics committee of the Medical University of Lucknow. All eligible patients were informed about the study and signed an informed consent form (ICF) from each subject before the beginning of the study.

Data collection

Personal interview and clinical examination

Interviews using structured questionnaires were used to collect the socio-demographic background and data on family history. Socio-economic status was assessed by Kuppuswamy’s socio-economic status (SES) scale.8 Subsequently, patients were thoroughly examined by medical doctors at both hospitals.

Assessment of clinical outcomes

Clinical outcomes were assessed at baseline and at the end of six-month-treatment. The following symptoms were clinically assessed including fever, cough, expectoration, chest pain breathlessness, wheezing, haemoptysis, dyspnea, night sweat, loss or improve of appetite and weight loss or gain.

Assessment of bacteriological outcomes

Bacteriological outcomes were assessed by RNTCP guidelines,9 2006, included AFB smear examination and grading, AFB culture and drug susceptibility test. All specimens were carried to the accredited Intermediate Reference Laboratory (IRL) at the Department of Microbiology, Medical University, Lucknow where further processing was done.

Specimen collection

The diagnosis of TB was done in accordance with the RNTCP guidelines,9 2006. At the time of
enrolment, three sputum specimens on two consecutive days from each patient were collected in properly labelled screw capped, sterile disposable plastic bottles after oral gargling with normal water. Thus, there were three samples: SPOT, EARLY MORNING and SPOT. Specimens contained mucoid or mucopurulent material with minimum amounts of oral or nasal material into the McCartney bottles and volume was of approximately 5ml.

**AFB smear examination and grading**

AFB smear examination was carried out by direct microscopy using the Ziehl Neelsen (ZN) method. Sputum smear result was examined and interpreted according to the AFB grading.10

**AFB culture and drug susceptibility test**

Culture examinations were done on all diagnostic specimens, regardless of AFB smear positivity. Sputum specimens from each patient were processed with sodium hydroxide (NaOH) method-Modified Petroff’s procedure and cultured on Lowenstein-Jensen (LJ) slopes.10 All inoculated LJ drug and control media were incubated at 37°C. All cultures were examined 48-72 hours after inoculation to detect gross contaminants. Thereafter, cultures were examined weekly, up to eight weeks on a specified day of the week. Typical colonies of *M. tuberculosis* were rough, crumbly, waxy, non-pigmented (buff-coloured) and slow-growers, i.e., only appeared two to three weeks after inoculation. The colony was confirmed by ZN staining. Detection time for MOTT was 25 days. *M. tuberculosis* positive strains were culture negative when they grew on p-nitro benzoate (PNB) containing medium. Only a few colonies of non-tuberculous *Mycobacteria* (NTM – often pigmented, with smooth morphology or PNB positive) were grown as visible colonies on PNB containing medium.10

Drug resistance was expressed in proportion method, where a strain was considered to be drug resistant if the number of colonies that grew on a drug containing medium was 1% or more of the colonies that grew on a control drug free medium. The control (drug free) medium showed good growth at least 50 to 100 colonies.10

**Assessment of radiological outcomes**

Radiological outcomes were assessed by chest x-ray examination. Chest radiographs (CXR) were made of all the patients at the time of diagnosis of TB at the end of six-month-treatment. Patients were evaluated by judging the site of lesions, zone of involvement, nature of the lesion (visible cavitary and non-cavitary area) in both lungs as well as classified as the extent of lesion having mild, moderate and far-advanced lesion according to American Thoracic Society classification.11 The chest x-rays (postero-anterior view) were appraised by a radiologist.

**Laboratory Definition**

MDR was defined as resistance to both isoniazid and rifampicin with or without resistance to other drugs.12

**Statistical Analysis**

The data collected was entered into Microsoft Excel and checked for any inconsistency. The descriptive statistics such as percentage and mean(±SD) were calculated. All the analysis was carried out by using SPSS 15.0 version.

**RESULTS**

A total of 185 newly diagnosed patients with pulmonary tuberculosis were recruited, in which 58.4% were males and 41.6% were females. The most frequent age group in the present study was 21-30 years consisting of 36.7% patients, followed by 26.4% patients in the age group of <21 years. The mean(±SD) age of the study population was 29(±12) years. Sputum positivity grade 3+ was most prevalent (35.1%). Majority of the patients were Muslims (53.5%). Most of the patients (78.9%) belonged to the upper lower income group.

It was found that the majority of the patients were non-vegetarian (69.1%) and 60.4% were smokers (Table 1).
Table 1: Characteristics and radiographic presentation of the tuberculosis patients

<table>
<thead>
<tr>
<th>Variables</th>
<th>n=185</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)*</td>
<td>29(±12)</td>
</tr>
<tr>
<td>Gender (n, %)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>108(58.4)</td>
</tr>
<tr>
<td>Female</td>
<td>77(41.6)</td>
</tr>
<tr>
<td>AFB positivity grade (n, %)</td>
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</tr>
<tr>
<td>1+</td>
<td>54(29.2)</td>
</tr>
<tr>
<td>2+</td>
<td>48(25.9)</td>
</tr>
<tr>
<td>3+</td>
<td>65(35.2)</td>
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<tr>
<td>Scanty</td>
<td>18(9.7)</td>
</tr>
<tr>
<td>Religion (n, %)</td>
<td></td>
</tr>
<tr>
<td>Hindu</td>
<td>86(46.5)</td>
</tr>
<tr>
<td>Muslims</td>
<td>99(53.5)</td>
</tr>
<tr>
<td>Socio-economic status (n, %)</td>
<td></td>
</tr>
<tr>
<td>Upper</td>
<td>1(0.5)</td>
</tr>
<tr>
<td>Upper Middle</td>
<td>3(1.6)</td>
</tr>
<tr>
<td>Lower Middle</td>
<td>34(18.8)</td>
</tr>
<tr>
<td>Upper Lower</td>
<td>146(78.6)</td>
</tr>
<tr>
<td>Lower</td>
<td>1(0.5)</td>
</tr>
<tr>
<td>Eating Habits (n, %)</td>
<td></td>
</tr>
<tr>
<td>Vegetarian</td>
<td>35(18.9)</td>
</tr>
<tr>
<td>Non-vegetarian</td>
<td>139(75.2)</td>
</tr>
<tr>
<td>Eggarian</td>
<td>11(5.9)</td>
</tr>
<tr>
<td>Family History of TB (n, %)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>43(23.3)</td>
</tr>
<tr>
<td>No</td>
<td>142(76.7)</td>
</tr>
<tr>
<td>Smoking (n, %)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>119(64.4)</td>
</tr>
<tr>
<td>No</td>
<td>66(35.6)</td>
</tr>
<tr>
<td>Chest Radiographic (Nature of lesion) (n, %)</td>
<td></td>
</tr>
<tr>
<td>Cavitary</td>
<td>64(34.5)</td>
</tr>
<tr>
<td>Non-cavitary</td>
<td>121(65.5)</td>
</tr>
<tr>
<td>Type of Lesions</td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>15(8.18)</td>
</tr>
<tr>
<td>Moderate</td>
<td>115(62.2)</td>
</tr>
<tr>
<td>Far Advanced</td>
<td>55(29.7)</td>
</tr>
</tbody>
</table>

Data were expressed as *mean ± standard deviation
During clinical assessment, most of the patients had persistent fever (98.9%), chronic cough (93.5%), weight loss (92.4%), and appetite loss (99.4%); other frequent symptoms were chest pain (77.8%), breathlessness (72.4%) and haemoptysis (28.6%). The prevalence of cavitary nature of lesion was found in the chest x-ray of 33.3% patients.

All 185 isolates were sputum smear positive for AFB of pulmonary TB. Among 185 isolates, 91.4% were culture positive, 3.7% were contaminated and 4.3% isolates indicated no growth of *Mycobacteria*. Among 91.4% culture positive isolates, 0.5% was MOTT (Table 2). Remaining 99.4% *M. tuberculosis* positive isolates were subjected to drug susceptibility testing (DST). In the DST among 169 strains, 78.6% strains were sensitive to all four anti-tubercular drugs and 21.3% strains were resistant to one or more drugs. Highest resistance was found in INH 18.3% either alone or in combination with other drugs (Table 3). Among new cases, four most frequent drug resistance patterns of 21.3% strains of *M. tuberculosis* from mono drug, double drug, triple drug and quadruple drug resistance were 5.9%, 10.7%, 2.4% and 2.4% respectively. MDR was observed in 4.7% isolates (Table 4).

### Table 2: Results of culture on LJ Medium among new sputum smear positive for AFB specimens

<table>
<thead>
<tr>
<th>Results of culture</th>
<th>Number</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth of <em>Mycobacteria</em></td>
<td>169</td>
<td>91.4</td>
</tr>
<tr>
<td><em>Mycobacterium</em> other than tuberculosis (MOTT)</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>Contamination</td>
<td>07</td>
<td>3.8</td>
</tr>
<tr>
<td>No growth of <em>Mycobacteria</em></td>
<td>08</td>
<td>4.3</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>185</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>

### Table 3: Sensitivity pattern of *M. tuberculosis* to four anti-tuberculosis drugs in LJ medium by proportion method (n=169)

<table>
<thead>
<tr>
<th>Name of drugs</th>
<th>Number of sensitive strains (%)</th>
<th>Number of resistant strains (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rifampicin</td>
<td>161(95.3)</td>
<td>8(4.7)</td>
</tr>
<tr>
<td>Isoniazid</td>
<td>138(81.7)</td>
<td>31(18.3)</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>152(89.9)</td>
<td>17(10.1)</td>
</tr>
<tr>
<td>Ethambutol</td>
<td>151(89.3)</td>
<td>18(10.7)</td>
</tr>
<tr>
<td>Sensitive to all drugs</td>
<td>133(78.6)</td>
<td>-</td>
</tr>
<tr>
<td>Resistance to any drug</td>
<td>-</td>
<td>36(21.3)</td>
</tr>
</tbody>
</table>
In 2011, 6.2 million cases of TB were notified by National TB Control Programmes and reported to WHO: 5.8 million were individuals newly diagnosed in 2011 and 0.4 million were previously diagnosed TB patients whose treatment regimen was changed. India and China accounted for 39% of notified cases of TB worldwide in 2011. Emergence and spread of drug resistant \textit{M. tuberculosis} is a serious threat to tuberculosis control programme because patients with drug-resistant bacilli respond less readily to therapy than those with sensitive bacilli, resulting in preferential spread of drug resistant bacilli in the community. Estimates of drug resistance in new cases carried out at the National Institute for Research in Tuberculosis (NIRT) (formerly known as Tuberculosis Research Centre), Chennai showed that primary resistance to INH was 15.0%, 11.8% to SM and 7.7% to both INH and SM, resistance to INH was reported varying by 3.2% in Pune and 32.9% in Kolar. Resistance to INH and RMP has been observed to increase over the past four decades. The present study estimated that the levels of resistance to INH, RMP, SM and EMB were 18.3%, 4.7%, 10.1% and 10.7% respectively. A study among the primary ATT drugs showed that the highest resistance was seen in pyrazinamide (PZA) (4.68%), followed by 3.5%, 2.9%, 3% and 2.1% respectively in RMP, INH, EMB and SM which entirely differ from our results. The overall incidence of initial drug resistance was 1.7-9% and MDR was reported only in 1.6% cases. Another study revealed that the maximum overall resistance was seen in PZA (6.6%), followed by 5.8%, 5.8%, 4.5% and 4.3% in RMP.

Table 4: Resistance pattern of 36 drug resistant strains of \textit{M. tuberculosis} to four anti-tuberculosis drugs

<table>
<thead>
<tr>
<th>Drug resistance pattern</th>
<th>Names of drugs</th>
<th>Number of resistant strains (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mono drug resistance</td>
<td>Isoniazid (INH)</td>
<td>6(16.6)</td>
<td>10(5.9)</td>
</tr>
<tr>
<td></td>
<td>Rifampicin (RMP)</td>
<td>0(0.0)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Streptomycin (SM)</td>
<td>1(2.7)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ethambutol (EMB)</td>
<td>3(8.3)</td>
<td></td>
</tr>
<tr>
<td>Double drug resistance</td>
<td>*INH+RMP</td>
<td>2(5.5)</td>
<td>18(10.7)</td>
</tr>
<tr>
<td></td>
<td>INH+SM</td>
<td>7(19.4)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>INH+EMB</td>
<td>8(22.2)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>EMB+SM</td>
<td>1(2.7)</td>
<td></td>
</tr>
<tr>
<td>Triple drug resistance</td>
<td>*INH+RMP+SM</td>
<td>2(5.5)</td>
<td>4(2.4)</td>
</tr>
<tr>
<td></td>
<td>*INH+RMP+EMB</td>
<td>0(0.0)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>INH+SM+EMB</td>
<td>2(5.5)</td>
<td></td>
</tr>
<tr>
<td>Quadruple drug resistance</td>
<td>*INH+RMP+SM+EMB</td>
<td>4(11.1)</td>
<td>4(2.4)</td>
</tr>
<tr>
<td>*MDR</td>
<td></td>
<td></td>
<td>8(4.7)</td>
</tr>
</tbody>
</table>

INH-Isoniazid, RMP-Rifampicin, SM-Streptomycin, EMB-Ethambutol*MDR: Multi-drug resistance: Resistance to both isoniazid and rifampicin with or without resistance to other drugs.
EMB, SM and INH respectively. Initial drug resistance to first line anti-tubercular drugs was found in 24.1% cases: single drug resistance in 21.3% and poly-drug resistance in 2.8% cases. There was no case of MDR in this study.

WHO/International Union Against Tuberculosis and Lung Disease global survey in the year 2000 showed that the prevalence of resistance to at least one anti-TB drug among new cases ranged from 1.7 to 36.9%. MDR-TB among new cases was 0.2% in Sri Lanka, 4.0% in Myanmar and 0.7% in Mayurbhanj District, Orissa. Another study conducted in Sindh among newly diagnosed cases showed that the resistance pattern of four most frequent drugs was: INH in 7.1% isolates, 4.1% to RMP, 3.5% to EMB and no case was resistant to SM. MDR-TB was observed in 5.9% isolates. Study in Turkey reported drug resistance pattern from SM, EMB and INH as 32.4%, 14.2% and 12.6% respectively and double INH+SM (8.2%), accounted for 67.4% of all resistant cases.

Resistance to two or three drugs is difficult to treat and often results in treatment failure. A major concern and one of the great biological interests in our study was the highest level of resistance to four first line drug combinations in comparison to INH+RMP alone.

MDR has been a topic of growing interest and posing a threat to the control of TB. The highest proportion of initial MDR-TB has been documented in 4.7% isolates in the present study. This situation is highly alarming in new patients and is higher than those studies reported by the RNTCP and Indian national figures in WHO global surveys. Data from studies conducted by the NIRT, Chennai and National TB Institute (NTI), Bangalore, have found MDR-TB levels less than 1% to 3% in new cases. A retrospective analysis of various randomized clinical trials conducted by the NIRT, Chennai with various rifampicin containing regimens in the initial intensive phase, and with and without rifampicin in the continuation phase, revealed an overall emergence of resistance to rifampicin in only 2% of patients, despite a high level (18%) of initial resistance to isoniazid, either alone or in combination with other anti-TB drugs. In 2008 (reported in 2010), in 27 high MDR-TB burden countries, 2.3% (1.8–2.8) MDR was estimated in new cases. In WHO, 2011, 2.1% was reported (1.5–2.7%) of new TB cases with MDR-TB in India.

In the present study, high incidence of initial MDR was seen. These patients had primary drug resistance who acquired infection from patients having drug resistance. In countries with low prevalence of initial multi-drug resistance, the current standardized treatment regimens for new cases appear to be adequate. However, in countries where the prevalence of initial-MDR exceeds 3%, we believe that it is urgent to strengthen capacity to perform drug sensitivity testing, or to re-examine these standardized regimens, given the unacceptably high rates of failure and relapse. The standardized re-treatment regimen requires equally urgent reassessment because of very poor treatment outcomes in all countries, but particularly in the countries with higher prevalence of MDR. Finding new approaches to treatment of new cases will consume enormous time, effort, and resources. Care must be taken not to worsen treatment outcomes by diverting scarce resources from adequate treatment of new cases.

However, our study has a few limitations. There might be a selection bias towards patients with no previous history of ATT and using a questionnaire to obtain data regarding family history of TB might have a recall bias.

CONCLUSION

This study reveals fairly a high rate of drug resistance (including MDR) among the new sputum smear positive pulmonary tuberculosis patients. The drug resistance pattern indicates the standard of TB treatment and reflects dissemination of MDR cases within the community. High initial MDR-TB will ultimately lead to a very desperate situation of TB management. A careful use of drugs, supervised standardized treatment, focused clinical, radiological and bacteriological follow-up (from accredited laboratories) are key factors in the successful management of MDR-TB. Since a more novel effective anti-TB drug is still a distant
dream, use of anti-TB drugs should be done judiciously under the standard practices by trained doctors. Without a prescription from a trained doctor, free availability of anti-TB drugs at medical stores must be banned by government. Misuse of quinolones and macrolides should be discouraged. Awareness and effective training of doctors regarding management of tuberculosis should be created. Community awareness programmes should be organized to decrease drug default by patients.

ACKNOWLEDGEMENTS

We gratefully acknowledge Dr. S.P Arya, and Dr. P.C. Gupta of Thakurgunj TB Hospital, Lucknow, U.P. for their valuable support in sample collection and useful suggestions. We appreciate the efforts of the DOTS workers (Mr. Sudheer Awasthi, Mr. Santosh Kumar), the cooperation of the patients and the staff at the DOTS Centres. The study was generously supported by the RNTCP through a research initiative grant.

REFERENCES


PSYCHOSOCIAL TRAUMA OF DIAGNOSIS: A QUALITATIVE STUDY ON RURAL TB PATIENTS’ EXPERIENCES IN NALGONDA DISTRICT, ANDHRA PRADESH

B Venkatraju¹ and Sheela Prasad²

(Received on 3.12.2012; Accepted after revision on 26.4.2013)

Summary

Background: The current tuberculosis (TB) control strategy in India largely ignores psychosocial needs of the patients. The present study was prompted by the recognition that, if TB treatment is to be culturally sensitive and effective, the psychosocial problems and issues need to be recognized and addressed.

Aims: The main aim of this study was to explore psychosocial problems and issues among rural patients being diagnosed with TB.

Methods: 110 respondents who had known about their TB diagnosis less than two months prior to conducting the interviews were recruited purposively from two selected rural TB units at Yadagirigutta and Chintapally in Nalgonda district in Andhra Pradesh (A.P.). Semi-structured interview schedule was used for the collection of primary data. A qualitative content analysis method was employed to analyze and interpret the data. Data analysis was carried out following multi-step procedure that consisted of data reduction, coding and identification of dominant themes.

Results: The diagnosis of TB was generally seen as a shocking and demoralizing experience, and raised a host of social and psychological problems among the patients. Six prominent themes emerged from the in-depth interviews with the respondents: i) worry, ii) disbelief, iii) embarrassment, iv) fear of death, v) fate, and vi) relief.

Conclusion: Effective care for TB requires a much broader approach beyond focusing on anti-tuberculosis drugs and diagnostic techniques. For medical care to be most effective and acceptable to patients, general practitioners should treat both illness and disease in their patients at the same time. Knowledge of the nature of psychosocial problems is crucial for the design of new approaches and methods to improve the quality of life of TB patients.

Key words: Tuberculosis, Worry, Disbelief, Illness, Psychosocial, Biomedical model

INTRODUCTION

In India, TB constitutes a major public health concern, and causes enormous economic and psychosocial burden1-3. In the state of Andhra Pradesh, TB poses a significant health burden with more than 100,000 new cases every year, more than half of them are infectious in nature. Nalgonda district is one of the highest TB burden districts (more than 5000 cases per year) in Andhra Pradesh⁴. Despite the introduction of TB control programmes since 1962 in India, TB still remains a leading killer of economically and reproductively active adults. TB continues to pose serious challenges to clinicians, public health professionals and health policy makers in India. A critical review of the evolution of TB control programmes in India suggests that TB control programmes largely focused on diagnosing and treating TB disease only, and the human and social aspect of care received minimal attention. No effort is made to evaluate patients’ social and psychological experiences under this programme. However, exploring psychosocial problems is particularly relevant in the case of TB, where patients and physicians have divergent perceptions, and concerns about TB care. According to Kleinman (1980)⁵, to provide care that effectively meets the patients’ and their family members’ psychosocial needs, a culturally sensitive clinician not only focuses on disease and its treatment, but on patients’ ideas about what is wrong with them, their fears about illness, and the impact illness has on their physical and psychosocial functioning. Review of literature suggests that there are a few qualitative studies on psychosocial experiences and feelings of rural TB patients upon learning that they were TB infected in...
the state of Andhra Pradesh. Understanding psychosocial problems is crucial for formulating policies, programmes and interventions that provide culturally sensitive supportive care to the patients. For instance, an awareness of psychosocial suffering experiences of patients enables the physician to personalize his/her approach to patient care, and to motivate, inspire or communicate with patients more effectively. The main objective of this study was to study psychosocial reactions of patients to the diagnosis of TB. The present study was prompted by the recognition that if TB treatment is to be culturally sensitive and effective, the psychosocial problems and issues need to be recognized and addressed.

MATERIAL AND METHODS

The field work for this study was conducted during the year 2008-2009 in two selected rural TB Units (Chintapally and Yadagirigutta) of Nalgonda district, Andhra Pradesh, South India. As the aim of the study was to highlight subjective experiences of patients, an explorative qualitative method was chosen for this study. Inclusion criteria of participants were age 18 years and above, and being the resident in the study area. Given the fact that many of the patients were illiterate, oral informed consent was obtained from them before administering the research instruments. Semi-structured interview schedule was used for the collection of data, and in-depth interviews were conducted face-to-face with the patients. The main interview question was ‘what are patient’s subjective experiences of being informed that they are TB infected?’ Interviews with the patients were conducted in native language (Telugu), and interviews were transcribed into English. Data was analysed based on the general guidelines of grounded theory\(^6\). Content analysis method was employed to analyze and interpret the data. Data analysis was carried out following multi-step procedure that consisted of data reduction, coding and identification of dominant themes.

RESULTS

110 TB patients were interviewed within two months of their registration at two TB units, and were recruited using purposive sampling methods. Of the total 110 patients, 72 (65.45%) were smear positive, 27 (24.5%) were smear negative and 11 (10%) were extra-pulmonary cases. The socio-demographic characteristics of the sample are summarized in Table 1.

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. of Patients</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18-25</td>
<td>14</td>
<td>12.8</td>
</tr>
<tr>
<td>26-35</td>
<td>22</td>
<td>20.0</td>
</tr>
<tr>
<td>36-45</td>
<td>26</td>
<td>23.6</td>
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<tr>
<td>46-55</td>
<td>28</td>
<td>25.4</td>
</tr>
<tr>
<td>56-65</td>
<td>16</td>
<td>14.6</td>
</tr>
<tr>
<td>66-72</td>
<td>4</td>
<td>3.6</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>81</td>
<td>73.6</td>
</tr>
<tr>
<td>Female</td>
<td>29</td>
<td>26.4</td>
</tr>
<tr>
<td>Marital Status</td>
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</tr>
<tr>
<td>Married</td>
<td>92</td>
<td>83.6</td>
</tr>
<tr>
<td>Widow/ Widower</td>
<td>10</td>
<td>9.1</td>
</tr>
<tr>
<td>Single</td>
<td>8</td>
<td>7.3</td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-literate</td>
<td>67</td>
<td>61.0</td>
</tr>
<tr>
<td>Primary</td>
<td>26</td>
<td>23.6</td>
</tr>
<tr>
<td>Secondary</td>
<td>14</td>
<td>12.7</td>
</tr>
<tr>
<td>College</td>
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<td>2.7</td>
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<tr>
<td>Occupation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Agriculture</td>
<td>38</td>
<td>34.5</td>
</tr>
<tr>
<td>Labour</td>
<td>33</td>
<td>30.1</td>
</tr>
<tr>
<td>Self-employed</td>
<td>24</td>
<td>21.8</td>
</tr>
<tr>
<td>Private employee</td>
<td>6</td>
<td>5.4</td>
</tr>
<tr>
<td>Student</td>
<td>2</td>
<td>1.8</td>
</tr>
<tr>
<td>Others</td>
<td>7</td>
<td>6.4</td>
</tr>
</tbody>
</table>

Table 1: Socio-demographic characteristics of the patients (n=110)
Respondents were asked to mention what they perceived was the single most troublesome psychosocial problem or issue upon being informed that they had TB. All the patients focused on one theme as the most important psychosocial concern. One of the possible reasons for this answer could be that patients may have interpreted the question they were asked as seeking a single, major concern, and obliged with a single concern, irrespective of what they thought. Six major themes emerged from the analysis of data which were considered to be of major importance in the lives of respondents: worry/depression (37.3%), disbelief/shock (23.6%), embarrassment/shame (16.4%), fear of dying (12.7%), fate/God’s act (9%), and relieved that it was just TB (0.9%). The initial psychosocial reactions to the diagnosis of TB are presented in Table 2.

### DISCUSSION

Study findings clearly suggest that patients are concerned more about psychosocial problems than patho-physiological problems associated with TB. The diagnosis of TB was generally seen as demoralizing experience, and it raised a host of social and psychological problems including depression, anxiety, low self-esteem, fear of death, loneliness, helplessness, shame, shock, fear of spreading disease to family members, social stigma or future of children. A fear of disease relapse is also the most common fear noted among the patients. Statements made by patients bear eloquent witness to the social and psychological ravages of the disease. In the following paragraphs some major psychosocial problems experienced by patients themselves are discussed in more detail below:

#### Worry/Depression

37.3% patients expressed worry/depression as the immediate reaction when they were informed of their TB infection. Loss of self esteem, fear of spreading the disease to family members, future of children, loss of family support, physical and sexual violence by partners, social discrimination, verbal abuse, ending of marital relationships, disownment, powerlessness, fear of social isolation, impact on marriage prospects or stigma were cited as major worries of having TB. Patient’s worries seem to be justified in light of various accounts told by them during the interview. Examples of patients’ worries are reflected in the following statements:

*I was worried about how the disclosure of my TB infection would affect my personal relationships within the family and community, particularly with my in-laws and husband. I was really worried about what their reactions would be. You know, I cried and cried.*

(32-year-old woman)

*It was an earth shattering experience. My major concern was my new born baby, aged 9 months. I was concerned for my young kid. Can I give milk to my son? I kept thinking what if my kid contracts the disease from me? Will the kid survive from this disease? Oh, my God, what did I do to deserve it? You know, I cried quite a lot by myself. This is the worst moment in my entire life.*

(29-year-old woman)

*You know, I got TB by using a plate that had been used by my late father, who died of TB. I am very much worried that my children may contract TB from me if they come in contact with my clothes, plates, glasses, bed sheets, bathroom or saliva.*

(58-year-old male)

---

**Table 2: Initial reaction to initial diagnosis among TB patients (n=110)**

<table>
<thead>
<tr>
<th>Reaction to Diagnosis</th>
<th>No. of Patients</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Worry/Depression</td>
<td>41</td>
<td>37.3</td>
</tr>
<tr>
<td>Disbelief/Shock</td>
<td>26</td>
<td>23.6</td>
</tr>
<tr>
<td>Embarrassment/Shame</td>
<td>18</td>
<td>16.4</td>
</tr>
<tr>
<td>Fear of death</td>
<td>14</td>
<td>12.7</td>
</tr>
<tr>
<td>Fate</td>
<td>10</td>
<td>9.1</td>
</tr>
<tr>
<td>Relief</td>
<td>1</td>
<td>0.9</td>
</tr>
</tbody>
</table>
My husband is an alcoholic, and often forces me to have sex with him. I am very much worried about his abusive nature. But, you know, it is very dangerous to have sex while suffering from this dreaded disease. Such an act (sex) could kill me and him. But, he will suspect my fidelity if I refuse to have sex with him. What can I do? You know, it is really shameful for me. This issue has become a big worry in my life

(43 year-old woman)

I am very much worried because my husband is a chronic alcoholic and is very abusive. He will neglect me badly if he comes to know about my condition. You know, I am staying in my mother's house ever since I was diagnosed with TB. I was worried for my poor children. I'm all they got and I was quite concerned about what will happen to them if I die.

(26-year-old woman)

If I go out, people will ask me several questions. I am anxious that they might ask me why I had become so thin and skinny. I am nervous that they might think that I am suffering from TB and AIDS

(36-year-old male)

If my disease status becomes public knowledge, then people may gossip about my health condition, and may spread the news around. I am afraid about my marriage prospects.

(19-year-old girl)

Disbelief/Shock

23.6% of respondents expressed disbelief on being told about their diagnosis of TB. When asked ‘Did you ever think that you could be at risk for TB disease?’, more than 90% of the patients said “No”. The patient narratives aptly demonstrate that past history of TB in the family, sexual promiscuity, and alcohol consumption constitute important predisposing risk factors for TB. Furthermore, many patients equated TB disease with chronic cough with sputum/blood, wastage of muscle and skeletal bones, skinny body, and as these symptoms did not match the initial symptoms experienced by these patients, they reported feelings of disbelief at hearing news of TB diagnosis. Such perception was echoed in the following accounts told by the patients:

I felt complete disbelief. I kept asking myself, why me? I thought that it would never happen to me. I never suspected that I can have TB for two important reasons. First, there was no one with TB in my family. Second, I didn't cough up blood/sputum, and I didn't develop skinny body. It was hard to accept (diagnosis). At first, I did not believe that I got TB. In fact, I went to another doctor. And then it was confirmed

(35-year-old male)

It came as a total disbelief. I was never a drinker and I don't smoke. I hadn't indulged in sex outside marriage. I was a fit person. How could I possibly get this disease? I kept thinking 'why me'? I always thought at the back of my mind that it wasn't TB. You know, this is really a shocking news for me. It took me sometime to accept that I have TB

(22-year-old male)

Embarrassment/Shame

16.3% patients reported that they became embarrassed (ijjath) upon discovering they had TB. Findings from the interview suggest that feelings of guilt, humiliation and shame were very common in afflicted patients. Infected individuals considered themselves suffering from a shameful disease. Illustrations of such beliefs included:

You know, in my society, people generally avoid TB patients because it is a dirty disease and highly infectious. People normally say that, Oh, this person has TB, and better not interact closely with him/her. This is really embarrassing.

(18 year-old girl)

If you've got heart disease or some other illnesses like that, people don't keep away from you. The minute they hear that you are a TB patient, they try to avoid you. For example, they may not give you water or food whenever you go to their home, and also they might not invite you for family functions. People think less of you and look down upon you, it is simply because of your low status in the community that they feel you got TB. I had restricted my social activities considerably, out of shame (ijjath) and fear that others would discover that I am a TB patient.

(26-year-old woman)
Fear of death

12.7% of respondents reported that they immediately sensed imminent death on learning the diagnosis. Interviews with the respondents revealed that almost all of them had seen or heard some patients dying from TB in the past. Such information has acted as a powerful force to promote fear of illness and death among the patients. Patients feared that there would be no one to support their children in the event of their death. An example of fear of death was very evident from the below account of a patient:

I immediately felt death. In the past I watched patients dying from this horrible disease in my village. It really terrifies me. But, I don’t want to die. I have the same dreams and aspirations as every father has. You know, things like sending children to school, taking better care of kids, etc. But, I felt I can’t fulfill my dreams, as I am physically and mentally very ill. I felt very guilty about that. I became thin and completely bedridden. Death is inevitable. I will never see my children again. I wonder, you know, what’s going to happen to my children after my death.

(33-year-old male labourer)

Fate/God’s act

9% of respondents turned to fatalism in accepting their diagnosis. These patients mentioned that some health issues are beyond human control on the basis of certain views about luck, fate or destiny. Elderly patients, in general, described a feeling of fatalism at hearing the diagnosis. They reported occurrence of TB as God’s will or the way God meant for them to die. Examples include:

I think it’s God’s will. There is nothing we can do about it. Can we? I have to die of something. To tell you the truth, if you’re going to get it, I think you’re going to get it. It is not in our hands. Death and disease are inevitable parts of life. Whatever is going to happen, will happen. We can’t do much about it, can we?

(65-year-old male)

Relief

One patient described a sense of relief upon learning the diagnosis after a long period of confusion and uncertainty. He mentioned that his whole family had been tormented for more than one year without a proper diagnosis. He said:

When I was diagnosed as having TB, I felt some kind of relief to learn that it was TB rather something worse (paralysis, cancer?). I suspected a tumour on my spine was something very serious. Luckily, it wasn’t as serious a disease as I thought to be. TB is a curable disease. It gives me a sense of relief and hope.

(55-year-old male)

CONCLUSION

In conclusion, it can be argued that in order to provide psychological support and quality care to the patients, health policy planners and health professionals need to recognize that factors such as fears, anxieties, and other social and emotional problems associated with TB are genuine and should be incorporated for clinical management. For instance, major emphasis on the treatment of disease without considering the illness dimension may cause dissatisfaction among the patients, may lead to non-compliance, self-medication, non-disclosure of TB infection, dissatisfaction with treatment, patient-physician miscommunication, delays in seeking treatment, or consultation with the unqualified practitioners who are more willing to deal with the illness dimension. TB control policies need to focus on the provision of psychosocial health services as an integral part of TB care. This study contributes in a modest way towards understanding the limitations of the biomedical model. The biomedical approach to TB treatment is extremely important, as long as its limitations are recognized. The findings of the study make a case for a new type of practitioner who is reflexive and culturally sensitive, caring and supportive. This is because biomedical model’s assumptions, such as physical reductionism, mind-body dualism and mechanical metaphor cannot provide a complete understanding of human suffering. To treat TB patients effectively, the medical
practitioners approach should be a holistic one, in the sense that the whole person should be taken into account, not simply the diseased organ of the human body which is the main focus of the biomedical practitioners. In other words, for medical care to be most effective and acceptable to patients, general practitioners should treat both illness and disease in their patients at the same time. Today, public health care providers and planners are challenged to employ innovative methods in treating TB patients. TB is more than just a biomedical phenomenon. It maintains its grip on those human populations already suffering from poverty, overcrowded living conditions, inadequate housing, malnutrition, and lack of access to medical care. Thus, any TB control programme needs to therefore move beyond ‘medicalization of the disease’, to include the socio-cultural and psychological dimensions that impact the disease and its treatment. Health policy planners and health care workers need to recognize that understanding the psychosocial world-view of patients would provide important inputs for any effective treatment and control of the disease. This qualitative study has one major limitation. The samples were not selected randomly, and hence, they are not statistically representative of populations beyond them. However, the findings of this study provide valuable information for planning culturally sensitive health care and education programmes for rural TB patients.

ACKNOWLEDGEMENTS

We wish to express our heartfelt thanks to the TB patients, their family members, villagers, traditional healers, health care workers and TB supervisors (Venkateswarlu, Anjamma), who participated in this study. Heartfelt thanks are due to Prof. Laxmi Lingam, Deputy Director, Tata Institute of Social Sciences, Hyderabad, for her support and constant encouragement for publication of articles and research work. We would like to express our thanks to Joint Director (TB), Andhra Pradesh and his staff who provided support and co-operation.

REFERENCES

TRENDS IN THE PREVALENCE OF PULMONARY TUBERCULOSIS OVER A PERIOD OF SEVEN AND HALF YEARS IN A RURAL COMMUNITY IN SOUTH INDIA WITH DOTS*

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Summary
Setting: Tiruvallur district In Tamil Nadu where DOTS was implemented by the State Government as the tuberculosis control measure in 1999, and monitored by the National Institute for Research in Tuberculosis for over five years.

Objective: To estimate trends in TB prevalence in a rural community with DOTS.

Design: Surveys of pulmonary tuberculosis were undertaken in representative samples of subjects aged ≥15 years (N = 83,000 – 92,000), initially and after two and half, five and seven and half years of implementation of DOTS. Sputa were collected from those with abnormal radiograph and/or presence of chest symptoms, and examined by direct smear and culture.

Results: The prevalence of culture-positive tuberculosis was 607, 454, 309 and 388 per 100,000 in the four surveys, and that of smear-positive tuberculosis was 326, 259, 168 and 180. In the first five years; annual decrease was 12.4% (95% CI 10.4 - 14.4%) for culture-positive tuberculosis, and 12.2% (95% CI 8.0–16.2) for smear-positive tuberculosis. This was, however, followed by a significant increase in the next two and half years. The average new smear-positive case-notification rate was 75 per 100,000 during first four years but declined to 49 in subsequent years. There were no methodological differences during this period and information on changes in socio-economic indicators and nutritional standards was unavailable.

Conclusion: Despite the average annual success rate (78%) in this tuberculosis unit being lower than the expected rate of 85%, the implementation of DOTS was followed by a substantial decrease in the prevalence of pulmonary tuberculosis over the seven and half year period. Our findings suggest that sustaining the high effectiveness of DOTS programme needs vigilant supervision.

Key words: Tuberculosis, DOTS, Prevalence trends

INTRODUCTION

The WHO 2012 Global Tuberculosis Control Report reveals that there were 1.4 million estimated deaths due to tuberculosis in 2011, of which 300,000 were in India.¹ In 1997, the ‘Directly Observed Treatment Supervised’ (DOTS)-based Revised National Tuberculosis Control Programme (RNTCP) was initiated in India.² This programme, as recommended by WHO, was implemented by the Tamil Nadu Government in the peri-urban district of Tiruvallur, south India, in 1999. To investigate the impact of DOTS implementation, periodic disease prevalence surveys were conducted. The findings of the baseline survey and the resurveys at two and half and five years have been reported earlier.³⁵ A further survey was conducted at seven and half years, and its findings are described here.

MATERIAL AND METHODS

Study area and population

Our study was conducted in a TB Unit (covering 500,000 predominantly rural population) in Tiruvallur area. A stratified cluster sampling design was employed ³. A simple random sample of 50 villages was selected from each of the five blocks, proportionate to the census population, and three towns were selected in a similar manner. In all the...

All persons aged ≥ 15 years (N = 83,000-92,000) in the four surveys were registered by door-to-door census. The resurvey activity comprises updating the census data through registration of new entrants (new born, settlers and persons missed in the previous survey) in the study population. Specially trained field investigators interviewed all persons in the selected sites at home. A quality check on symptom screening was done by a supervisor on a random sample of 5% of subjects.

All persons were also screened by a chest radiograph (MMR X-ray) for tuberculosis. The radiograph was read independently by two readers and, in case of disagreement, by a third reader. For those with an abnormal chest radiograph and/or chest symptoms or previously diagnosed cases in the earlier surveys, two sputum samples were collected and examined by fluorescent microscopy and culture for *Mycobacterium tuberculosis*. All study subjects were informed of the purpose of the survey, and their written consent was obtained. The Institutional Ethics Committee approved the study.

Treatment in study area

All TB patients diagnosed were treated as per the national policy. National technical guidelines were followed.

Case notification rate

The projected populations were estimated from the 2001 census population and decadal growth rate of 35.3% of Tiruvallur district. Thereafter, the case notification rate (CNR), defined in the RNTCP as the number of newly reported smear-positive TB cases per 100,000 population, was determined.

Treatment success

This was defined as the percentage of new smear-positive PTB cases registered under RNTCP for treatment who were cured or had completed the full course of treatment.

Estimation of the number of cases in subjects with no sputum/radiograph

The number of sputum-positive cases among those who did not have sputum collected was estimated from the nature of the radiographic abnormality by utilizing the probability of a positive finding in the appropriate radiographic category in the corresponding survey. To estimate the number of cases among those with no radiograph, the relative risk (RR) of a person with no radiograph having chest symptoms (compared to a person with a radiograph) was taken as a proxy for the RR of this person being a case of tuberculosis. This risk was homogenous in the four surveys (P > 0.3), and the common estimate was 0.6 for males and 0.4 for females. Details of both adjustments have been published earlier.4,5

Data analysis and Statistical methods

The population in each selected cluster was stratified by age (15-34, 35-54, ≥ 55 years) and sex, the prevalence estimated, and standardized by the ‘Direct’ method, using the baseline survey population in the same cluster as the ‘Standard’. The overall prevalence for each survey was then computed as a weighted average of the cluster prevalences, the weight being the corresponding population size. Next, the variance of the prevalence was estimated allowing for varying size of the clusters and stratification by blocks. Finally, a weighted regression (linear and quadratic) of prevalence on time was undertaken, and the univariate and multivariate correlation (r and R) were determined, using the SPSS version 14.0 (SPSS version 14.0 Chicago, IL, USA). Fuller details of data analytical methods have been described in earlier reports.3,5,8

RESULTS

Numbers in study and proportions investigated

Eligible subjects in the four surveys varied from 83,000 to 92,000. Males constituted 49% of the study sample in all the surveys (Table 1). As regards age, there were fewer young adults (15-34 years) in the resurveys at five and seven and half...
years (48%, 47%) than at the baseline and first resurvey (50%). The proportion investigated for various examinations was consistently high - about 90% for chest radiography and for symptom inquiry, and at least 95% for sputum examination.

**Cases of pulmonary tuberculosis detected**

The numbers of culture-positive cases detected in the four surveys were 457, 344, 253 and 332, respectively (Table 1). Of these, 80% to 83% were males (P = 0.7). The age profile also was similar in the four surveys (P = 0.6).

The corresponding numbers of smear-positive cases detected in the four surveys were 245, 196, 136 and 155, respectively (Table 1). The proportion of males varied between 79% and 85% (P = 0.7). Again, the age profile was similar in the four surveys (P = 0.7).

**Table 1**: Number of eligible persons examined and number of tuberculosis cases detected

<table>
<thead>
<tr>
<th></th>
<th>Baseline Survey</th>
<th>2½-year survey</th>
<th>5-year survey</th>
<th>7½-year survey</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.  %</td>
<td>No.  %</td>
<td>No.  %</td>
<td>No.  %</td>
</tr>
<tr>
<td>Number examined</td>
<td>83425 100</td>
<td>85474 100</td>
<td>89413 100</td>
<td>92255 100</td>
</tr>
<tr>
<td>Male</td>
<td>40848 49.0</td>
<td>41607 48.7</td>
<td>43477 48.6</td>
<td>44996 48.8</td>
</tr>
<tr>
<td>Female</td>
<td>42577 51.0</td>
<td>43867 51.3</td>
<td>45936 51.4</td>
<td>47259 51.2</td>
</tr>
<tr>
<td>15 - 34 years</td>
<td>42118 50.5</td>
<td>43138 50.5</td>
<td>43044 48.1</td>
<td>43702 47.4</td>
</tr>
<tr>
<td>35 - 54 years</td>
<td>27141 32.5</td>
<td>28199 33.0</td>
<td>30567 34.2</td>
<td>32480 35.2</td>
</tr>
<tr>
<td>&gt; 55 years</td>
<td>14166 17.0</td>
<td>14137 16.5</td>
<td>15802 17.7</td>
<td>16073 17.4</td>
</tr>
<tr>
<td>Culture-positive cases</td>
<td>457 100</td>
<td>344 100</td>
<td>253 100</td>
<td>332 100</td>
</tr>
<tr>
<td>Male</td>
<td>381 83</td>
<td>276 80</td>
<td>204 81</td>
<td>276 83</td>
</tr>
<tr>
<td>Female</td>
<td>76 17</td>
<td>68 20</td>
<td>49 19</td>
<td>56 17</td>
</tr>
<tr>
<td>15 - 34 years</td>
<td>73 16</td>
<td>63 18</td>
<td>35 14</td>
<td>46 14</td>
</tr>
<tr>
<td>35 - 54 years</td>
<td>178 39</td>
<td>137 40</td>
<td>99 39</td>
<td>142 43</td>
</tr>
<tr>
<td>&gt; 55 years</td>
<td>206 45</td>
<td>144 42</td>
<td>119 47</td>
<td>144 43</td>
</tr>
<tr>
<td>Smear-positive cases</td>
<td>245 100</td>
<td>196 100</td>
<td>136 100</td>
<td>155 100</td>
</tr>
<tr>
<td>Male</td>
<td>209 85</td>
<td>165 84</td>
<td>109 80</td>
<td>127 82</td>
</tr>
<tr>
<td>Female</td>
<td>36 15</td>
<td>31 16</td>
<td>27 20</td>
<td>28 18</td>
</tr>
<tr>
<td>15 - 34 years</td>
<td>37 15</td>
<td>39 20</td>
<td>19 14</td>
<td>27 17</td>
</tr>
<tr>
<td>35 - 54 years</td>
<td>101 41</td>
<td>78 40</td>
<td>60 44</td>
<td>70 45</td>
</tr>
<tr>
<td>&gt; 55 years</td>
<td>107 44</td>
<td>79 40</td>
<td>57 42</td>
<td>58 37</td>
</tr>
</tbody>
</table>
Prevalence of pulmonary tuberculosis

The prevalence of culture-positive tuberculosis was 607 per 100,000 at the baseline survey and decreased significantly to 454, 309 and 388 per 100,000 at two and half, five and seven and half years (Table 2). Regression analysis showed that a linear model was inadequate to explain the variation in prevalence with \( r^2 = 0.59 \), and that a quadratic model improved the fit significantly (\( P < 0.001 \)) and substantially with \( R^2 = 0.93 \) (Fig. 1). The findings with smear-positive tuberculosis showed the same pattern (Fig. 1), the prevalence being 326, 259, 168 and 180 per 100,000 (Table 2); and the corresponding values of \( r^2 \) and \( R^2 \) were 0.81 and 0.95, respectively, the quadratic fit being significantly better (\( P=0.02 \)).

Confirming the appropriateness of the quadratic model, further analysis showed that the prevalence of culture-positive tuberculosis declined steadily in the first five years by 12.4% per annum.

Table 2: Prevalence of culture-positive/smear-positive tuberculosis by sex and age (per 1,00,000 population)

<table>
<thead>
<tr>
<th>Pulmonary tuberculosis</th>
<th>Group</th>
<th>Baseline Survey</th>
<th>2.5 years*</th>
<th>5 years*</th>
<th>7½ years*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture-positive</td>
<td>Total</td>
<td>607</td>
<td>454</td>
<td>309</td>
<td>388</td>
</tr>
<tr>
<td></td>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>1043</td>
<td>752</td>
<td>513</td>
<td>665</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>189</td>
<td>168</td>
<td>114</td>
<td>122</td>
</tr>
<tr>
<td></td>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>15 - 34</td>
<td>194</td>
<td>168</td>
<td>95</td>
<td>118</td>
</tr>
<tr>
<td></td>
<td>35 - 54</td>
<td>742</td>
<td>546</td>
<td>360</td>
<td>487</td>
</tr>
<tr>
<td></td>
<td>= 55</td>
<td>1576</td>
<td>1129</td>
<td>849</td>
<td>999</td>
</tr>
<tr>
<td>Smear-positive</td>
<td>Total</td>
<td>326</td>
<td>259</td>
<td>168</td>
<td>180</td>
</tr>
<tr>
<td></td>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>572</td>
<td>448</td>
<td>278</td>
<td>305</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>89</td>
<td>77</td>
<td>62</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>15 - 34</td>
<td>99</td>
<td>102</td>
<td>51</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td>35 - 54</td>
<td>420</td>
<td>312</td>
<td>220</td>
<td>236</td>
</tr>
<tr>
<td></td>
<td>= 55</td>
<td>817</td>
<td>623</td>
<td>416</td>
<td>410</td>
</tr>
</tbody>
</table>

Table 2: Prevalence of culture-positive/smear-positive tuberculosis by sex and age (per 1,00,000 population)

* The prevalence at 2½, 5 and 7½ years was standardized by sex and age to the baseline survey population.
Figure 1: Model-fitting to the prevalence of culture-positive (C+)/smear-positive (S+) PTB per 100,000 population

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(95% CI: 10.4 - 14.4%), but increased significantly thereafter to 388 per 100,000 at seven and half years (P < 0.005). This pattern was seen in both sexes and in all three age-groups. Similar findings were observed for the prevalence of smear-positive tuberculosis, namely, a significant decrease from 326 to 168 per 100,000 at five years at the rate of 12.2% (95% CI: 8.0 - 16.2%) per annum, followed by a slight increase to 180 per 100,000 at seven and half years (P = 0.3). Next, analysis was undertaken by radiographic and chest symptom status (Table 3). In all the groups, the same pattern was seen.

Had the declining survey prevalence in the first five years persisted, only 127 smear-positive cases should have been expected at seven and half years, but the actual number observed was 180 (Fig. 2). The corresponding figures for culture-positive tuberculosis are also shown in Fig. 2.

Table 3: Prevalence of sputum-positive tuberculosis by chest symptom status and radiographic status

<table>
<thead>
<tr>
<th></th>
<th>Abnormal radiograph with chest symptoms</th>
<th>Abnormal radiograph with no chest symptoms</th>
<th>Normal radiograph with chest symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Specimen</td>
<td>Culture-positive</td>
<td>Smear-positive</td>
</tr>
<tr>
<td>Baseline survey</td>
<td>1030</td>
<td>165</td>
<td>16.0</td>
</tr>
<tr>
<td>Resurvey at 2% years</td>
<td>1251</td>
<td>163</td>
<td>13.0</td>
</tr>
<tr>
<td>Resurvey at 5% years</td>
<td>1317</td>
<td>120</td>
<td>9.1</td>
</tr>
<tr>
<td>Resurvey at 7% years</td>
<td>1131</td>
<td>144</td>
<td>12.7</td>
</tr>
<tr>
<td>Independent survey at 7% years</td>
<td>457</td>
<td>49</td>
<td>10.7</td>
</tr>
</tbody>
</table>

* Excluded from analysis as the numbers are very small.

Figure 2: Prevalence of culture-positive/smear-positive tuberculosis and Smear-positive Case Notification Rate (per 1000,000 population) in Tiruvallur district, where the DOTS strategy was initiated in 1999 (the dotted line indicates the extrapolated prevalence at the seven and half year survey, assuming that the decreasing trend observed in the first five years would persist)
positive cases were 228 and 388, respectively. Over the seven and half year period, the observed average annual decrease was 5.8% (from 607 to 388) for culture-positive tuberculosis and 7.6% (from 326 to 180) for smear-positive tuberculosis.

Standards of taking symptom history / reading radiographs / laboratory investigations

The proportion of subjects eligible for sputum examination was very similar in the four surveys (12%, 13%, 13%, and 11%). The average agreement between duplicate readers in identifying a radiographic abnormality (among those with an abnormality by one or other reader) was 70%, 69%, 63% and 65%, respectively. As regards bacteriological standards, the proportion with a smear-positive culture-negative result was stable, namely, 2.5, 2.1, 1.9 and 2.0 per thousand in the four surveys (P = 0.6); furthermore, among those with a positive smear result, the proportions not confirmed by culture were 12.3%, 15.8%, 22.1% and 19.4%, respectively (P > 0.05). Finally, the proportion with contaminated culture was 4% or less in all surveys except the first where it was 6%. These findings indicate that, the clinical, radiographic and bacteriological standards were all stable over the seven and half year period of follow-up.

RNTCP performance

The RNTCP case-finding performance in this area during the period of study is presented in Table 4. The newly notified smear-positive cases which ranged from 386 to 449 per annum until 2004 (average 420) declined between 2005 and 2008 to an average of 272 (P< 0.001), a decrease of 35%. The corresponding Case Notification Rates (CNRs) were 75 and 49 per 100,000 (P< 0.001). The success rate varied from 73% to 86% (average: 78%), but showed no consistent trend; the averages in the two periods were similar, namely, 79% and 76%, respectively (P=0.36).

DISCUSSION

This report summarizes the situation at the start of the DOTS-based RNTCP programme in Tiruvallur district in south India, and makes comparisons between a series of consecutive disease prevalence surveys conducted in the area. The prevalence of tuberculosis nearly halved in the first five years, the annual decline being 12.4% for culture-positive tuberculosis and 12.2% for smear-positive tuberculosis. This was substantially more than 2.3% and 2.5% observed over three decades in the pre-DOTS era in the same area. It has also been reported that the prevalence of tuberculous infection among
unvaccinated children aged 1-9 years declined during this period from 19.4% to 11.4%, at the rate of 5.2% per annum (95% CI: 3.6-6.8%). There were no methodological differences during this period and information on changes in socio-economic indicators and nutritional standards was unavailable. It is concluded that the substantial declines observed are largely due to the implementation of the DOTS strategy under RNTCP, coupled with efficient case-finding in the community.

However, in the next two and half years, the prevalence increased to 388 per 100,000 for culture-positive cases and to 180 for smear-positive cases. Confirmation of the higher prevalence came from the finding of an independent random sample survey at seven and half years (on 41773 representative subjects from 39 other villages in the DOTS area), which yielded a culture-positive prevalence of 363 and smear-positive prevalence of 201 per 100,000; the corresponding values standardized to the baseline population were 340 and 184, respectively. It could be argued that the prevalence at five years happened to be unusually low and that at seven and half years was rather high, and that both are consistent with an explanation of a ‘steady’ decrease over the seven and half-year period. This explanation may, however, be rejected as a model-fitting exercise showed that a linear model was unable to explain the variation in the prevalence.

Detailed analysis was undertaken to check whether the increased prevalence in the last survey could be due to changes in the screening standards adopted, the bacteriological procedures employed, or variations in the sex-age composition of the population studied. These showed that the proportion of subjects eligible for sputum examination was similar in the four surveys, and so was the average agreement between readers in screening for radiographic abnormality. Further, the frequency of smear-positive culture-negative results and the proportion of specimens yielding a contaminated culture were fairly stable throughout. The gender profile was also constant, and although there were some differences in the age composition, with fewer young adults (15-34 years) in the five-year and seven and half-year surveys, these were allowed for by the statistical technique of standardization. Taken together, these findings indicate that the significant increase in prevalence between five and seven and half years was not due to any differences in methodology.

An increase in the incidence could be a possible explanation (the incidence was not measured in this study), but this is unlikely as the potential risk factors for tuberculosis such as tobacco smoking, alcohol use, biomass use, lower socio-economic status, HIV prevalence and MDR TB in the community have not been reported to have changed from the earlier five-year survey period. Other theories for the increase at seven and half years are that a large outbreak of tuberculosis might have occurred during the seven and half-year survey or that the immigration patterns had changed during this period. No evidence was, however, available on either aspect.

Another hypothesis is an increase in the average duration of illness which could have resulted from a number of operational factors involved in the implementation of the control programme. These factors include greater proportion of undetected cases remaining in the community, as suggested by the decreasing CNRs in the later years of the study period. It is likely that such undetected cases persisted in the community and were only discovered at a later survey, resulting in increased survey prevalence at seven and half years (Figure 2). Other possible causes are increased default and greater irregularity in treated cases, but information on these aspects was not available. In this context, it might be relevant to point out that the NIRT supervised the implementation in the first five years, but thereafter the Tamil Nadu Government took over. The STO and STLS from the research organization were withdrawn, and State government personnel were appointed in their place.

Considering experiences elsewhere, DOTS was implemented during 1991-2000 in approximately half of China’s population, and the decline in the smear-positive prevalence of pulmonary tuberculosis was 5.7% per annum. In the Philippines where DOTS was initiated in 1997, a national sample survey conducted 10 years later demonstrated a 31% reduction in culture-positive prevalence, and a 27% reduction in smear-positive prevalence;11 the latter corresponds to an average annual decrease of 3.1% compared with 7.6% in our study area and 5.7% in China. In New

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York city, a reduction of 21% in new cases was reported over two years, important contributory factors being implementation of supervised treatment and improved infection-control measures. A nationwide programme in Peru showed that decline in tuberculosis incidence almost doubled between 1991 and 1999 through the implementation of DOTS. Lastly, a community-based DOTS approach in Baltimore resulted in the incidence declining from 36 to 17 per 100,000 in 11 years.

One-time baseline prevalence surveys have recently been undertaken in a rural population near Bangalore and in Madhya Pradesh tribals; the estimates were 152 and 207 per 100,000, respectively, for prevalence of culture-positive tuberculosis based on symptom screening alone, and 198 per 100,000 in Bangalore when radiographic screening was also taken into account.

Limitations

Detailed data about performance indicators were not available to verify if the increased prevalence at seven and half years could be attributed to poorer programme implementation. Information on socio-economic change in the community was also not available and so its possible impact could not be assessed. Finally, since HIV infection and MDR TB were not highly prevalent in the study area, our conclusion cannot be generalized to all areas, especially to those with a high prevalence of HIV infection or MDR TB.

CONCLUSION

Despite the average annual success rate (78%) in this tuberculosis unit being lower than the expected rate of 85%, the implementation of DOTS was followed by a substantial decrease in the prevalence of pulmonary tuberculosis over five years in a rural population, but this was partially off-set by an increase in the next two and half years. Although the average annual decline over the seven and half-year period was still significant, our findings suggest that sustaining the high effectiveness of DOTS programme needs vigilant supervision.

REFERENCES

POST-OPERATIVE SINUS FORMATION DUE TO *MYCOBACTERIUM ABSCESSUS*: A CASE REPORT

Mehvash Haider, Priyanka Banerjee, Tavleen Jaggi, Jasmin Husain, Bibhabati Mishra, Archana Thakur, Vineeta Dogra and Poonam Loomba

(Received on 18.9.2012; Accepted after revision on 15.4.2013)

**Summary:** *Mycobacterium abscessus* is ubiquitously found rapidly growing mycobacteria. Although it is an uncommon pathogen, it has been known to cause cutaneous infection following inoculation, minor trauma or surgery. This communication reports an immuno-competent patient developing multiple sinuses due to *Mycobacterium abscessus* in the post-operative period. [Indian J Tuberc 2013; 60: 177-179]

**Key words:** Non-tuberculous mycobacteria, Infectious Skin Disease, Infection

**INTRODUCTION**

The species of rapidly growing mycobacteria (RGM) capable of producing disease in humans consists primarily of the *Mycobacterium fortuitum* group, the *M. chelonae/abscessus* group, and the *M. smegmatis* group. *Mycobacterium abscessus* has been associated with chronic lung infection, localized post-traumatic wound infection, surgical wound infection, chronic otitis media and catheter infections in normal hosts and disseminated skin infections in immuno-compromised hosts. A case of post-operative cutaneous infection by *Mycobacterium abscessus* in a young female is reported here.

**CLINICAL RECORD**

A 40-year-old immuno-competent female underwent laproscopic cholecystectomy for acute cholecystitis. The operation was uneventful. Three weeks later, she developed port site granuloma with persistent seropurulent discharge (Figure 1). Empirical oral antibiotics were started but provided no relief. In view of no response to antibiotic therapy, the wound was explored three months post-surgery and a biopsy was taken. Histopathological examination of the biopsy reported an acute on chronic non-specific inflammation but bacterial culture was negative. The seropurulent discharge persisted. Multiple pus cultures were sent and antibiotics were administered empirically. As there was no improvement with antibiotics, empirical four-drug Anti Tuberculosis Treatment (ATT) was started. Ultrasonograpy and CT abdomen showed soft tissue edema but no intraperitoneal

**Figure 1:** Port site infection
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communication or collection was seen. The wound was re-explored again after three months of ATT during which a pus collection was found in rectus sheath extending up to falciform ligament, which was drained. Cultures grew skin flora. Tissue biopsy showed granulomatous inflammation but bacterial cultures were sterile. Fungal culture was also sterile. Few acid fast bacilli were seen on Ziehl Neelsen staining of pus (Figure 2). PCR for mycobacteria came out to be negative. Rapid mycobacterial culture yielded Mycobacteria other than Tuberculosis (MOTT) on BacT/Alert MB 3D automatic culture system on two separate occasions. The isolates were identified by Hains test to be Mycobacterium abscessus. Mycobacterium abscessus was sensitive to Amikacin, Clarithromycin and Linezolid. ATT was stopped and patient started on i/v Amikacin for one month along with Clarithromycin, after which Clarithromycin alone was continued. The patient was HIV negative and lung fields were clear on chest X-ray. Therapy was monitored with ESR, hscRP, KFT and CT scan. The discharging sinuses improved and healed completely by the end of six months of Clarithromycin therapy.

DISCUSSION

*M. abscessus* belongs to the group of rapidly growing non-tuberculous mycobacteria which is characterized by a rapid growth (within seven days) on the culture. The organism is ubiquitously present in soil, decaying vegetation, and water (e.g., natural water, sewage water, drinking water tanks, and tap water). *M. abscessus* causes a wide range of clinical diseases including skin and soft-tissue infection, keratitis, osteomyelitis, pulmonary infection, and disseminated infection. Skin and soft-tissue infection usually follows penetrating trauma and typically occurs in immunocompetent individuals. There are several reports of outbreaks of *M. abscessus* infections caused by non-sterile techniques or contaminated material, following surgery, liposuction, foreign body implantation, mesotherapy, acupuncture, and soft tissue augmentation. Although it is difficult to definitely ascertain the source of infection in the present case, the temporal association between surgery and infection is highly suggestive that infection is iatrogenic. The clinical presentations of cutaneous *M. abscessus* infection ranges from asymptomatic to tender erythematos violaceous nodules and plaques, cellulitis, abscesses, ulcer, and sinus with serosanguinous discharge. As the symptoms are relatively mild and indolent, clinical diagnosis of mycobacteriosis is often delayed as in the present case. In contrast to other pyogenic bacterial infections with a shorter incubation period, infections due to rapidly growing mycobacteria have longer incubation periods (several days to several months). It took around six months from initial manifestation to diagnosis, which was also observed in a previous study. This suggests that careful monitoring is required for early diagnosis and appropriate treatment.

The treatment of such infection depends on the extent of the disease and the underlying immune status of the host. *M. abscessus* is usually resistant to conventional anti-tuberculous drugs, and it is generally susceptible to parenteral therapy with amikacin, cefoxitin, imipenem and to oral medication with Clarithromycin. As treatment usually extends for three to six months, oral Clarithromycin is considered
to be the first-line agent for localized \textit{M. abscessus} infection\textsuperscript{6}. It is not clear as to how long the treatment should be continued, but as with other mycobacterial infections, many authors feel that treatment should be given for six months, and especially in immuno-compromised patients\textsuperscript{3}.

There was a delay in establishing diagnosis in our patient and this is contributed in part to her relatively mild and indolent symptoms as well as the persistently sterile pyogenic cultures. \textbf{This case illustrates the need to consider atypical mycobacterial infections in patients with persistent cutaneous infection, especially in those with a history of antecedent trauma, persistently sterile cultures, relatively mild symptoms and poor response to standard treatment. In such cases, skin biopsy for histology and appropriate microbiological studies are essential.}

\textbf{ACKNOWLEDGEMENTS}

We would like to acknowledge the help of Dr. Jagdeep for providing the detailed history of the patient.

\textbf{REFERENCES}

CRYPTOCOCCAL MENINGITIS ASSOCIATED WITH TUBERCULOSIS IN HIV INFECTED PATIENTS

Urvinderpal Singh, Aditi, Pooja Aneja, B K Kapoor, S P Singh and Sukhpreet Singh Purewal

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Summary: Opportunistic infections are common complications of advanced immuno-deficiency in individuals with Human Immunodeficiency Virus (HIV) infection. Following involvement of the lung, the central nervous system (CNS) is the second most commonly affected organ. We report two cases of concurrent cryptococcal meningitis and tuberculosis (TB) in HIV infected persons. A high suspicion of multiple opportunistic infections should be kept in mind in HIV seropositive individuals.

Key words: Human Immunodeficiency Virus, Cryptococcal Meningitis, Tuberculosis.

INTRODUCTION

Tuberculosis (TB) is the most common opportunistic infection in patients with Human Immunodeficiency Virus (HIV). The estimated relative risk of HIV-infected individuals developing TB is 20.6 compared to HIV uninfected, in populations with a generalized HIV epidemic. Cryptococcosis occurs worldwide and mostly affects the immuno-deficient individuals. The condition has been reported more frequently since the emergence of AIDS. Cryptococcosis is the most common life threatening fungal infection in Acquired Immunodeficiency Syndrome (AIDS) with meningitis being the most common manifestation. Because of increased immunosuppression due to HIV, we are coming across more cases harbouring multiple opportunistic infections such as tuberculosis and cryptococcosis. Overlapping of the symptoms and delay in diagnosis is the main cause for increased mortality in such cases, thus a report of these cases.

CASE REPORT

Case 1: A 30-year-old male, known case of Pulmonary Tuberculosis was admitted with fever, severe headache, mental changes and increasing drowsiness of three weeks’ duration. He was on continuation phase of anti-tubercular treatment. The patient was anaemic and emaciated, examination of the chest elicited bilateral vesicular breathing and coarse crepitations. CNS examination revealed the patient to be disoriented in time, place and person but responsive to verbal orders. Neck rigidity was appreciated. Laboratory evaluation revealed a hemoglobin of 10.5gm/dl, white blood cell (WBC) count of 12,500/cmm with 84% neutrophils and platelets 2,07,000/cmm. His urinalysis, liver, renal function tests and electrolytes were normal. Sputum was negative for AFB. As a standard protocol, he was tested for HIV and came out to be positive with a CD4+ count of 97 cells/µl. ELISA test for HBsAg and Anti HCV was negative. His x-ray chest revealed bilateral heterogeneous opacities (Fig. 1). Fundoscopy was normal. Contrast Enhanced Computerized Tomography (CECT) scan of the brain was normal. Cerebrospinal fluid (CSF) analysis revealed a cell count of 64 cells/cumm (100% lymphocytes), proteins 34mg/dl, sugar 31mg/dl and ADA was 5U/L. Gram and Ziehl-Neelsen (ZN) stain did not reveal any bacterial infection. CSF for Indian ink stain was positive for Cryptococcus neoformans (Fig. 2). Thus, patient was diagnosed as a case of cryptococcal meningitis. Alongwith anti-tubercular treatment, antifungal treatment was started with amphotericin B 50mg IV infusion/day at a dose of 1mg/kg/daily and fluconazole 400mg PO daily. Patient showed significant improvement after two
weeks of antifungal therapy. Antiretroviral therapy (ART) was initiated after patient’s condition stabilized. Patient was discharged in satisfactory condition after three weeks of stay in our hospital. Patient is on regular follow up and his general condition is stable.

**Case 2:** A 30-year-old married male was admitted in our hospital with severe headache, giddiness and painful sensitivity to light for the past three days, which was sudden in onset. Past history revealed that the patient was taking anti-tubercular treatment (ATT) since past two months for abdominal tuberculosis on the basis of contrast enhanced computed tomography (CECT) abdomen (Fig. 3) which showed lymphadenopathy, thickening of the ileocecal wall, ascitis and cytological evaluation of ascitic fluid. Patient was also showing signs of symptomatic improvement prior to this episode. On examination, the patient was anaemic and dehydrated. CNS examination showed the patient to be disorientated and neck rigidity was appreciated. Examination of the abdomen revealed diffuse tenderness. Other than a haemoglobin of 8.0gm/dl, the laboratory evaluation did not reveal any other abnormality. ELISA test for HIV 1 and 2 came out to

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**Fig 1:** X-ray chest PA view showing bilateral heterogenous opacities.

**Fig 2:** CSF sediment smear showing cryptococcal cells with capsule (Indian ink preparation at 40x magnification)

**Fig 3:** CECT abdomen showing ileocecal thickening (arrow) with lymphadenopathy (arrow heads)
be positive with a CD4+ cell count of 7 cells/µl. However, ELISA test for HBsAg and Anti HCV was negative. Electrocardiography (ECG) of the patient did not reveal any abnormality other than sinus tachycardia. Fundoscopy also revealed no abnormality. Ultrasonography (USG) of the abdomen showed multiple lymphadenopathy with minimal ascites. Due to gradual deterioration in patient’s condition, lumbar puncture was performed. CSF analysis revealed 52 cells/cmm in CSF with lymphocytic pleocytosis (100% lymphocytes), raised protein (82 mg/dl) and low sugar levels (36 mg/dl). Gram and ZN staining did not reveal any bacterial infection. India ink smear of CSF sediment showed budding cryptococci with a capsule. The patient was diagnosed as a case of cryptococcal meningitis along with tuberculosis and HIV. The patient was advised antifungal treatment but patient left the hospital against medical advice and could not be followed up.

**DISCUSSION**

Both our cases were taking treatment for tuberculosis and their condition deteriorated during treatment and were admitted with suspicion of tubercular meningitis and subsequently diagnosed as being HIV positive and also suffering from cryptococcal meningitis. Another important fact is that in both the cases at the time of initiation of treatment for tuberculosis, screening for HIV was not done which is the protocol these days and if HIV infection had been detected earlier and ART initiated, meningitis due to cryptococcus neoformans may have been prevented.

HIV infection, which was first reported in India in the state of Tamil Nadu in 1986, has since spread to the entire country. Opportunistic infections are common complications of advanced immunodeficiency in individuals with HIV infection. Following involvement of the lung, the central nervous system is the second most commonly affected organ. Tuberculosis is the most common opportunistic infection in HIV patients in India and these individuals are at increased risk of all forms of extrapulmonary tuberculosis, including tuberculous meningitis. Bhagwan et al have highlighted the occurrence of tuberculous meningitis in patients already receiving antituberculous therapy.

Cryptococcosis, one of the AIDS defining infections, considered as “sleeping disease” became an “awakening giant” within a couple of years and has now been predicted as the “Mycosis of the future”. Cryptococcal meningitis, a more serious form of meningitis, has been reported as the most common opportunistic infection of CNS of Indian patients with HIV infection. Symptoms include headache, stiff neck, fever and painful sensitivity to light. Untreated cryptococcal meningitis is a disease associated with 100% mortality. Despite there being case reports of cryptococcal meningitis along with concurrent pulmonary tuberculosis, cryptococcal meningitis can be and is misdiagnosed as tuberculous meningitis, as reported in a few studies, especially in patients who are undergoing treatment for pulmonary tuberculosis. In both our cases also initially tubercular meningitis was suspected.

The CD4+ T cell count is the best indicator of the immediate state of immunologic competence and also the strongest predictor of HIV-related complications in these patients. Cryptococcal infection was the major opportunistic infection and a major cause of death in HIV-infected patients with CD4+ cell count <100 cells/µl in the pre-highly active antiretroviral therapy era. The CD4+ cell count in our cases was also very low 97 cells/µl and 7 cells/µl respectively.

The diagnostic dilemma in both the cases was compounded as both the cases were taking treatment for tuberculosis. It has been stated that patients receiving antituberculous therapy were more likely to have tubercular meningitis, whereas Levy et al have suggested that in the presence of multiple opportunistic infections, the clinical findings of cryptococcal infection may get overlapped and confused with the findings of the other opportunistic infections such as tuberculosis as in our cases.

By reporting these cases, we intend to create an awareness amongst clinicians that all cases diagnosed as suffering from tuberculosis must be screened for HIV and secondly, a high index of suspicion and laboratory work up are the need of the hour to diagnose and treat multiple opportunistic infections to improve survival in HIV patients.
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Tuberculosis of the gastrointestinal tract (GIT) occurs as a primary lesion or secondary to a focus of tuberculosis elsewhere in the body, most commonly in the lungs. Tuberculosis can affect any part of the GIT from the oesophagus to the anal canal. Two main types are – the tuberculous ulcer and the rarer hypertrophic type which is generally found at the ileocecal junction, less commonly in the colon or rectum. Tuberculosis of bowel distal to ileocecal junction is rare and is seldom considered as a differential diagnosis of rectal stricture (2%). We report a case of rectal tuberculosis presenting with rectal prolapse and masquerading as malignancy, clinically, radiologically as well as on colonoscopy. The diagnosis was confirmed by repeated histopathological examination. The patient underwent definitive surgery along with anti-tuberculous therapy. [Indian J Tuberc 2013; 60: 184-185]
The patient was started on anti-tuberculous therapy and was scheduled for an exploratory laparotomy for treatment of rectal prolapse as well as obstructive rectal lesion. Intraoperatively, a mass was palpated in the rectum approximately 9cm from the anal verge with thickening of the proximal rectal wall, with infiltration of the mesorectum. The rectal mass was resected with a proximal and distal 5 cm margin and primary anastomosis done with silk (single layer intermittent). Tubercles were found in the mesentery of the small intestine with two passable strictures 30 cm and 60 cm proximal to the ileocecal junction. The distal stricture was exteriorised as a covering loop ileostomy. The diagnosis was confirmed by histopathology. The patient had an uneventful post-operative course. Patient was operated for ileostomy closure two months following initial surgery. The patient had an uncomplicated post-operative period and returned to activities of daily routine with no complaints on follow up after two months. Follow up examination following completion of anti-tuberculous therapy at six months post-operatively revealed no complaints related to rectal prolapse or constipation. Clinical examination and abdominal ultrasonography were normal.

**DISCUSSION**

Tuberculosis of the GIT occurs either as a primary lesion in areas consuming unpasteurised milk or secondary to tuberculous foci elsewhere in the body, most commonly lung. 70% of primary gastrointestinal tuberculosis tends to be hypertrophic or hyperplastic, while secondary tuberculosis is generally ulcerative in nature.

Hypertrophic or hyperplastic tuberculosis is uncommon in the colon and rarely seen in the rectum. Anorectal tuberculosis is responsible for less than 2% of abdominal tuberculosis. There are six morphological types of anorectal tuberculosis, viz. 1. Fistula in ano 2. Ulcer with undermined edges 3. Stricture 4. Multiple small mucosal ulcers 5. Lupoid form with submucosal nodule and mucosal ulceration 6. Verrucous form with multiple warty excrences.

Differential diagnosis includes carcinoma, lymphoma and granulomatous conditions like Crohn’s disease, syphilis, lymphogranuloma venereum histoplasmosis, actinomycosis and cytomegalovirus infection.

Rectal tuberculosis can present as a malignant lesion clinically, radiologically as well as endoscopically. Histopathology provides the only means of definitive diagnosis. Anti-tuberculous therapy has changed the dismal prognosis of abdominal tuberculosis and has made surgical intervention safe and curative. Controversy exists in the efficacy of chemotherapy in a hypertrophic or hyperplastic lesion. Surgical intervention is indicated if a) symptoms of intestinal obstruction are present b) stenosis persists three-six months after chemotherapy c) lesion is difficult to differentiate from malignancy d) malignancy and tuberculosis co-exist.

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**REFERENCES**

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GENERAL

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

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(c) the diagnosis in each case has been confirmed;
(d) the chest radiograph is accompanied by brief clinical account, not exceeding 500 words, and five references

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**ABSTRACTS**

**Trends in the annual risk of tuberculous infection in India**


The study was conducted in twenty-four districts in India. The aim was to evaluate trends in annual risk of tuberculous infection (ARTI) in each of four geographically defined zones in the country. Two rounds of house-based tuberculin surveys were conducted eight-nine years apart among children aged one-nine years in statistically selected clusters during 2000-2003 and 2009-2010 (Surveys I and II). Altogether, 1,84,992 children were tested with 1 tuberculin unit (TU) of purified protein derivative (PPD) RT23 with Tween 80 in Survey I and 69,496 children with 2TU dose of PPD in Survey II. The maximum transverse diameter of induration was measured about 72 hours after test administration. ARTI was computed from the prevalence of infection estimated using the mirror-image method. Estimated ARTI rates in different zones varied between 1.1% and 1.9% in Survey I and 0.6% and 1.2% in Survey II. The ARTI declined by respectively 6.1% and 11.7% per year in the north and west zones; no decline was observed in the south and east zones. National level estimates were respectively 1.5% and 1.0%, with a decline of 4.5% per year in the intervening period. Although a decline in ARTI was observed in two of the four zones and at national level, the current ARTI of about 1% in three zones suggests that further intensification of TB control activities is required.

**Six-Minute-Walk Test in Chronic Obstructive Pulmonary Disease: Minimal Clinically Important Difference for Death or Hospitalization**


Outcomes other than spirometry are required to assess non-bronchodilator therapies for chronic obstructive pulmonary disease. Estimates of the minimal clinically important difference for the 6-minute-walk distance (6MWD) have been derived from narrow cohorts using non-blinded intervention. The objective was to determine minimum clinically important difference for change in 6MWD over one year as a function of mortality and first hospitalization in an observational cohort of patients with COPD. Data from the ECLIPSE cohort were used (n = 2,112). Death or first hospitalization was index event; we measured change in 6MWD in the 12-month period before the event and related change in 6MWD to lung function and St. George’s Respiratory Questionnaire (health status). Of subjects with change in the 6MWD data, 94 died, and 323 were hospitalized. 6MWD fell by 29.7 m (SD, 82.9 m) more among those who died than among survivors (P < 0.001). A reduction in distance of more than 30 m conferred a hazard ratio of 1.93 (95% confidence interval, 1.29–2.90; P = 0.001) for death. No significant difference was observed for first hospitalization. Weak relationships only were observed with change in lung function or health status. A reduction in the 6MWD of 30 m or more is associated with increased risk of death but not hospitalization due to exacerbation in patients with chronic obstructive pulmonary disease and represents a clinically significant minimally important difference.

**Obesity-Associated Severe Asthma Represents a Distinct Clinical Phenotype: Analysis of the British Thoracic Society Difficult Asthma Registry Patient Cohort According to BMI**

David Gibeon, Kannangara Batuwita, Michelle Osmond, Lian G. Heaney, Chris E. Brightling, Rob Niven, Adel Mansur, Rekha Chaudhuri, Christine E. Bucknall, Anthony
Obesity has emerged as a risk factor for the development of asthma and it may also influence asthma control and airway inflammation. However, the role of obesity in severe asthma remains unclear. Thus, our objective was to explore the association between obesity (defined by BMI) and severe asthma. Data from the British Thoracic Society Difficult Asthma Registry were used to compare patient demographics, disease characteristics, and health-care utilization among three BMI categories (normal weight: 18.5-24.99, overweight: 25-29.99, obese: ≥ 30) in a well-characterized group of adults with severe asthma. The study population consisted of 666 patients with severe asthma; the group had a median BMI of 29.8 (interquartile range, 22.5-34.0). The obese group exhibited greater asthma medication requirements in terms of maintenance corticosteroid therapy (48.9% vs 40.4% and 34.5% in the overweight and normal-weight groups, respectively), steroid burst therapy, and short-acting β2-agonist use per day. Significant differences were seen with gastroesophageal reflux disease (53.9% vs 48.1% and 39.7% in the overweight and normal weight groups, respectively) and proton pump inhibitor use. Bone density scores were higher in the obese group, while pulmonary function testing revealed a reduced FVC and elevated carbon monoxide transfer coefficient. Serum IgE levels decreased with increasing BMI and the obese group was more likely to report eczema, but less likely to have a history of nasal polyps. Patients with severe asthma display particular characteristics according to BMI that support the view that obesity-associated severe asthma may represent a distinct clinical phenotype.

Estimating the tuberculosis burden in resource-limited countries: a capture-recapture study in Yemen


The lack of applicable population-based methods to measure tuberculosis (TB) incidence rates directly at country level emphasises the global need to generate robust TB surveillance data to ascertain trends in disease burden and to assess the performance of TB control programmes in the context of the United Nations Millenium Development Goals and World Health Organization targets for TB control. The aim of the study was to estimate the incidence of TB cases (all forms) and sputum smear-positive disease, and the level of under-reporting of TB in Yemen in 2010. Methodology used was record-linkage and three-source capture-recapture analysis of data collected through active prospective longitudinal surveillance within the public and private non-National Tuberculosis Programme sector in twelve Yemeni governorates, selected by stratified cluster random sampling. For all TB cases, the estimated ratio of notified to incident cases and completeness of case ascertainment after record linkage, i.e., the ratio of detected to incident cases, was respectively 71% (95%CI 64-80) and 75% (95%CI 68-85). For sputum smear-positive TB cases, these ratios were respectively 67% (95%CI 58-75) and 76% (95%CI 66-84). We estimate that there were 13,082 (95%CI 11610-14513) TB cases in Yemen in 2010. Under-reporting of TB in Yemen is estimated at 29% (95%CI 20-36).

Annual risk of tuberculous infection among schoolchildren in Bhutan


It was a school-based survey in the mountainous nation of Bhutan. Aim was to estimate the annual risk of tuberculous infection (ARTI) among children aged six-eight years. A national-level tuberculin survey was carried out among children attending 64 schools selected by two-stage cluster sampling. The study population comprised children without and with Bacillus Calmette-Guérin (BCG) scar. Tuberculin testing was performed using two tuberculin units of purified protein derivative RT23. The maximum transverse diameter of induration was measured at 48-72 hours. Of 6087 satisfactorily test-read children, 82% had a BCG scar. The frequency
distribution of tuberculin reaction sizes in all children (with and without BCG scar) did not reveal the mode for tuberculous reactions. The mode seen at 17 mm among children without BCG scar was applied to estimate the prevalence of infection among all children using the mirror-image method. Estimation was also undertaken by shifting the mode by 1 mm on either side. The ARTI computed from the prevalence thus estimated varied between 0.2% and 0.7%. There was no difference in the prevalence of infection by BCG scar status, implying that the estimated ARTI was not influenced by BCG-induced tuberculin sensitivity. The ARTI has declined in Bhutan compared to the 1991 survey estimate of 1.9%.

**Effect of rifampicin and isoniazid on the steady state pharmacokinetics of moxifloxacin**


Moxifloxacin (MFX) is reported to have a promising antimycobacterial activity, and has a potential to shorten tuberculosis (TB) treatment. We undertook this study to examine the influence of rifampicin (RMP) and isoniazid (INH) on the steady state pharmacokinetics of MFX individually in healthy individuals. A baseline pharmacokinetic study of MFX (400 mg once daily) was conducted in 36 healthy adults and repeated after one week of daily MFX with either RMP (450/600 mg) (n = 18) or INH (300 mg) (n = 18). Plasma MFX concentrations were determined by a validated HPLC method. Plasma peak concentration and exposure of MFX was significantly lower and plasma clearance significantly higher when combined with RMP (P<0.0001). The Cmax to MIC and AUC0-12 to MIC ratios of MFX were significantly lower during concomitant RMP (P<0.0001). INH had no significant effect on the pharmacokinetics of MFX. Concomitant RMP administration caused a significant decrease in Cmax and AUC0-12 of MFX, the mean decreases being 26 and 29 per cent, respectively. It is uncertain whether this decrease would affect the treatment efficacy of MFX. Prospective studies in TB patients are needed to correlate MFX pharmacokinetics with treatment outcomes.

**Activity of 5-chloro-pyrazinamide in mice infected with Mycobacterium tuberculosis or Mycobacterium bovis**


Pyrazinamide is an essential component of first line anti-tuberculosis regimen as well as most of the second line regimens. This drug has a unique sterilizing activity against *Mycobacterium tuberculosis*. Its unique role in tuberculosis treatment has led to the search and development of its structural analogues. One such analogue is 5-chloro-pyrazinamide (5-CI-PZA) that has been tested under *in vitro* conditions against *M. tuberculosis*. The present study was designed with an aim to assess the activity of 5-CI-PZA, alone and in combination with first-line drugs, against murine tuberculosis. The minimum inhibitory concentration (MIC) of 5-CI-PZA in Middlebrook 7H9 broth (neutral pH) and the inhibitory titre of serum from mice that received a 300 mg/kg oral dose of 5-CI-PZA 30 min before cardiac puncture were determined. To test the tolerability of orally administered 5-CI-PZA, uninfected mice received doses up to 300 mg/kg for two weeks. Four weeks after low-dose aerosol infection either with *M. tuberculosis* or *M. bovis*, mice were treated five days/week with 5-CI-PZA, at doses ranging from 37.5 to 150 mg/kg, either alone or in combination with isoniazid and rifampicin. Antimicrobial activity was assessed by colony-forming unit counts in lungs after four and eight weeks of treatment. The MIC of 5-CI-PZA against *M. tuberculosis* was between 12.5 and 25 µg/ml and the serum inhibitory titre was 1:4. Under the same experimental conditions, the MIC of pyrazinamide was > 100 µg/ml and mouse serum had no inhibitory activity after a 300 mg/kg dose; 5-CI-PZA was well tolerated in uninfected and
infected mice up to 300 and 150 mg/kg, respectively. While PZA alone and in combination exhibited its usual antimicrobial activity in mice infected with *M. tuberculosis* and no activity in mice infected with *M. bovis*, 5-CI-PZA exhibited antimicrobial activity neither in mice infected with *M. tuberculosis* nor in mice infected with *M. bovis*. Our findings showed that 5-CI-PZA at doses up to 150 mg/kg was not active in chronic murine TB model. Further studies need to be done to understand the mechanism and mode of inactivation in murine model of tuberculosis.

**Rifampicin plus isoniazid for the prevention of tuberculosis in an immigrant population**


The study was done in an immigrant population to compare the tolerance, adherence and effectiveness of two approaches for the treatment of latent tuberculosis infection (LTBI): six months of isoniazid (6H) vs three months of isoniazid plus rifampicin (3RH). Participants were enrolled in a controlled, randomised clinical trial in Barcelona, Spain, from April 2001 to April 2005. Monthly follow-up was done to assess tolerance, side effects and adherence. Effectiveness was evaluated at five years. In the 590 subjects enrolled, the rate of adherence was greater in the 3RH than in the 6H arm (72% vs 52.4%, *P* = 0.001). No differences between study arms were observed with respect to hepatotoxicity or side effects. Variables associated with non-adherence were diagnosis by screening (OR 1.88, 95% CI 1.26-2.82, *P* = 0.001), illegal immigration status (OR 1.48, 95% CI 1.01-2.15, *P* = 0.03), unemployment (OR 1.91, 95% CI 1.28-2.85, *P* = 0.0008), illiteracy (OR 1.73, 95% CI 1.04-2.88, *P* = 0.02), lack of family support (OR 3.7, 95% CI 2.54-5.4, *P* = 0.001) and the six-month treatment regimen (OR 2.45, 95% CI 1.68-3.57, *P* = 0.0001). None of the patients who completed either treatment developed tuberculosis. The 3RH regimen facilitates adherence to LTBI treatment and offers a safe, well-tolerated and effective alternative.

**Improving screening and chemoprophylaxis among child contacts in India’s RNTCP: a pilot study**


While contact screening and chemoprophylaxis is recommended by India’s Revised National Tuberculosis Control Programme for asymptomatic children aged <6 years who are household contacts of smear-positive pulmonary tuberculosis (PTB) patients, implementation is suboptimal. The objective was to evaluate the effectiveness of an isoniazid preventive therapy (IPT) register and card in improving the adherence of health care workers (HCWs) to programmatic guidelines. This prospective study was conducted in two Tuberculosis Units in South India. Child contacts of smear-positive PTB patients initiated on treatment between November 2009 and January 2010 were screened, and IPT was initiated in asymptomatic children. HCWs were trained in the use of the IPT register and card. The process was evaluated using patient and HCW interviews. Of 87 children identified aged <6 years, 71 (82%) were traced by HCWs; 53 were screened for TB and initiated on IPT, and 39 completed treatment. HCWs expressed satisfaction with the use of the IPT card and register, saying that it helped them to remember to complete required tasks. In a programme setting, with HCW training and introduction of specific documentation (IPT card and register), implementation of contact tracing and chemoprophylaxis for child contacts improved from 19% to 61%.

**Health care index score and risk of death following tuberculosis diagnosis in HIV-positive patients**

The aim was to assess health care utilisation for patients co-infected with TB and HIV (TB-HIV), and to develop a weighted health care index (HCI) score based on commonly used interventions and compare it with patient outcome. A total of 1061 HIV patients diagnosed with TB in four regions, Central/Northern, Southern and Eastern Europe and Argentina, between January 2004 and December 2006 were enrolled in the TB-HIV study. A weighted HCI score (range 0-5), based on independent prognostic factors identified in multivariable Cox models and the final score, included performance of TB drug susceptibility testing (DST), an initial TB regimen containing a rifamycin, isoniazid and pyrazinamide, and start of combination antiretroviral treatment (cART). The mean HCI score was highest in Central/Northern Europe (3.2, 95%CI 3.1-3.3) and the lowest in Eastern Europe (1.6, 95%CI 1.5-1.7). The cumulative probability of death one year after TB diagnosis decreased from 39% (95%CI 31-48) among patients with an HCI score of 0 to 9% (95%CI 6-13) among those with a score of ≥ 4. In an adjusted Cox model, a 1-unit increase in the HCI score was associated with 27% reduced mortality (relative hazard 0.73, 95%CI 0.64-0.84). Our results suggest that DST, standard antituberculosis treatment and early cART may improve outcome for TB-HIV patients. The proposed HCI score provides a tool for future research and monitoring of the management of TB-HIV patients. The highest HCI score may serve as a benchmark to assess TB-HIV management, encouraging continuous health care improvement.

**Therapeutic drug monitoring in the treatment of tuberculosis: a retrospective analysis**


The study was conducted in Tuberculosis (TB) in-patient treatment unit in Vancouver, Canada. The aim was to examine the results of therapeutic drug monitoring (TDM) in antituberculosis treatment. We performed a retrospective analysis of TDM data from 2000 to 2010. All in-patients treated for TB with TDM performed during their treatment course were included. TDM was performed on 52 patients in 76 treatment episodes from 2000 to 2010. Overall, 103/213 (48.4%) drug levels measured were low, and 5/213 (2.3%) were high. At least one drug level was low in 47/52 (90.3%) patients. Initial serum levels were low in respectively 76.6% and 68.4% of isoniazid (INH) and rifampicin (RMP) levels. In contrast, only 2.9% of initial pyrazinamide levels were low. Five patients with a susceptible strain on initial presentation later developed drug-resistant disease, with all five patients demonstrating at least one low drug level and two demonstrating multiple low levels. Dose adjustments were made in response to 26 INH and RMP levels, with variable serum responses. In this population with high rates of treatment failure and acquired resistance, we demonstrate that most patients had low drug levels. Prospective studies are required to examine the relationship between drug levels and clinical outcomes.

**Tuberculous lymphadenopathy: a multicentre operational study of six-month thrice weekly directly observed treatment**


The study was conducted in eight operational locations for the Revised National Tuberculosis Control Programme in six Indian states. The aim was to assess the six-month efficacy of an intermittent thrice-weekly directly observed treatment (DOT) regimen for tuberculous
peripheral adenopathy and the need for prolongation of treatment to nine months for non-resolution of lymphadenopathy. Patients aged >5 years with tuberculous lymphadenopathy were included in the study. Patients were evaluated for resolution at repeat visits following treatment. Those with poor resolution at six months were randomised to extended treatment up to nine months or observation without additional treatment. Resolution of lymphadenopathy was observed at the end of six months in 517/551 (93.8%) patients. There was a significant difference in response among patients with and those without the presence of systemic symptoms. There was no association between treatment response and number, size, site, consistency and matting of lymphadenopathy. No differences in response were seen in the remaining 34 patients with or without extended treatment. The operational efficacy of 6-month thrice-weekly DOT for peripheral tubercular lymphadenopathy was satisfactory. There was no evidence of additional benefits of prolonging treatment to nine months.

Development and validation of a tuberculosis prognostic score for smear-positive in-patients in Japan


No scoring system has ever been used to estimate the prognosis of individual tuberculosis (TB) patients. The aim was to develop and validate a tuberculosis prognostic score. This retrospective cohort study conducted in Japan comprised the development (n = 179; mean age 65.9 ± 18.8 years) and validation (n = 244; mean age 64.3 ± 20.1 years) of a tuberculosis prognostic score among patients with newly diagnosed smear-positive non-multidrug-resistant pulmonary tuberculosis without human immunodeficiency virus infection. The score (raw score) was defined by modifying a logistic regression formula using known risk factors as independent variables and in-patient death as a dependent variable. The raw score was calculated as follows: age (years) + (oxygen requirement, 10 points) - 20 × albumin (g/dl) + (activity of daily living: independent, 0 point; semi-dependent, five points; totally dependent, 10 points). The raw scores were grouped into risk groups 1 (raw score < -30) to 5 (raw score ≥ 60) using 30-point intervals. Every increase in risk group was equivalent to a 7.3-fold increase in the odds ratio for in-hospital death (P < 0.001). The area under the receiver operating characteristics curve by risk group for in-patient death was 0.875 (P < 0.001). In this study we were able to develop and validate a tuberculosis prognostic score.

Interferon-γ ELISPOT as a Biomarker of Treatment Efficacy in Latent Tuberculosis Infection: A Clinical Trial


Biomarkers that can be used to evaluate new interventions against latent tuberculosis infection (LTBI) and predict reactivation TB disease are urgently required. The aim was to evaluate ESAT-6 and CFP-10 (EC) IFN-γ ELISPOT as a biomarker for treatment efficacy in LTBI. This was a randomized, blinded, and placebo-controlled trial of INH in EC ELISPOT and Mantoux test positive participants. Participants received a six-month course of 900 mg INH twice weekly or a matching placebo. INH acetylator genotypes were determined and urine tested for INH metabolites to confirm adherence. The proportion of positive responders for CFP-10 and ESAT-6 between treatment arms was compared using mixed effects logistic regression models. A Tweedie (compound Poisson) model was fitted to allow for zero inflation and overdispersion of quantitative response. The proportions of EC ELISPOT-positive subjects reduced over time (P < 0.001) but did not differ by study arm (P = 0.36). Median spot-forming units for ESAT-6 and CFP-10 also declined...
significantly with time (P < 0.001) but did not differ by study arm (P = 0.74 and 0.71, respectively). There was no evidence of an interaction between acetylator status and INH treatment with respect to ELISPOT results over time. In contacts with LTBI, INH therapy plays no role in observed decreases in *Mycobacterium tuberculosis* antigen–specific T-cell responses over time. IFN-γ ELISPOT is probably not a useful biomarker of treatment efficacy in LTBI.

Use of recombinant purified protein derivative (PPD) antigens as specific skin test for tuberculosis


Purified protein derivative (PPD) is currently the only available skin test reagent used worldwide for the diagnosis of tuberculosis (TB). The aim of this study was to develop a *Mycobacterium tuberculosis* specific skin test reagent, without false positive results due to Bacillus Calmette-Guerin (BCG) vaccination using recombinant antigens. Proteins in PPD IC-65 were analyzed by tandem mass spectrometry and compared to proteins in *M. tuberculosis* culture filtrate; 54 proteins were found in common. Top candidates MPT64, ESAT 6, and CFP 10 were overexpressed in Escherichia coli expression strains and purified as recombinant proteins. To formulate optimal immunodiagnostic PPD cocktails, the antigens were evaluated by skin testing guinea pigs sensitized with *M. tuberculosis* H37Rv and BCG. For single antigens and a cocktail mixture of these antigens, best results were obtained using 3 µg/0.1 ml, equivalent to 105 TU (tuberculin units). Each animal was simultaneously tested with PPD IC- 65, 2 TU/0.1 ml, as reference. Reactivity of the multi-antigen cocktail was greater than that of any single antigen. The skin test results were between 34.3 and 76.6 per cent the level of reactivity compared to that of the reference when single antigens were tested and 124 per cent the level of reactivity compared to the reference for the multi-antigen cocktail. Our results showed that this specific cocktail could represent a potential candidate for a new skin diagnostic test for TB.

Point-of-care Xpert® MTB/RIF for smear-negative tuberculosis suspects at a primary care clinic in South Africa


Aim was to assess the clinical utility and cost of point-of-care Xpert® MTB/RIF for the diagnosis of smear-negative tuberculosis (TB). It was a cohort study of smear-negative TB suspects at a South African primary care clinic. Participants provided one sputum sample for fluorescent smear microscopy and culture and an additional sample for Xpert. Outcomes of interest were TB diagnosis, linkage to care, patient and provider costs. Among 199 smear-negative TB suspects, 16 were positive by Xpert, 15 by culture and seven by microscopy. All cases identified by Xpert began anti-tuberculosis treatment the same or next day; only one of five Xpert-negative culture-positive cases started treatment after 34 days. Xpert at point of care offered similar diagnostic yield but a faster turnaround time than smear and culture performed at a centralized laboratory. Compared to smear plus culture, Xpert (at US9.98 per cartridge) was US3 less expensive per valid result (US21 vs US24) and only US6 more costly per case identified (US266 vs US260). Xpert is an effective method of diagnosing smear-negative TB. It is cost saving for patients, especially if performed at point of care, but it is costly for health care providers. Data-driven studies are needed to determine its cost-effectiveness in resource-poor settings with diverse diagnostic practices.

Safety of long-term isoniazid preventive therapy in children with HIV: a comparison of two dosing schedules

The study was conducted in two pediatric hospitals in Cape Town, South Africa. The aim was to investigate the incidence of and risk factors for severe liver injury in human immunodeficiency virus (HIV) infected children receiving long-term isoniazid preventive therapy (IPT). Randomised trial of IPT or placebo given daily or thrice weekly to HIV-infected children aged ≥8 weeks; placebo was discontinued early. Alanine transaminase (ALT) was measured at baseline, six-monthly and during illness: an increase of ≥10 times the upper limit of normal defined severe liver injury. Of 324 children enrolled, 297 (91.6%) received IPT (559.1 person-years [py]). Baseline median age was 23 months (interquartile range [IQR] 9.5-48.6) and median CD4%, 20% (IQR 13.6-26.9). A total of 207 (63.9%) children received combination antiretroviral therapy: 19 developed severe liver injury, 16 while receiving IPT. Among these, there were eight cases of viral hepatitis (five with hepatitis A), two antiretroviral-induced liver injuries and one case of abdominal tuberculosis. IPT-related severe liver injury occurred in 1.7% (5/297, 0.78/100 py). No child developed hepatic failure; one died of an unrelated cause. All surviving children subsequently tolerated IPT. This study suggests that long-term IPT has a low toxicity risk in HIV-infected children. In the absence of chronic viral hepatitis, IPT can be safely re-introduced following recovery from liver injury.

Epidemiology and clinical significance of non-tuberculous mycobacteria isolated from pulmonary specimens


The study was conducted in a tertiary university medical centre in northern Israel. The aim was to evaluate the clinical significance of non-tuberculous mycobacteria (NTM) isolated from pulmonary specimens. Clinical and microbiological data were collected from patient files. Cases were classified as definite, probable and possible NTM. Between 2004 and 2010, 215 cases with respiratory isolates of NTM were identified. *Mycobacterium xenopi* was the most common species (n = 84, 39.1%), followed by *M. simiae* (n = 52, 24.2%). A total of 170 (79.1%) cases were classified as possible and 24 (11.2%) as probable NTM. Only 21 (9.8%) cases were considered definite NTM, the majority of which were *M. kansasii* and *M. avium complex*. *M. xenopi* and *M. simiae* are the most prevalent species of NTM isolated from respiratory samples in northern Israel. However, most of these isolates represent colonisation. Of the relatively small number of clinically significant isolates, *M. kansasii* and *M. avium complex* were the most common.