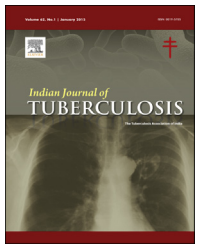
INDIAN JOURNAL OF TUBERCULOSIS

EDITORIAL BOARD

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.journals.elsevier.com/indian-journal-of-tuberculosis/



Editorial

TB control requires new tools, policies, and delivery models

Despite the global scale-up of the DOTS strategy, reduction in TB incidence has been disappointingly modest.[1] India is a good illustration. Although the Revised National TB Control Programme (RNTCP) covers the entire population and has met the 2015 targets, India continues to report over 2 million cases every year, and accounts for a third of the 3 million 'missing cases'.[1]

Mathematical models suggest that a major reason behind the observed lack of rapid reduction in TB incidence is the inability of programmes to rapidly diagnose and treat TB, before transmission occurs.[2] In India, an average TB patient is diagnosed after a delay of about 2 months, and after having seen three different providers.[3] This underscores the importance of early diagnosis, of engaging private and informal sectors where patients seek care, and suggests that systematic screening (or active case finding) may be necessary to identify missing cases.

To control TB in India and elsewhere, we need:

a. New tools (e.g. new diagnostics and drugs)
b. New policies (e.g. Standards for TB Care in India)
c. New delivery models (e.g. public-private mix [PPM] to engage private sector)
d. Substantially higher allocation of resources for TB control

1. New tools

With the incidence of TB declining very slowly, it is difficult to imagine elimination of TB by 2050 with the kind of TB tests, drugs and vaccine used in most high TB burden countries. For example, most high burden countries still rely on the insensitive sputum smear microscopy.

TB elimination will require substantially better tools. Thankfully, new, accurate diagnostics for TB are finally here and steadily being scaled up. The Xpert MTB/RIF test (Cepheid Inc., Sunnyvale, CA), is now being scaled up for TB diagnosis (pulmonary as well as extrapulmonary) and drug-resistance detection and over 10 million tests have been used in the public sector in high burden countries.[4] In India, this technology is being used in the RNTCP as a rapid drug-susceptibility test, along with other WHO-endorsed tools like line probe assays and liquid cultures. In the private sector, these WHO-approved tests are now more affordable and accessible via the Initiative for Promoting Affordable and Quality TB Tests (IPAQT www.ipaqt.org).[5]

Progress has also been made with TB drugs. Bedaquiline, a new drug to treat adults with MDR-TB, is the first new TB drug approved in over 40 years. Other new TB drugs (e.g. delamanid) or combinations (e.g. moxifloxacin-containing regimens; combinations containing PA-824, moxifloxacin and pyrazinamide) are expected in the near future. Shortening TB treatment will increase cure rates, improve adherence, and reduce the risk of drug resistance.

2. New policies

In March 2014, two major standards were published – the 3rd edition of the International Standards for TB Care (ISTC),[6] and the first edition of the Standards for TB Care in India (STCI).[7] These policy documents are based on the most current evidence, and already incorporate new tools like Xpert MTB/RIF and newer WHO recommendations on treatment (e.g. acceptance of both daily and thrice-weekly intermittent regimens). These standards aim to inform physicians about the best approaches to TB detection, treatment and follow-up, and their acceptance and widespread use should reduce mismanagement of TB.[8]

The impact of these new policies, of course, will depend on how widely they are disseminated and used.[9] In India, available evidence suggests that most private practitioners do not follow international standards.[10] Thus, it is important to educate the large number of private practitioners about STCI, and to monitor whether they are following the standards.

3. New delivery models

New tools and new policies will obviously need to reach patients who need them the most. This brings up the relevance of new business models and delivery innovations that can make quality care more affordable and accessible to patients at the base of the pyramid (BOP).[11]

TB patients need a complete and patient-centric solution, regardless of where they seek care (public or private).[12] Engagement of the private sector for TB control is a key area

where newer PPM models are urgently needed. As articulated by Ratnavelu and Pai, there are many good reasons to work with the private sector for TB control in India.[13]

First, half of all patients with TB seek care in the private and informal sectors, and private practitioners are often the first contact care providers. Many patients begin seeking care in the informal private sector, including chemists and unqualified practitioners. So, if we want to diagnose TB early and prevent further transmission, then engagement of such first-contact private providers is the important. For example, India has over 7 lakh chemists, and many of them directly dispense medications, without prescriptions, for persons with chest symptoms. If chemists can be engaged, they could become a great source of active case finding.

Second, there is plenty of evidence that quality of TB care in the private sector is suboptimal.[14] Private doctors prefer blood tests for TB that have not been recommended by ISTC or STCI.[15] Even if diagnosis is made correctly, TB treatment in the private sector is highly variable with a variety of irrational drug regimens, formulations and dosages.[10,16,17] So, it is important for private practitioners to follow international and national guidelines and use the correct drugs and regimens.

Third, even if the correct TB treatment is started, adherence is not guaranteed. In fact, private practitioners find it difficult to ensure treatment completion among their patients.[10] Thus, in the private sector, there is a need create systems to support patients during therapy.

Fourth, engagement of the private sector is necessary to increase rates of TB case notification. Since 2012, it is mandatory for all TB cases in the country to be notified to the public health authorities. Unfortunately, most private practitioners and private hospitals still do not notify TB cases. Fifth and last, engagement of the private sector is critical to detect drug-resistance and ensure that all patients with drug-resistant disease have access to free second-line treatment that is available in the public sector.

In India, there are several examples of innovative models in healthcare aimed at the BOP segment – from artificial limbs, to affordable cataract and heart surgeries.[11] There are novel models in the area of TB care as well, including World Health Partners, Operation ASHA, and Initiative for Promoting Affordable and Quality TB Tests (IPAQT).[12] These models have used product and process innovations to serve the BOP market.

Currently, a Private Provider Interface Agency (PPIA) model is being tried out in two urban cities in India, to assess whether interface agencies can aggregate and incentivize private providers, educate them on STCI, improve quality of care and increase case notifications.[12] Lessons from this pilot should inform larger-scale PPM initiatives in India.

4. Increased resources for TB control

Lastly, for implementing new tools, policies and delivery approaches, we need much more resources for TB control.[18] In particular, the RNTCP requires a substantially higher budget, if it has to deliver on the objectives laid out in the National Strategic Plan.[19] Expenditure on health itself needs to be increased, given how little India spends on health. After all, without adequate resources, no country can tackle TB. Policy makers and politicians need to realize that TB control will result in substantial cost savings down the line, and bring significant economic benefits to the country.[20]

Conflicts of interest

The author has none to declare.

REFERENCES

1. World Health Organization. *Global Tuberculosis Control: WHO Report 2013*. Geneva: WHO; 2013.
2. Dye C, Williams BG. The population dynamics and control of tuberculosis. *Science*. 2010;328:856–861.
3. Sreeramareddy CT, Qin ZZ, Satyanarayana S, Subbaraman R, Pai M. Delays in diagnosis and treatment of pulmonary tuberculosis in India: a systematic review. *Int J Tuberc Lung Dis*. 2014;18:255–266.
4. Weyer K, Mirzayev F, Migliori GB, et al. Rapid molecular TB diagnosis: evidence, policy-making and global implementation of Xpert®MTB/RIF. *Eur Resp J*. 2013;42:252–271.
5. Pai M. Promoting affordable and quality tuberculosis testing in India. *J laboratory physicians*. 2013;5:1–4.
6. TB CARE I. *International Standards for Tuberculosis Care*. 3rd ed.; 2014 [Accessed March 2014]. www.istcweb.org.
7. Central TB. *Division–Ministry of Health and Family Welfare & WHO Country Office for India. Standards of TB Care in India*. New Delhi, India: Ministry of Health and Family Welfare; 2014.
8. Pai M, Satyanarayana S, Hopewell PC. Improving quality of tuberculosis care in India. *Ind J Tuberc*. 2014;61:1–7.
9. Pai M. Improving the quality of tuberculosis care: we need standards and strategies to translate them into practice. *J Epi Glob Health*. 2014;4:77–80.
10. Achanta S, Jaju J, Kumar A, et al. Tuberculosis management practices by private practitioners in Andhra Pradesh, India. *PLoS One*. 2013; 13;8:e71119.
11. Prahalad CK. *The Fortune at the Bottom of the Pyramid* [5th Anniversary Edition]. New Jersey, USA: Wharton School of Publishing; 2010.
12. Pai M, Yadav P, Anupindi R. Tuberculosis control needs a complete and patient-centric solution. *Lancet Glob Health*. 2014;2:e189–e190.
13. Ratnavelu VK, Pai M. *TB Control: Five Key Reasons to Engage the Private Sector*. The Hindu; 2014. http://www.thehindu.com/sci-tech/health/medicine-and-research/tb-control-five-key-reasons-to-engage-the-private-sector/article5805559.ece [19 March 2014].
14. Bhargava A, Pinto LM, Pai M. Mismanagement of tuberculosis in India: causes, consequences, and the way forward. *Hypothesis*. 2011;9:1–13.
15. Jarosawlski S, Pai M. Why are inaccurate tuberculosis serological tests widely used in the Indian private healthcare sector? A root-cause analysis. *J Epidemiol Glob Health*. 2012;2:39–50.
16. Udwadia ZF, Pinto LM, Uplekar MW. Tuberculosis management by private practitioners in Mumbai, India: has anything changed in two decades? *PLoS ONE*. 2010;5:e12023.

17. Mishra G, Mulani J. Tuberculosis prescription practices in private and public sector in India. *Natl J Integr Res Med*. 2013;4:71–78.
18. Laxminarayan R, Nandi A. *Tuberculosis control in India: more bang for bucks than simply saving lives. Ideas for India*. New Delhi: International Growth Centre; 2013. http://ideasforindia.in/article.aspx?article_id=147.
19. Sachdeva KS, Kumar A, Dewan P, Kumar A, Satyanarayana S. New vision for revised National Tuberculosis Control Programme (RNTCP): universal access - "Reaching the un-reached". *Indian J Med Res*. 2012;135:690–694.
20. Laxminarayan R, Klein EY, Darley S, Adeyi O. Global investments in TB control: economic benefits. *Health Aff (Millwood)*. 2009;28:w730–w742.

Madhukar Pai*
McGill International TB Centre, Department of Epidemiology & Biostatistics, McGill University, Montreal, Quebec, Canada

*Dept of Epidemiology & Biostatistics, 1020 Pine Ave West, Montreal, QC H3A 1A2, Canada. Tel.: +1 514 398 5422; fax: +1 514 398 4503.
E-mail address: madhukar.pai@mcgill.ca

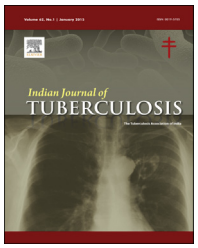
Available online 6 March 2015

http://dx.doi.org/10.1016/j.ijtb.2015.02.001

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.journals.elsevier.com/indian-journal-of-tuberculosis/



View Point

Mycobacterium Tuberculosis Biofilm – A new perspective

Niranjan Nayak

Professor & Head, Department of Microbiology, Santosh Medical College and Hospital, Ghaziabad, Uttar Pradesh, India

Tuberculosis today remains a major global health problem. According to the WHO estimates for the year 2012, about 8.6 million people developed TB and almost 1.3 million died of the disease, including 320,000 deaths among HIV positive patients due to co-infection with *Mycobacterium tuberculosis*. Out of those asymptomatically infected, approximately 5–10% visit clinics with symptoms of active tuberculosis, and a vast majority of the deaths are due to multi drug resistant strains.[1,2] It is therefore clear that while the existing anti-TB drug regimen has been effective in decreasing the mortality rate, it has been inadequate in reducing the global burden of the disease.

Thus two critical approaches have been defined in terms of TB control measures: (a) to predict and prevent the conversion of asymptomatic infection to active TB, and (b) to develop a shorter and more effective therapeutic regime for active disease. Accomplishing these goals has been difficult because of our limited understanding of the mechanisms adopted by *M. tuberculosis* to persist in the host despite the host immunity and antibiotic administration.

The persistence mechanism in *M. tuberculosis*, though, remains largely unclear, yet it is known that persistence of most, if not all, microbial species is facilitated by growth and existence as surface associated organized communities called biofilms.[3,4] Several bacterial species are now known to adopt this sessile mode of growth in the form of biofilms while residing in the host.[5,6] Even many bacteria in their biofilm mode of growth harbour drug tolerant forms. Thus it is reasonable to know if biofilms could also be accountable for the *in vivo* persistence of *M. tuberculosis*; and, if so, what could be the clinical and therapeutic implications of such biofilm formation by the organism.

A biofilm, by definition, is a structured community of bacterial cells, enclosed in a self-produced polymeric matrix and adherent to an inert or living surface. Biofilms, in general, constitute a protected mode of growth that allows survival in a hostile environment. Truly, in most natural environments, biofilm formation (i.e. association with surface structure) is the prevailing life-style, rather than the free floating form. Surface association is an efficient way of living in a micro-environment as a means of protection from being swept away by current. At the other extreme, the planktonic or free swimming microbial phase is primarily a mode of translocation from one surface to another. Formation of biofilm is a very complex process encompassing three major steps: (a) initial attachment and micro-colony formation (b) maturation of attached bacteria into a differentiated structure and (c) detachment and dispersal of planktonic cells from the biofilm. However, under the purview of this discussion, it is needless to emphasize the details of these steps. Nevertheless, growing evidences document that the persistence of most, if not all, microbial species in general is achieved through their ability to grow in self-organized surface associated sessile communities called biofilms.[3,4,7] Besides, several long term colonizers in humans, like *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis* grow as biofilms in tissues or on indwelling medical devices.[8,9]

While the long term persistence of *M. tuberculosis* against host immune system and antibiotics has striking similarity with the chronic infections due to biofilm forming pathogens, it remains unclear if the tubercle bacilli form biofilms in the tissues of the diseased individual. It is, however, a well known observation that surfactant free liquid cultures of all mycobacterial species develop pellicles at the liquid–air interface,[10] although the growth characteristics and persistence of the pathogens in these multicellular communities have not been closely scrutinized.

E-mail address: niruni2000@yahoo.com.
http://dx.doi.org/10.1016/j.ijtb.2015.02.028

Interestingly, such growth pattern of *Mycobacteria* had frequently been noted in the literature as aggregations of cells driven by their surface hydrophobicity. However, the recent emerging concept of microbial persistence in biofilms led several groups to investigate the detergent free *in vitro* growth of mycobacterial species from the perspective of organized multi-cellular structures[11] In one of the first genetic studies of such surface associated growth of *Mycobacteria*, Kolter and colleagues[12] observed that a mutant of *Mycobacterium smegmatis* deficient in the biosynthesis of acetylated glycopeptidolipid was unable to attach and grow on an abiotic surface, thus demonstrating a specific genetic factor involved in the process. Other researchers[13] subsequently reported that a mutation in one of the genes in *M. smegmatis* specifically retarded the maturation stages of pellicle formation. This maturation defect was linked to defective synthesis of mycolic acid as a consequence of the alteration of the gene encoding the enzyme involved in mycolic acid biosynthesis. Therefore the previously observed high extra-cellular free mycolic acid content during the maturation of *M. smegmatis* pellicles might be consistent with the obvious waxy appearance of the structure, and thus could constitute the component of the biofilm extracellular matrix (ECM) as seen in biofilms formed by other bacteria.

It was also noted that these mycobacterial species not only had specific genetic requirement for forming the pellicle, but also produced abundant free mycolic acid in the structure, which harboured a large number of drug tolerant bacilli.[10] Taken together, these *in vitro* studies strongly supported the view that surface associated multi-cellular structures of *Mycobacteria* had all the characteristics of biofilm, developing through distinct growth phases, having specific genetic requirements and conferring high tolerance to antibiotics.

Although, formation of biofilm *in vivo* by *M. tuberculosis* remains controversial, evidences from animal experiments suggested the presence of biofilm like structures on the acellular rim adjacent to the edge of the primary central necrotic lesions in infected guinea pigs. These structures designated as necrosis associated extra-cellular clusters very well exhibited persistence of the organisms and drug tolerance.[14]

In other diseased conditions such as cystic fibrosis and Urinary Tract Infection (UTI), biofilms formed by *P. aeruginosa* and *Escherichia coli* respectively provided an important reservoir of cells that could repopulate colonized sites upon omission of drug therapy.[15] As discussed above, correlation between biofilm formation and bacterial persistence was proposed with sufficient data and evidence in cystic fibrosis, UTI and in cases of indwelling device related infections.[3–6] However, question still remains as to whether *M. tuberculosis* could form drug tolerant biofilms in the host. If so, it raises the possibility of *M. tuberculosis* biofilm formation as a potential new target for drugs that facilitate the use of current antituberculosis antibiotics administered in ultra-short regimens.

The situation may be somewhat similar to the recalcitrant nature of the organisms like *P. aeruginosa*, *E. coli and S. epidermidis* in conditions like cystic fibrosis, UTI and medical device associated infections respectively. In the above mentioned clinical situations and possibly in *M. tuberculosis* biofilm associated *in vivo* conditions, the major riddle, that could arise because of the tendency of the bacterial population to grow in the sessile mode, would be the survival advantage of the population of cells inside the extra-cellular matrix of the biofilm as persisters.[16] In models of biofilms studied, it was shown earlier that an initial treatment with antibiotics killed planktonic cells and most of the biofilm cells. The immune system killed planktonic pesisters, whereas the biofilm persisters were well-protected from the host defence due to their exopolysaccharide matrix.[16]

What really could happen *in vivo* was extrapolated from the *in vitro* studies, and would be of interest.[17] Planktonic and biofilm cells are known to co-exist at the site of infection. Whenever these cells are exposed to antibiotics, the planktonic and surface biofilm cells are quickly inactivated because these are actively growing cells and such actively growing cells are invariably susceptible to antibiotics. The number of antibiotic molecules entering the cells is likely to be greater than that actually needed for inactivating the cells. The excess of antibiotic molecules that have entered the cells and which are not engaged in cell inactivation are probably destroyed by antibiotic degrading enzymes or are involved in a non-specific interaction with other cellular components. This gives rise to a significant reduction in the number of antibiotic molecules that are available to kill the biofilm cells embedded in the most interior thick glycocalyx matrix.

Those antibiotic molecules which do not find chance to interact with the planktonic and surface biofilm cells continue their journey to reach the embedded cells. The glycocalyx produced by the biofilm cells (exopolysaccharide) is negatively charged and is known to function as an ion exchange resin which is capable of binding a large number of antibiotic molecules which are attempting to reach the embedded cells. Antibiotic degrading enzymes (elaborated by certain bacteria) may also be trapped inside the matrix so that the incoming antibiotic molecules can be made ineffective.

Furthermore the embedded cells are not actively engaged in cell division, are slow growing and are smaller in size as opposed to the actively growing planktonic and surface biofilm cells as mentioned above. Slow growing cells are generally less susceptible to antibiotics presumably because their membranes are less permeable. Yet another speculative mechanism of antibiotic resistance is that a sub-population of micro-organisms in a biofilm forms a unique and highly protected phenotypic state; a cell differentiation somewhat similar to the formation of dormant spores.

Lastly the osmotic environment within the biofilm is altered, leading to induction of an osmotic stress response. Such a response could contribute to antibiotic resistance by changing the relative proportions of molecules on the bacterial surface responsible for permeability to antibiotics. In addition, the anaerobic niche in the deeper layers of the biofilm and the low pH due to accumulation of acidic metabolic wastes may contribute to direct antagonism of many aminoglycosides and other antibiotics. Whatever is the mechanism, the overall reduction in the populations of planktonic and surface cells curtails the clinical symptoms and the patient may temporarily feel better. At that point of time, clinician may decide to terminate the treatment, as a result of which the biofilm cells sense the change in the environment and revert back to cell division, producing a recurrent infection.

Clinically biofilm is quite relevant because this mode of growth is associated with chronicity of the disease along with the persistence of the organism with its recalcitrant nature to

respond to various antibiotics. Clinical and public health microbiologists' recognition that microbial biofilms are ubiquitous in nature has resulted in the study of a number of infectious disease processes from a biofilm perspective. Cystic fibrosis, native valve endocarditis, otitis media, periodontitis, and chronic prostatitis, all appear to be caused by biofilm forming bacteria. A spectrum of indwelling medical devices had been shown to harbor biofilms, resulting in substantial increase in the rate of device associated infections. The list of human infections both device related and nondevice related, due to biofilm forming bacteria is expanding day by day with more and more state of the art investigations on various aspects of infectious diseases. Though evidences in favor of *in vivo* biofilm formation as a means of survival and persistence for *M. tuberculosis* are scanty, a lot of insight is needed to be focused in order to find a solution to the riddle of *M. tuberculosis* biofilm.

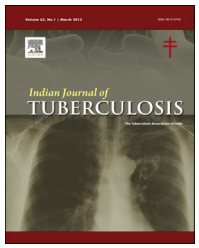
REFERENCES

1. Dye C, Lonnroth K, Jaramilo E, Williams BG, Raviglione M. Trends in tuberculosis incidence and their determinants in 134 countries. *Bull WHO*. 2009;87:683–691.
2. Harrington M. From HIV to tuberculosis and back again: a tale of activism in two pandemics. *Clin Infect Dis*. 2010;50:S260–S266.
3. Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of persistent infections. *Science*. 1999;284:1318–1322.
4. Fux CA, Costerton JW, Stewart PS, Stoodley P. Survival strategies of infectious biofilms. *Trends Microbiol*. 2005;13:34–40.
5. Nayak N, Satpathy G, Nag HL, Venkatesh P, Nag TC, Prasad S. Slime production is essential for the adherence of Staphylococcus epidermidis in implant related infections. *J Hosp Infect*. 2011;77:153–156.
6. Prasad S, Nayak N, Satpathy G, et al. Molecular and phenotypic characterization of *Staphylococcus epidermidis* in implant related infections. *Indian J Med Res*. 2012;136:95–102.
7. Kolter R, Greenberg EP. Microbial sciences: the superficial life of microbes. *Nature*. 2006;441:300–302.
8. O'Gara JP, Humphreys H. *Staphylococcus epidermidis* biofilms: importance and implications. *J Med Microbiol*. 2001;50:582–587.
9. Watnick P, Kolter R. Biofilm: the city of microbes. *J Bacteriol*. 2000;182:2675–2679.
10. Ojha AK, Baughn AD, Sambandan D, et al. Growth of *Mycobacterium tuberculosis* biofilms containing free mycolic acids and harbouring drug tolerant bacteria. *Mol Microbiol*. 2008;69:164–174.
11. Carter GM, Wu M, Drummond DC, Bermudez JE. Characterization of biofilm formation by clinical isolates of *Mycobacterium avium*. *J Med Microbiol*. 2003;52:747–752.
12. Recht J, Kolter R. Glycopeptidolipid acetylation affects sliding motility and biofilm formation in *Mycobacterium smegmatis*. *J Bacteriol*. 2001;183:5718–5724.
13. Ojha A, Anand M, Bhatt A, Kremer L, Jacobs Jr WR, Hatful GF. GroEL1: a dedicated chaperone involved in mycolic acid biosynthesis during biofilm formation in mycobacteria. *Cell*. 2005;123:861–873.
14. Vega-Dominguez PJ, Pedroza-Roldian C, Flores-Valdez MA. New evidences strengthening the need for considering *M. tuberculosis* biofilms in drug development pipelines. *J Mycobac Dis*. 2014;4:2.
15. Robert ME, Stewart PS. Modelling protection from antimicrobial agents in biofilms through the formation of persister cells. *Microbiology*. 2005;151:75–80.
16. Lewis K. Persister cells. *Annu Rev Microbiol*. 2010;64:357–372.
17. Anwar H, Strap JL, Costerton W. Establishment of aging biofilms: possible mechanism of bacterial resistance to antimicrobial therapy. *Antimicob Agents Chemother*. 1992;36:1347–1351.

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.journals.elsevier.com/indian-journal-of-tuberculosis/



Review Article

Endobronchial tuberculosis

Pyng Lee [a,b,*]

[a] *Associate Professor, National University of Singapore, Singapore*
[b] *Director of Interventional Pulmonology, Senior Consultant, Division of Respiratory and Critical Care Medicine, National University Hospital, 1E Kent Ridge Road 119228, Singapore*

ARTICLE INFO

Article history:
Available online 18 March 2015

Keywords:
Pulmonary tuberculosis
Endobronchial tuberculosis
Bronchoscopy
Surgery
Airway stricture

ABSTRACT

Endobronchial tuberculosis refers to tuberculous infection of the tracheobronchial tree. Diagnosis requires a high index of suspicion since symptoms are attributed to co-existing pulmonary tuberculosis and airway lesions are not detectable on chest radiograph. While computed tomography and bronchoscopy are useful for the evaluation of tracheobronchial stenosis or obstruction, goals of treatment remain in the eradication of tubercle bacilli and prevention of airway stenosis. Corticosteroids may halt progression of active disease to fibro-stenotic stage, however if tracheobronchial stenosis causing post-obstructive pneumonia, atelectasis and dyspnea has occurred, airway patency must be restored mechanically by surgery or bronchoscopic techniques.

1. Introduction

Involvement of the trachea and major bronchi by tuberculosis was first described by Morton in 1698.[1] Endobronchial tuberculosis (EBTB) defined as tuberculous infection of the tracheobronchial tree is not uncommon. EBTB is found to account for 42% of 1000 autopsies of patients with tuberculosis,[2] and affects 10–38.8% of living patients undergoing rigid bronchoscopy.[3–5]

After the introduction of effective anti-tuberculous drugs, interest in pulmonary tuberculosis (PTB) eclipses the study of EBTB. However EBTB continues to be a major public health problem because its diagnosis is often delayed, and airway stenosis with its attendant complications of post-obstructive pneumonia, atelectasis, hemoptysis, wheezing and dyspnea can develop during the course of treatment.[6–8] Owing to HIV infection, poverty, ageing population, migration, multi-drug resistance, failure in health systems and rise in diabetes, a resurgence in tuberculosis is observed which accounts for 8.8 million new cases and 1.8 million TB related deaths each year.[9,10] It is also likely that HIV may be associated with a higher incidence of EBTB.[11,12]

The pathogenesis of EBTB is not fully understood and is thought to arise from direct implantation of the tubercle bacilli onto the tracheobronchial tree from adjacent pulmonary parenchymal lesion. This theory is supported by finding tuberculosis affecting the bronchus opposite to the airway that drains the tuberculous cavity. Another cause is direct airway infiltration by adjacent tuberculous mediastinal lymph node more commonly seen in children while lymphatic and hematogenous spread is rare.[13–15] The clinical course of EBTB can be variable and complex, dependent on the interaction of

* Division of Respiratory and Critical Care Medicine, National University Hospital, 1E Kent Ridge Road 119228, Singapore. Tel.: +65 67723941; fax: +65 67723968.
E-mail address: pyng_lee@nuhs.edu.sg.
http://dx.doi.org/10.1016/j.ijtb.2015.02.002
0019-5707/

mycobacteria with host immunity and anti-tuberculous drugs.[16,17]

2. Clinical features and radiology

EBTB appears to occur more frequently in women in their second and third decades of life even though they have a lower incidence of pulmonary TB.[6,13,16,17] One explanation is implantation of mycobacteria from infected sputum occurs more frequently in females as they do not expectorate sputum well due to sociocultural circumstances. Clinical features depend on the type and stage of EBTB. Some are asymptomatic while most complain of productive cough, fever, hemoptysis, hoarseness, chest pain and generalised weakness.[6] Wheezing can be detected by auscultation in a third of patients erroneously managed as asthma with steroids, and decreased air-entry in a quarter.[8,18–20]

Diagnosis of EBTB is difficult based on symptoms since they can occur as part of pulmonary TB or other respiratory diseases. CXR can be normal as these lesions are not detectable unless airway obstruction has occurred causing distal atelectasis (Fig. 1). Interestingly the lower or middle lung lobes are affected slightly more often than upper lobes which would favour the direct implantation theory of EBTB by gravity.[6,8,16,18] Pleural effusions and military tuberculosis may be observed.[14,21] CT is more useful in demonstrating bronchial wall irregularities and lymphadenopathy associated with bronchial lesion, and 3D CT reconstruction for degree and extent of tracheobronchial stenosis especially if surgery or bronchoscopic intervention is planned (Fig. 2).[22,23]

Sputum smear for acid fast bacilli (AFB) is positive in 17% and increases to 79% when combined with bronchoscopic specimens.[6,18] This finding is unexpected as EBTB is presumed to yield higher sputum AFB smear positivity. However sputum expectoration may be difficult due to mucus entrapment by proximal granulation tissue, or mucosal ulceration may be necessary for positive AFB smear. PCR for mycobacteria tuberculosis is increasingly applied to improve the diagnosis of EBTB.[24,25]

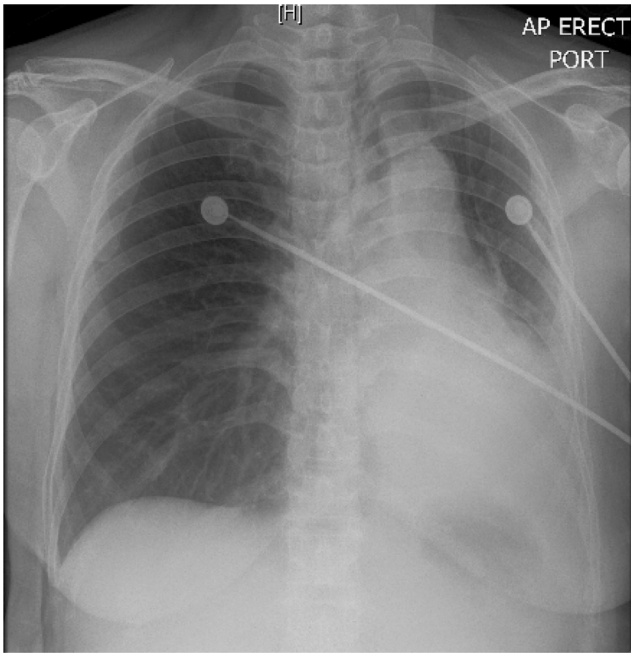


Fig. 1 – CXR showing left lower lobe collapse.

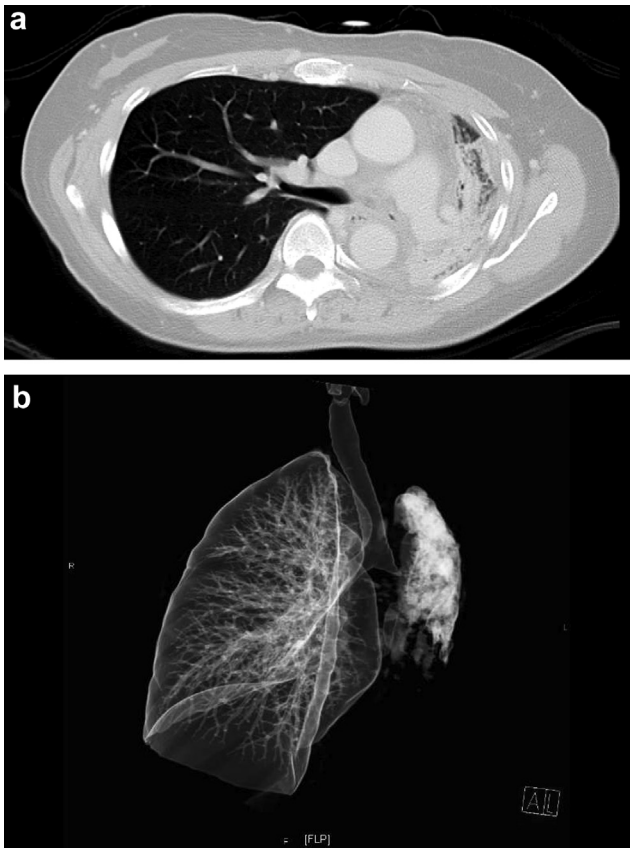


Fig. 2 – (a): CT scan of left main bronchial stricture, distal lingular and lower lobe collapse. (b): 3D CT reconstruction showing LMB stricture with left lung collapse.

3. Bronchoscopy and histopathology

EBTB affects the trachea, main bronchi and upper bronchi (Fig. 3). Biopsy specimens are definitive for EBTB if caseating granulomas or AFBs are present, or if they show non-caseating granulomas and Langhan's giant cells for which sarcoidosis, fungal or granulomatous diseases are excluded. Chung and co-workers classified EBTB into 7 categories (% prevalence): non-specific bronchitis (8%), actively caseating (43%), granular (11%), edematous hyperemic (14%), ulcerative (3%), tumorous (10.5%), and fibrostenotic (10.5%). In this study, serial bronchoscopy was performed from the diagnosis of EBTB to completion of anti-tuberculous treatment and actively caseating, edematous-hyperemic, tumorous and fibrostenotic lesions demonstrated higher risk of progression to tracheobronchial stenosis, usually within 3 months.[6,26]

The classification of EBTB can be explained pathologically by disease progression. The initial lesion is characterised by erythema and lymphocytic infiltration which corresponds to

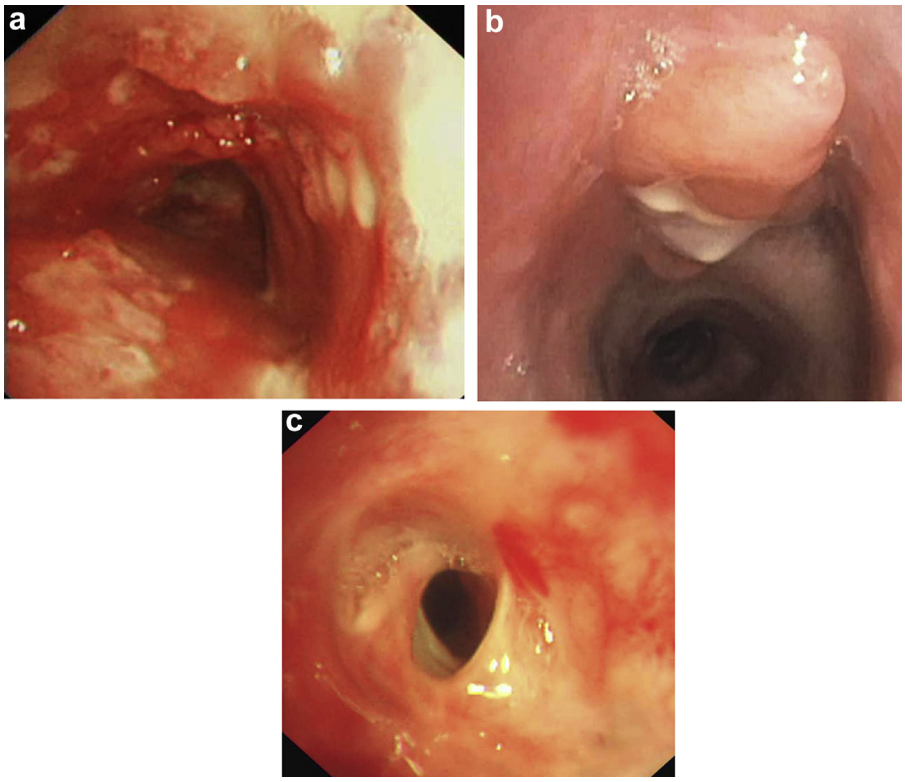


Fig. 3 – (a): Actively caseating EBTB of trachea and main carina. (b): Tumorous EBTB of right upper lobe. (c): Fibrostenotic EBTB of left main bronchus.

non-specific bronchitis. As the disease advances submucosal tubercles develop giving it a granular appearance (granular) while marked mucosal edema describes the edematous-hyperemic type. It can undergo caseous necrosis (actively caseating) or becomes ulcerative if the inflammation continues. The actively caseating or ulcerative lesion can either evolve to hyperplastic iniflammatory polp (tumorous type) or heal by fibrostenosis.[15,27,28] Associated intrathoracic tyberculous lymph node can erode and protrude into the airway akin to tumorous EBTB.[11,14,28] Rikimaru and coworkers further divided the ulcerative type into active (Stage A), healing (Stage H) and scarring (stage S). Only Stage A lesions were observed before anti-tuberculous treatment. During 1 and 2 months of therapy 76% of ulcerative lesions were in Stage A or H, and thereafter 63% were in Stage S of which one-third of patients developed inflammatory polyps.[29]

4. Treatment

Active and fibrous subtypes must be differentiated. Fibrous disease is considered inactive TB but it can lead to bronchial stenosis which can be a challenging sequel to manage during or after treatment of EBTB.

4.1. *Active EBTB*

In active EBTB, the most important goal of treatment is in the eradication of tubercle bacilli without selecting for drug-resistant mycobacteria. The second most important goal is in the prevention of tracheobronchial stenosis. Chemotherapy eradicates tubercle bacilli except for multi-drug resistant TB while the sequel of tracheobronchial stricture is atelectasis with dyspnea or obstructive pneumonia. Tracheobronchial strictures can develop despite prompt anti-tuberculous therapy,[6,8,16,26] and previously topical silver nitrate application has been attempted for ulcerative EBTB[3,30] and electrosurgery via rigid bronchoscopy for tumorous or polypoidal lesions.[31] A recent systematic review and meta-analysis concludes that steroids could be effective in reducing mortality for all forms of tuberculosis including PTB.[32] However, the role of corticosteroids in preventing fibrostenosis consequent to EBTB remains controversial. In 2 prospective, randomized, placebo-controlled studies of children with endobronchial obstruction from enlarged tuberculous hilar lymph nodes demonstrated significant improvement in the group treated with steroids.[33,34] There was only 1 randomized study in adults which did not show any difference in the rate of bronchial strictures between the steroid-treated and placebo groups. It was a small study, and timing of initiation of systemic steroids could contribute to the failure since there were other case reports which showed favourable response to both systemic and endoscopic injection of steroids.[35]

Shim recommends steroids for the edematous-hyperemic, actively caseating and tumorous types as these tend to progress to tracheobronchial stenoses. Prednisolone at 1 mg/kg is prescribed for 4–6 weeks followed by slow taper for the same

duration.[36] In 1963 Nemir and colleagues observed that short course prednisone of less than 4 months was an effective adjunct to the anti-tuberculous therapy for EBTB.[37] Song and coworkers also observed good response if steroids were initiated within 3 months of symptoms and concluded that steroids were beneficial in early phase EBTB but had no impact on bronchial stenosis.[38] Rikumaru and coworkers also observed that heal time for ulcerative EBTB was shorter, and bronchial stenosis less severe if patients were treated with twice daily aerosol therapy of streptomycin 100 mg, dexamethasone 0.5 mg and naphazoline 0.1 mg in addition to anti-tuberculous therapy.[39] Um and coworkers found that age >45 years, fibrostenotic subtype and >90 days between symptom onset to the initiation of anti-tuberculosis chemotherapy were independent predictors of persistent airway stenosis, and oral corticosteroids (prednisolone equivalent ≥30 mg/d) did not reduce the frequency of airway stenosis.[21] It is therefore apparent that steroids do not affect regression of the fibrostenotic lesions but can ameliorate inflammation and edema if administered in the early course of EBTB.

4.2. *Fibrous EBTB*

An important sequel of EBTB is bronchial stenosis which causes atelectasis and obstructive pneumonia. Patients present with dyspnea and wheezing. As steroids are unable to reverse tracheobronchial stenosis, airway patency must be restored by surgery or bronchoscopic intervention. Surgical resection of an atelectatic lung with stenotic main stem bronchus (pneumonectomy) has been normal practice but lung sparing surgery such as sleeve resection, carina resection and end-to-end anastomosis are increasingly performed.[40–42] Bronchoscopic techniques that include laser, electrosurgery, argon plasma coagulation, cryotherapy and balloon bronchoplasty (Fig. 4) have been applied singly or in combination to restore airway patency.[43–52] Silicon stents are deployed following airway recanalization and dilatation as adjunct to the management of complex strictures[53–55] while metallic stents should be avoided since they are difficult to remove due to airway epithelization.[55,56] Complications consequent to dilatation techniques and stenting include airway perforation, stent migration and stent related obstructing granuloma, which can cause subcutaneous emphysema, pneumothorax, pneumomediastinum, mediastinitis, dyspnea, and hemoptysis.[56] We reported a patient who received silicon stent for post-TB complex stricture developed obstructing granuloma that was successfully treated with laser and topical mitomycin C application.[57]

It is indeed challenging to determine who would respond to interventional procedures or surgery. In fact Lee and coworkers reported that only 30% experienced successful re-expansion defined as recovery of lung volume >80% of estimated original volume. These responders were younger with median age 22 years versus 34 years for non-responders. Presence of parenchymal calcification as well as bronchiectasis within the atelectasis showed higher tendency for failure whilst mucus plugging, extent of airway narrowing, volume loss on CT and endobronchial TB activity at the time of intervention did not affect lung re-expansion.[58]

5. Conclusion

Diagnosis of EBTB is often delayed as it is difficult to detect on CXR. Symptoms of hemoptysis, wheezing and dyspnea as well as CXR finding of atelectasis should alert the physician of EBTB. EBTB is divided into 7 categories based on bronchoscopic appearances, and actively caseating, edematous-hyperemic, tumorous and fibrostenotic lesions demonstrate higher risk of progression to tracheobronchial stenosis. Airway strictures occur in up to two thirds of EBTB and steroids when instituted early can prevent progression to tracheobronchial stenosis. Aerosol therapy comprising of streptomycin and corticosteroid is also an effective adjunct to anti-tuberculous treatment. 3D reconstruction CT is not only useful in the planning of bronchoscopic intervention or surgery it can also be a means to follow EBTB during therapy instead of serial bronchoscopy. Patients with airway strictures consequent to EBTB will require surgery or bronchoscopic procedures that may include a combination of tools such as laser, electrocautery, argon plasma coagulation or cryotherapy, balloon bronchoplasty or stent.

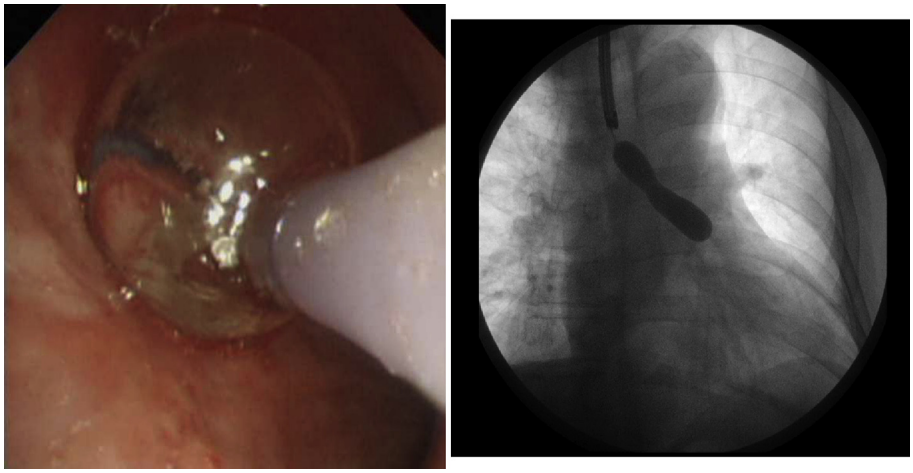


Fig. 4 – Balloon Bronchoplasty of left main bronchus with fluoroscopy.

Conflicts of interest

The author has none to declare.

REFERENCES

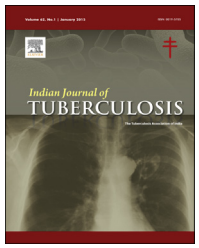
1. Morlock HV, Hudson EH. Bronchoscopy in pulmonary tuberculosis. *Br Med J*. 1939;1:381–383.
2. Auerbach O. Tuberculosis of the trachea and major bronchi. *Am Rev Tuberc*. 1949;60:604–620.
3. Judd AR. Tuberculous tracheobronchitis. *J Thorac Surg*. 1947;16:512–523.
4. MacRae DM, Hiltz JE, Quinlan JJ. Bronchoscopy in a santorium. *Am Rev Tuberc*. 1950;61:355–368.
5. Jokinen K, Palva T, Nuutinen J. Bronchial findings in pulmonary tuberculosis. *Clin Otolaryngol*. 1997;2:139–148.
6. Lee JH, Park SS, Lee DH, et al. Endobronchial tuberculosis: clinical and bronchoscopic features in 121 cases. *Chest*. 1992;102:990–994.
7. Albert RK, Petty TL. Endobronchial tuberculosis progressing to bronchial stenosis. *Chest*. 1976;70:537–539.
8. Hoheisel G, Chan BK, Chan CH, et al. Endobronchial tuberculosis: diagnostic features and therapeutic outcome. *Respir Med*. 1994;88:593–597.
9. Dye C, Watt CJ, Bleed DM, et al. Evolution of tuberculosis control and prospects for reducing tuberculosis incidence, prevalence and deaths globally. *J Am Med Assoc*. 2005;293:2767–2775.
10. World Health Organization. *47th World Health Assembly: Provisional Agenda Item 19. Tuberculosis Programme- Progress Report by the Director-General*. Document WHA47/1994/A47/12. Geneva, Switzerland: World Health Organization; 1994.
11. Judson MA, Sahn SA. Endobronchial lesion in HIV infected individuals. *Chest*. 1994;105:1314–1323.
12. Calpe JL, Chiner E, Larramendi CH. Endobronchial tuberculosis in HIV infected patients. *AIDS*. 1995;9:59–64.
13. Smart J. Endobronchial tuberculosis. *Br J Tuberc Dis Chest*. 1951;45:61–68.
14. Matthews JI, Matarese SL, Carpenter JL. Endobronchial tuberculosis simulating lung cancer. *Chest*. 1984;86:642–644.
15. Smith LS, Schillaci RF, Sarlin RF. Endobronchial tuberculosis: serial fiberoptic bronchoscopy and natural history. *Chest*. 1987;91:644–647.
16. Kim YH, Kim HT, Lee KS, et al. Serial fiberoptic bronchoscopic observations of endobronchial tuberculosis before and early after antituberculous chemotherapy. *Chest*. 1993;103:673–677.
17. Chan HS, Pang JA. Effect of corticosteroids on deterioration of endobronchial tuberculosis during chemotherapy. *Chest*. 1989;96:1195–1196.
18. Ip MS, So SY, Lam WK, Mok CK. Endobronchial tuberculosis revisited. *Chest*. 1986;89:727–730.
19. Kurasawa T, Kuze F, Kawai M, et al. Diagnosis and management of endobronchial tuberculosis. *Intern Med*. 1992;31:593–598.
20. Williams DJ, York EL, Norbert EJ, et al. Endobronchial tuberculosis presenting as asthma. *Chest*. 1988;93:836–838.
21. Rikumaru T, Kinosita M, Yano H, et al. Diagnostic features and therapeutic outcome of erosive and ulcerous endobronchial tuberculosis. *Int J Tuberc Lung Dis*. 1998;2:558–562.
22. Lacrosse M, Trigaux JP, vanBeers BE, et al. 3D spiral CT of the tracheobronchial tree. *J Coput Assist Tomogr*. 1995;19:341–347.
23. Lee KS, Yoon JH, Kim TK, et al. Evaluation of tracheobronchial disease with helical CT with multiplanar and three-dimensional reconstruction: correlation with bronchoscopy. *Radiographics*. 1997;17:555–567.
24. Lee SH, Kim SW, Lee S. Rapid detection of mycobacterium tuberculosis using a novel ultra-fast chip-type real-time PCR system. *Chest*. 2014;146:1319–1326. http://dx.doi.org/10.1378/chest.14–0626 [Epub ahead of print].
25. Kim CH, Woo H, Hyun IG, et al. A comparison between the efficiency of the Xpert MTB/RIF assay and nested PCR in identifying mycobacterium tuberculosis during routine clinical practice. *J Thorac Dis*. 2014;6:625–631.
26. Chung HS, Lee JH. Bronchoscopic assessment of the evolution of endobronchial tuberculosis. *Chest*. 2000;117:385–392.
27. Salkin D, Cadden AV, Edson RC. The natural history of tuberculous tracheobronchitis. *Am Rev Tuberc*. 1943;47:351–359.
28. Medlar EM. The behaviour of pulmonary tuberculosis lesions: a pathological study. *Am Rev Tuberc*. 1955;71:1–244.
29. Rikimaru T, Tanaka Y, Ichikawa Y, et al. Endoscopic classification of tracheobronchial tuberculosis with healing processes. *Chest*. 1994;105:318–319.
30. Sharp JC, Gorham CB. Routine bronchoscopy in tuberculosis. *Am Rev Tuberc*. 1940;41:708–718.
31. Packard JS, Davison FW. Treatment of tuberculous tracheobronchitis. *Am Rev Tuberc*. 1938;38:758–768.
32. Critchley JA, Young F, Orton L, et al. Corticosteroids for prevention of mortality in people with tuberculosis: a systematic review and meta-analysis. *Lancet Infect Dis*. 2013;13:223–237.
33. Nemir RL, Cardiba FV, Toledo R. Prednisone as an adjunct in the chemotherapy of lymph node-bronchial tuberculosis in childhood: a double-blind study. *Am Rev Respir Dis*. 1967;95:402–410.
34. Topper M, Malfrost A, Derde MP, et al. Corticosteroids in primary tuberculosis with bronchial obstruction. *Arch Dis Child*. 1990;65:1222–1226.
35. Park IW, Choi BW, Hue S. Prospective study of corticosteroid as an adjuct in the treatment of endobronchial tuberculosis in adults. *Respirology*. 1997;2:275–281.
36. Shim YS. Endobronchial tuberculosis. *Respirology*. 1996;1:95–106.
37. Nemir RL, Cardona J, Lacoius A, et al. Prednisone therapy as an adjunct in the treatment of lymph node, bronchial tuberculosis in childhood. A double blind study. *Am Rev Respir Dis*. 1963;88:189–198.
38. Song JH, Han SK, Heo IM. Clinical study on endobronchial tuberculosis. *Tuberc Respir Dis*. 1985;32:276–282.
39. Um SW, Yoon YS, Lee SM, et al. Predictors of persistent airway stenosis in patients with endobronchial tuberculosis. *Int J Tuberc Lung Dis*. 2008;12:57–62.
40. Nakamoto K, Tse CY, Hong B, et al. Carina resection for stenotic tuberculous tracheitis. *Thorax*. 1988;43:492–493.
41. Bisson A, Bonnette P, el Kadi NB, et al. Tracheal sleeve resection for iatrogenic stenoses (subglottic laryngeal and tracheal). *J Thorac Cardiovasc Surg*. 1992;104:882–887.
42. Lei Y, Tian-Hui Z, Ming H, et al. Analysis of the surgical treatment of endobronchial tuberculosis (EBTB). *Surg Today*. 2014;44:1434–1437 [Epub ahead of print].
43. Liu AC, Mehta AC, Golish JA. Upper airway obstruction due to tuberculosis: treatment by photocoagulation. *Postgrad Med*. 1985;78:275–278.
44. Dumon JF, Reboud E, Garbe L, et al. Treatment of tracheobronchial lesions by laser photoresction. *Chest*. 1982;81:278–284.
45. Tong MC, van Hasselt CA. Tuberculous tracheobronchial strictures: clinicopathological features and management with

the bronchoscopic carbon dioxide laser. *Eur Arch Otorhinolaryngol*. 1993;250:110–114.
46. Hooper RG, Jackson FN. Endobronchial electrocautery. *Chest*. 1985;87:712–714.
47. Morice RC, Ece T, Ece F, et al. Endobronchial argon plasma coagulation for treatment of hemoptysis and neoplastic airway obstruction. *Chest*. 2001;119:781–787.
48. Jin F, Mu D, Xie Y, et al. Application of bronchoscopic argon plasma coagulation in the treatment of tumorous endobronchial tuberculosis: historical controlled trial. *J Thorac Cardiovasc Surg*. 2013;145:1650–1653.
49. Mathur PN, Wolf KM, Busk MF, et al. Fiberoptic bronchoscopic cryotherapy in the management of tracheobronchial obstruction. *Chest*. 1996;110:718–723.
50. Nakamura K, Terada N, Ohi M, et al. Tuberculous bronchial stenosis: treatment with balloon bronchoplasty. *AJR Am J Roentgenol*. 1991;157:1187–1188.
51. Sheski FD, Mathur PN. Long-term results of fiberoptic bronchoscopic balloon dilation in the management of benign tracheobronchial stenosis. *Chest*. 1998;114:796–800.
52. Mehta AC, Lee FY, Cordasco EM, et al. Concentric tracheal and subglottic stenosis. Management using the Nd-YAG laser for mucosal sparing followed by gentle dilatation. *Chest*. 1993;104:673–677.
53. Dumon JF. A dedicated tracheobronchial stent. *Chest*. 1990;97:328–332.
54. Petrous M, Kaplan D, Goldstraw P. Bronchoscopic diathermy resection and stent insertion: a cost effective treatment for tracheobronchial obstruction. *Thorax*. 1993;48:1156–1159.
55. Iwamoto Y, Miyazawa T, Kurimoto N, et al. Interventional bronchoscopy in the management of airway stenosis due to tracheobronchial tuberculosis. *Chest*. 2004:1344–1352.
56. Lee P, Kupeli E, Mehta AC. Airway stents. *Clin Chest Med*. 2010;31:141–150.
57. Penafiel A, Lee P, Hsu A, Eng P. Topical Mitomycin-C for obstructing endobronchial granuloma. *Ann Thorac Surg*. 2006;82:22–23.
58. Lee JY, Chin AY, Kim TS, et al. CT scan features as predictors of patient outcome after bronchial intervention in endobronchial TB. *Chest*. 2010;138:380–385.

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.journals.elsevier.com/indian-journal-of-tuberculosis/



Review Article

Detection of drug resistance in *Mycobacterium tuberculosis*: Methods, principles and applications

*Anamika Gupta, Shampa Anupurba**

Department of Microbiology, Institute of Medical Sciences, Banaras Hindu University, Varanasi 221005, India

ARTICLE INFO

Article history:
Available online 20 March 2015

Keywords:
Drug resistant TB
Drug susceptibility testing
Tuberculosis
Turnaround time

ABSTRACT

The growing emergence of multidrug resistant tuberculosis (MDR-TB) strains is obstructing efforts for the control and management of TB. Proper management of MDR-TB relies on early recognition of drug resistance followed by timely treatment initiation. Several diagnostic methods, both phenotypic and molecular, have been developed in last few years for rapid identification of drug resistant (DR)-TB. Revised national tuberculosis control programmes (RNTPs) may find it tough to choose from the puzzling variety of rapid tests. Here, we present an outline of the available methods, discussing their basis, advantages and deficiencies.

1. Introduction

Mycobacterium tuberculosis (MTB) strains that were resistant to streptomycin (STR) appeared just after the introduction of the drug for treatment of tuberculosis (TB) in 1944.[1] The potent antitubercular action of isoniazid (INH) was first observed in the year 1951. Shortly after the introduction of INH the first resistant strains were isolated from patients treated by INH monotherapy.[2] The most effective first line antutubercular drug rifampicin (RIF) was introduced in 1967 and resistance to it has also emerged.[3] Together with INH, RIF is the backbone of TB treatment. The strain resistant with RIF and INH is called multidrug resistant (MDR) strains and greatly hampers the prospects for successful chemotherapy.[3] MDR and extensively drug resistant TB (XDR-TB) is currently the most severe form of bacterial as well as mycobacterial resistance and greatly obstruct the TB control programs. Laboratory services for sufficient and timely diagnosis of MDR/XDR-TB must be build up to fill up the gap between vision and actual implementation.[4] The MDR/XDR phenotype is caused by sequential accumulation of mutations in different genes involved in individual drug resistance (Table 1).[1]

2. Methods for the detection of drug resistance *in M. tuberculosis*

Rapid detection of DR/MDR/XDR isolates allows initiation of the appropriate treatment in patients and also surveillance of drug resistance.

3. Phenotypic methods

Three conventional methods the proportion method, absolute concentration method (MIC method) and the resistance ratio method have been efficiently used since long time for determining DST of MTB against first line anti-TB drugs *i.e.*, RIF, INH, STR and ethambutol (EMB) with the recommended critical concentrations of 40 μg/mL, 0.2 μg/mL, 4 μg/mL and 2 μg/

* *Corresponding author*. Tel.: +91 542 2368655; fax: +91 542 2367568.
E-mail address: shampa_anupurba@yahoo.co.in (S. Anupurba).
http://dx.doi.org/10.1016/j.ijtb.2015.02.003
0019-5707/

Table 1 – Description of some most important drugs, their mode of action and related genes with the proportion of drug-resistant *M. tuberculosis* isolates showing resistance-conferring mutations in the respective genes.

Drug	Discovered in	MIC μg/ml	Related genes	Gene function	Role	Mechanism of action	Mutation frequency %
Isoniazid	1952	0.02–0.2	*katG*	Catalase-peroxidase	Pro-drug conversion	Inhibition of mycolic acid biosynthesis	50–95
			inhA	Enoyl ACP reductase	Drug target	and other multiple effects	8–43
Rifampicin	1966	0.05–1	*rpoB*	β subunit of RNA polymerase	Drug target	Inhibition of RNA synthesis	95–100
Ethambutol	1961	1–5	*embB*	Arabinosyl transferase	Drug target	Inhibition of arabinogalactan synthesis	47–65
Streptomycin	1944	2–8	*rpsL*	S12 ribosomal protein	Drug target	Inhibition of protein synthesis	52–59
			rrs	16S rRNA	Drug target		8–21
			gidB	rRNA methyltransferase (G527 in 530 loop)	Drug target		?
Pyrazinamide	1952	16–50 (pH 5.5)	*pncA*	Nicotinamidase/pyrazi-namidase	Pro-drug conversion	Depletion of membrane energy or activated to pyrazinoic acid, which is bacterialcidal	72–97
Amikacin/Kanamycin	1957	2–4	*Rrs*	16S rRNA	Drug target	Inhibition of protein synthesis	76
				16S rRNA			
Capreomycin	1960		*tlyA*	2′-O-methyltransferase			
Quinolones	1963	0.5–2.5	*gyrA*	DNA gyrase subunit A	Drug target	Inhibition of DNA gyrase	75–94
			gyrB	DNA gyrase subunit B			
Ethionamide	1956	2.5–10	*etaA*/*ethA*	Flavin monooxygenase	Prodrug conversion	Inhibition of mycolic acid synthesis	37
			inhA		Drug target		56
PAS (1946)	1946	1–8	*thyA*	Thymidylate synthase	Drug activation?	Inhibition of folic acid and iron metabolism?	36

Modified from Zhang et al [4]. MIC = minimum inhibitory concentration; ACP = acyl carrier protein; PAS = para-aminosalicylic acid.

mL respectively.[5,6] However, the DST of second-line drugs is not easy even the critical concentrations are not as clear cut as for first line drugs.[7]

3.1. *Absolute concentration method*

In this test a standardized inoculum is grown on drug free media and media containing graded concentrations of anti-TB drugs to be tested. Several concentrations of each drug are tested, and resistance is expressed in terms of the lowest concentration of the drug that restrains growth; *i.e.* minimal inhibitory concentration (MIC). It is greatly affected by inoculums size and by the viability of the organisms. This is an indirect method on solid media with a turnaround time (TAT) of 4 weeks.[5,6]

3.2. *Resistance ratio method*

In this test a parallel set of media containing graded concentration of the drug are inoculated with a standard strain of tubercle bacilli (H37Rv). Resistance is expressed as the ratio of the MIC of the test strain divided by the MIC for the standard strain in the same set with a TAT of 4 weeks. This test is also greatly affected by the inoculums size as well as the viability of the strains. Further, any variation in the susceptibility of the standard strain also affects the resistance ratio of the test strain.[8]

3.3. *Proportion method*

The proportion method determines the percentage of growth of a standardized inoculum on a drug-free medium against growth on culture media containing the critical concentration of an anti-TB drug. Its TAT is between 4 to 6 weeks and can be used both as direct or indirect method.[5,8] Proficiency testing for DST is implemented in the Supranational Reference Laboratory Network, which was established by the WHO and IUATLD. Results from nine rounds of proficiency testing carried out between 1994 and 2002 showed higher sensitivities and specificities for INH and RIF than for EMB and STR testing (cumulative sensitivities: 99% for INH, 97% for RMP, 91% for STR and 89% for EMB; cumulative specificities: 98% for INH, 97% for RIF, and 94% for both EMB and STR).[9] Therefore, tests need to be carefully standardized and quality controlled.

4. Rapid methods

Conventional phenotypic methods although reliable and accurate, are time-consuming, cumbersome and have prolonged TATs. To overcome this drawback, numerous new techniques (commercial/manual) have become evaluated with the aim of more rapid detection of resistance.

4.1. *Liquid culture-based methods*

4.1.1. *BACTEC radiometric method (BACTEC- 460)*

It uses an enriched Middlebrook 7H9 liquid medium containing ^{14}C-labeled palmitic acid as the sole carbon source (12B vial). Growth of the mycobacteria and consumption of the labeled fatty acid will produce $^{14}CO_2$, which is detected inside the 12B vial by the BACTEC apparatus and expressed as a growth index. When the Growth index (GI) of the control reaches ˃30 the results can be interpreted by comparing the increase in GI from the previous day in the control vial with that in the drug vial. The following formula can be used to interpret results: GI control > GI drug = susceptible; GI control < GI drug = resistant. The method has been successfully used for DST of MTB against first and second-line anti-TB drugs.[10–12] Apart from being advantageous in shorter TAT *i.e.,* 5–10 days, precautions for the handling and waste disposal of radioactive substances render this system fraught with risk, impractical and expensive.

4.1.2. *BacT/alert 3D*

The BacT/Alert 3D or MB/BacT system (bioMerieux, Durham, NC, USA) is a nonradiometric, fully automated, continuously monitoring liquid culture system which uses advanced colorimetric detection of carbon dioxide reduction and a sophisticated computer algorithm. Carbon dioxide released into the medium by actively growing mycobacteria is detected through a liquid emulsion sensor (LES) containing a colorimetric indicator embedded at the bottom of culture vials which undergoes a color change from blue to yellow. Color changes are monitored by a reflectometric detection unit contained inside each incubating drawer of the instrument. It was introduced for the primary isolation as well as for susceptibility testing of mycobacteria. BacT/Alert 3D with the computerized data management system is an acceptable method for diagnosis of MTB with some disadvantages like prone to contamination, longer turnaround time [diagnosis: 17 days (DST range 17–30), requirement of an expensive and non-robust machine, complications and cumbersomeness.[13,14]

4.1.3. *The mycobacterial growth indicator tube (MGIT) (Becton Dickinson, Sparks, MD)*

It is based on fluorescence detection of mycobacterial growth in a tube containing a modified Middlebrook 7H9 medium together with a fluorescence quenching-based oxygen sensor embedded at the bottom of the tube. During bacterial growth within the tube, the free oxygen is utilized and is replaced with carbon dioxide. Consumption of oxygen in the medium produces fluorescence when illuminated by a UV lamp. The intensity of fluorescence is directly proportional to the extent of oxygen depletion. The instrument interprets results at the time when the growth unit (GU) in growth control reaches 400 (within 4–13 days). At this point, the GU values of the drug vial are evaluated. If the GU of the drug tube is less than 100 then it will be considered as susceptible and if the GU of the drug tube is 100 or more then the isolate will be considered resistant to that particular drug.[15,16] Many studies have now been published on the application of the MGIT system for the rapid detection of resistance to first and second line anti-TB drugs and very good correlation was found between MGIT system and conventional methods.[17,18] Uses of liquid media are technically limited because they are prone to contamination. The maintenance of this machine must be necessary, which requires very frequent technical support from the company.

4.1.4. *Versa TREK system/ESP II system*

Versa Trek system formerly known as ESP culture system II (Trek Diagnostic systems, West Lake, OH), is a non-radiometric and fully automated continuous monitoring technique. This system detects the growth of tubercle bacilli by measuring pressure changes inside the culture vial *i.e.* either gas production or gas consumption due to microbial growth. A DST kit is available for testing INH, RIF and EMB. A PZA test kit is pending US FDA clearance but, it is not in wide use due to its cumbersomeness and long TAT which is 14–30 days.[7,19]

4.1.5. *Microscopic-observation drug-susceptibility assay (MODS)*

It is based on the observation of the characteristic cord formation of MTB that is visualized microscopically in liquid medium with the use of an inverted microscope. Tests in several laboratories have demonstrated high concordance for INH (97%), RIF (100%) and fluoroquinolones (100%) compared with reference standard techniques,[20] but lower concordance for EMB (95%) and STR (92%).[21] The technique needed no more than 7 days for obtaining DST results.

4.1.6. *Colorimetric redox indicator methods*

Colorimetric tests are based on the reduction of the colored redox indicator added to the culture medium after MTB has been exposed *in vitro* to different antibiotics. Resistance is distinguished by a change in color of the indicator, which is directly proportional to the number of viable mycobacteria in the culture medium.[22] Mainly two dyes i.e., resazurin, an oxidation–reduction indicator dye, which is marketed as alamarBlue®, and tetrazolium bromide 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-[2] H-tetrazoliumbromid (MTT), an indicator which is reduced by dehydrogenases of living cells have been applied for DST of MTB. The MICs were obtained after 1–2 weeks of incubation.

Alamar blue (Trek Diagnostics, Ohio, USA) reagent can be used to detect drug resistance in MTB. The reagent is blue in the oxidized state but changes to pink when reduced. Alamar blue has been tested in several studies to detect drug resistance in MTB and to assess the activity of anti-mycobacterial drugs. The performance was good with an overall accuracy of 97% compared to the agar proportion method for the detection of resistance to INH, RIF, EMB and STR.[23–25]

MB Redox system (Heipha Diagnostika Biotest, Heidelberg, Germany) is based on reduction of tetrazolium salt or MTT. MTT is a yellow compound that, when reduced by metabolically active cells produce crystals of insoluble purple MTT formazan. This can be easily visualized or measured with a spectrophotometer after solubilization.[26] The MTT test has also been applied in the detection of resistance to RIF and other anti-TB drugs with good results.[27,28]

4.2. *Solid-culture based methods*

4.2.1. *Mycobacteriophage-based methods*

Pha B Assay (phage-amplified biologically) based on the ability of MTB to support the growth of an infecting mycobacteriophage has gained wider application. The extracellular phages are killed by a virucidal solution and the number of endogenous phages, representing the original number of viable MTB bacilli, is then determined by counting phages in a rapidly-growing mycobacterium, such as *M. smegmatis*.[29,30] **The *in house* phage amplification test** and the commercially available ***FastPlaque TB* assay** (Biotech Laboratories Ltd, Ipswich, UK) have been tested for the detection of RIF resistance both in MTB isolates and directly on TB suspected clinical specimens. The TAT is 2 days for both the assays.[30] **The luciferase reporter phage method** is based on the efficient production of a light signal by viable mycobacteria infected with specific reporter phages expressing the firefly luciferase gene. Light production is dependent on phage infection, expression of the luciferase gene, and the level of cellular ATP.[31] Signals can be detected within minutes after the infection. MTB isolates susceptible to INH or RIF, result in extinction of light production, while drug-resistant strains continue to produce light. Phage-based assays appear to have high sensitivity, but variable and slightly lower specificity. The median time to detection of bacteria was 7 days.[30,32]

4.2.2. *Microcolony method/Thin layer method*

This method relies on the visual detection of MTB microcolonies on solid medium. The Thin Layer 7H11 agar (TL7H11) or micro colony method has been used for the rapid detection of multidrug resistance directly from sputum samples. This employs observation of micro-colonies of MTB with the help of a microscope, on a thin layer of 7H11 agar plate. The TL7H11/INH/RIF has been shown in preliminary studies to be accurate for the detection of MDR-TB as compared to the reference proportion method, with results available in one week.[33]

4.2.3. *E-test (commercially available as AB BIODISK)*

The E-test is based on determination of drug susceptibility using strips containing gradients of impregnated antibiotics. There are reports about a high rate of false resistance by this method when compared with BACTEC or conventional LJ proportion methods. Susceptibility test results can be read in 5–10 days after application of the E-Test strip.[34]

4.2.4. *TK medium*

TK medium is a newly developed commercial culture medium containing dye indicators. It is used for the diagnosis and DST of MTB.[35] The metabolic activity of the mycobacteria is detected by color changes of medium. Initially the medium is red, it turns yellow if TB is present and becomes green if the sample is contaminated. The colour changes are visible earlier in comparison to solid medium such as LJ-medium, and thus reduce the time for producing result by about half (from roughly 6 weeks to about 3 weeks).[35]

4.2.5. *The nitrate reductase assay (NRA)*

NRA is based on the capacity of MTB to reduce nitrate to nitrite, which is detected by adding a chemical reagent (Griess reagent) to the culture medium (Fig. 1). The test uses conventional LJ media with the same methodology and the same concentration of antimicrobial drugs that is used for the proportion method and additionally nitrate substrate. Reading of the results after induction of the color change could be performed within 7–14

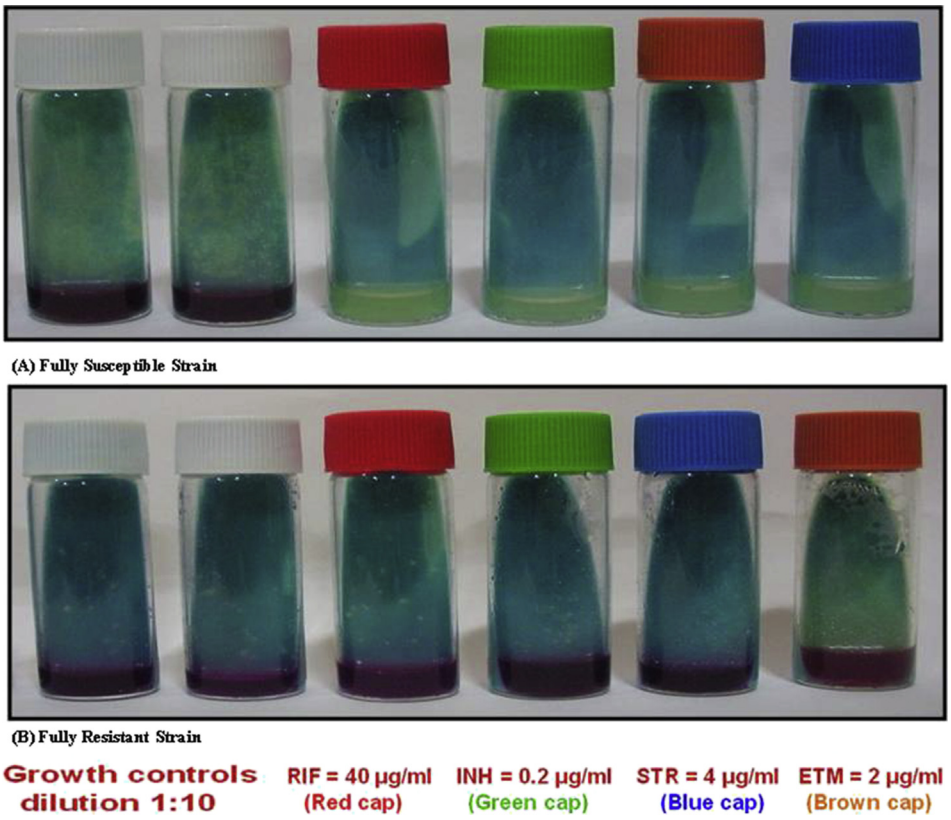


Fig. 1 – Examples of NRA results. A strain is considered resistant to a certain drug if there is a color change in the drug-containing tube greater than that in the 1:10-diluted (1:10 dil.) control tube (A) Fully susceptible strain; (B) strain resistant to all four tested drugs.

days of incubation. The sensitivities and specificities of direct and indirect NRA for the detection of INH, RIF, STR and EMB resistance are comparable to the conventional methods.[36–39]

4.3. *Chromatographic method*

4.3.1. *Mycolic acid index susceptibility testing*

This is a modification of the original mycolic acid analysis by HPLC where a coumarin compound is used as a fluorescent derivatizing agent of mycolic acid instead of *p*-bromophenacyl bromide. The drug sensitivity is assessed by measuring the total area under mycolic acid (TAMA) chromatographic peaks of a culture of MTB, and this area has a very good correlation with log CFU per milliliter. Depending on the signal and quantification of this procedure, drug susceptibility pattern can be carried out as a rapid method. Susceptibility test results in the form of chromatograms of the mycolic acid pattern can be read in 5–10 days.[40]

5. Genotypic methods

Generally, these are polymerase chain reaction (PCR)-based methods targeting well characterized resistance-related mutations. Different molecular DST methods for TB are discussed below:

5.1. *Deoxyribonucleic acid (DNA) sequencing*

DNA sequencing is the gold standard molecular method as it visualizes the whole nucleotide sequences of the DNA of interest. TAT is less i.e., 10–12 h but sometimes the mutations detected by sequencing could not be related to drug resistance as some mutations could be silent. Unfortunately, sequencing is not as easily applicable for routine identification of drug resistance mutations as it is for identification of mycobacterial species: many different genes may be involved for a single drug, as is the case in INH resistance, or the mutations may be scattered in a large segment of the gene. This implies that, in order to obtain complete drug-resistance data for a single MTB isolate, a range of sequencing reactions needs to be performed or the whole genome should be sequenced. However, for targeted use such as *rpoB* analysis, where mutations associated with RIF resistance are concentrated in a very short segment of the gene, PCR-based sequencing is a useful technique.[41] Sequencing was performed several years ago by manual procedures (conventional Sanger's method), but nowadays; it is performed with automatic sequencers *i.e.,* the Illumina automatic sequencer.[42,43] Both are based on the same principle of chain termination but the speed and length of readout are better in the latter, as it can produce a staggering 1 GB of nucleotide per run.

5.2. *Pyrosequencing*

Pyrosequencing applies the DNA sequencing-by-synthesis principle, and is mostly used for short read sequencing and SNP analysis. It has been used for the detection of drug-resistance in MTB as well.[44,45] In a study from Sweden,[46] authors have developed a pyrosequencing method for simultaneous detection of mutations associated with resistance to RIF, INH, EMB, amikacin, kanamycin, capreomycin, and ofloxacin. The method demonstrated high specificity (100%) and sensitivity (94.6%) for detection of MDR MTB as well as high specificity (99.3%) and sensitivity (86.9%) for detection of XDR MTB. In another study, it detected relevant mutations in the rpoB gene for 96.7% of RIF-resistant isolates, in katG for 64% of INH resistant isolates, and in gyrA for 70% of ofloxacin-resistant isolates.[47] Pyrosequencing has gained attention over the conventional sequencing method mainly because of its improved TAT. The entire procedure, from DNA extraction to the availability of results can be accomplished within 6 h. However, the inherent problems associated with pyrosequencing are mainly the length of DNA sequence that can be sequenced that made it impossible to replace the conventional method. Pyrosequencing allows sequencing a string of up to 20–50 nucleotides. If a longer DNA fragment is to be analyzed, the conventional Sanger's method or Illumina is preferable.[44]

5.3. *Multiplex allele-specific PCR (MAS-PCR)*

MAS-PCR assay was first described by Mokrousov et al[48] for the detection of *embB*306 mutations in EMB resistant MTB strains. Further it was also successfully applied for *katG315*[49] and *rpoB*[50] mutation detections. MAS-PCR is based just on conventional PCR followed by agarose gel electrophoresis without any supplementary sequence analysis. TAT of the assay is only 6–7 h. It uses two outer primers that flank a region under study and invariably anneal on the conserved DNA targets, plus wild-type-allele-specific inner primers that stops in its 3′ end at the targeted codon and amplifies wild-type allele specific fragments. An alteration of the base that corresponds to the 3'-end of the specific primer causes the primer template mismatch that prevents polymerase to extend the primer and results in non amplification of the indicative fragment. A number of studies have been carried out to evaluate the performance of MAS-PCR assay[51–53] and found excellent sensitivity and specificity for the detection of first line as well as second line drug resistance in MTB.[51–53]

5.4. *PCR SSCP (PCR-single strand conformation polymorphism)*

The SSCP technique is capable of identifying most sequence variations in a single strand of DNA. Under non-denaturing conditions a single strand of DNA will adopt a conformation (presumably dependent on internal base-pairing between short segments by fold back) that is uniquely dependent on its sequence composition. This conformation will usually be different if even a single base is changed. Most conformations seem to alter the physical configuration or size sufficiently that, even though the variant sequence has the same charge, the configuration-to-charge (size-to-charge) ratio is different enough to be detectable as a mobility difference upon electrophoresis through a retarding matrix such as acrylamide gel. Typically, the duplexes will be from the same PCR reaction for samples with possible genotypic differences. In combination with PCR, SSCP has been applied for the detection of resistance to RIF, INH, STR, and ciprofloxacin.[54] PCR-SSCP is useful for rapid detection of mutation in the clinical isolates of MTB with 10–14 h TAT and is able to detect 95% of the resistant isolates.[55]

5.5. *PCR hetero-duplex formation (PCR HDF)*

Williams et al[56] described this assay to characterize mutation in *rpoB* gene of RIF resistant *M. tuberculosis* isolates. This assay is performed by mixing amplified DNA from the test organisms and susceptible control strains to obtain hybrid complementary DNA. If a resistant strain is present, the mutation will produce a heteroduplex which has different electrophoretic mobility compared with the homoduplex hybrid (wild type sequence). The method takes approximately 6 h to produce the result.[56]

5.6. *Solid-phase hybridization assays*

In this test, first the resistance specific region of the clinical strain is amplified using labeled primers then the resulting amplicon is hybridized with the immobilized probe and visualized by a colorimetric reaction or fluorescence signal. If a mutation is present in the target region of interest, the amplicon will not hybridize with the probe representing the wild type sequence, but only with probes representing the complementary strand of that specific mutation. Line probe assays (LiPAs) and DNA microarrays (DNA biochips) are the most widely known techniques using immobilized probes on a solid support.

LiPAs is based on DNA extraction, multiplex PCR and reverse hybridization with a TAT of approximately 5 h. Currently there are two commercially available tests that were endorsed by WHO: GenoType® MTBDR and MTBDRplus (Hain Life science, Nehren, Germany) and INNO-LiPA Rif.TB (LiPA) (Inogenetics, Ghent, Belgium). They can detect MTB-complex (MTBC)-specific DNA and genetic mutations associated with drug resistance from smear positive sputum specimens or culture isolates, after DNA extraction and PCR amplification.[57] In a systematic review and meta-analysis including 15 studies using LiPA, 14 studies reported sensitivity between 82% and 100% for correctly identifying RIF resistance among MTBC isolates, with a specificity ranging from 92% to 100%.[58] Twelve of these studies reported a sensitivity of 95% or more, and 5 of them even reached 100% sensitivity. Of the 4 studies testing LiPA directly on clinical specimens, reported sensitivity estimates were consistently high (80%–100%), with a specificity of 100%.[59] In a separate systematic meta analysis of studies on direct DST, the pooled sensitivities for Genotype® MTBDR were 99% and 98%, and for the new Genotype® MTBDRplus 99% and 99%.[60] GenoType MTBDR assay detects the resistance to both INH and RIF simultaneously, and thus can detect MDR-TB.[61–63] Recently, the application of the new GenoType MTBDRsl (Hain Lifescience, Nehren, Germany) for detection of MTBC resistant to second-line drugs was reported with excellent sensitivity for the detection of fluoroquinolone, amikacin and

capreomycin resistance among MTBC isolates.[64] The main advantage of LPAs is that they can be performed directly on smear-positive sputum samples. Most of the laboratories now use LiPAs as the primary method for DST on cultured isolates of MTB, instead of phenotypic DST. The use of LiPA improved instrumentation for analysis and documentation of results. The disadvantages of LPAs are that they required multiple probes to cover overlapping regions and even with LiPA silent mutations may result in false predication of resistance. Further, they are labor intensive and require highly trained personnel and dedicated laboratory space and equipment.[61,62]

DNA microarrays analyze several genetic markers in a single hybridization step due to the immobilization of a large set of oligonucleotides at a precisely defined spot on a polyacrylamide gel or glass carrier.[65] The fluorescence-labeled DNA amplicon can hybridize to the microarray oligonucleotides. The resulting fluorescence intensity between the different positions in the microarray defines the pattern of mutations of the clinical strain. Such an approach has previously been used for the successful detection of *rpoB* mutations conferring RIF resistance[54] and *pnc*A mutations for PZA resistance.[66] A recent report of DNA microarray analyzed culture isolates and clinical samples for mutations in seven genes related to five anti-TB drugs (INH, RIF, STR, kanamycin and EMB). The report indicated a high sensitivity (90%) for all five drugs.[67] In this study specificities for RIF and EMB were nearly 90%, but the specificity for INH (60%) and kanamycin (67%) was not satisfactory. More recently, further attempts were started for rapid detection of resistance to fluoroquinolones by microarrays.[58] TAT is approximately 5–7 h.

5.7. *Real-time PCR techniques*

Real-time PCR techniques monitor DNA amplification reaction directly while it is occurring *i.e.*, in real time. They are based on hybridization of amplified nucleic acids with fluorescent-labeled probes spanning DNA regions of interest. The increase in fluorescence signal is monitored on-line and directly proportional to the amount of amplified product. Different types of probes have been used including the TaqMan probe,[68] fluorescence resonance energy transfer (FRET) probes,[69] molecular beacons[70] and bioprobes.[71] Although, real-time PCR was initially developed for MTBC strains, more recently it has been successfully applied directly on clinical samples. The main advantages of real-time PCR techniques are the speed of the test and a lower risk of contamination. Real-time PCR techniques have been applied to MTB strains and, also, directly to clinical samples.[68] Results are generally obtained in an average of 1.5–2.0 h after DNA extraction. Real-time PCR could eventually be implemented in reference laboratories with the required capacity to properly set up the technique and in settings where it can contribute to the management of TB patients.

5.8. *PCR restriction fragment length polymorphism (PCR-RFLP)*

PCR-RFLP is a simple, reliable, useful and robust technique for the rapid identification of drug resistant MTB. It has potential application for rapid diagnosis for INH resistance due to *katG* S315utation.[72] A study from northwestern region of Russia used *Hap*II to detect the Ser→Thr in *katG* gene, associated with INH resistance. This analysis revealed a 93.6% prevalence of the *katG* S315T mutation in strains. The design of this PCR-RFLP assay allowed the rapid and unambiguous identification of the *katG* 315ACC mutant allele.[72] A study from South China region, reported that PCR-RFLP of the *katG* amplicons by *Msp*I digestion identified 51% of INH-resistant MTB with the Ser→Thr phenotype and codon 463 was a polymorphic site with no linkage to INH resistance.[73] Results are normally obtained in 8–10 h after DNA extraction.

5.9. *Multiplex-PCR*

Herrera-León et al,[74] described a multiplex PCR to detect a AGC→ACC (serine to threonine) mutation in the *katG* gene and a $-15^{C\text{-}to\text{-}T}$ substitution ($inhA^{C\text{-}15T}$) at the 5′ end of a presumed ribosome binding site in the promoter of the *mabA-inhA* operon. They analyzed INH-resistant MTB isolates by PCR-RFLP (using the *katG* gene), DNA sequencing, and the newly developed multiplex PCR. The analysis revealed that 68.7% of the isolates carried one or both of the mutations. This finding suggests that with further development this multiplex PCR will be able to detect the majority of the INH-resistant MTB clinical isolates from countries where high frequencies of similar mutations occur. Results are normally obtained in 7–8 h after DNA extraction.

5.10. *PCR-reverse cross-blot hybridization assay*

The PCR-reverse cross-blot hybridization was described by Kox et al[75] for various genotypes of drug-resistant strains. Briefly, the oligonucleotide probes were subjected to the tailing reactions with dTTP to permit the efficient capture of the PCR products in the reverse cross-blot hybridization assay. Then, they were blotted on top of a positively charged nylon membrane. The hybridized PCR products were detected by incubation with streptavidin-alkaline phosphatase and a colour substrate. Mutations responsible for INH resistance were successfully identified by PCR-reverse cross-blot hybridization assay in 82.9% of strains tested by reverse hybridization with *katG*, *inhA*, and *ahpC* gene probes. TAT is 10–12 h. But this genotypic technique could not detect all phenotypic resistance to INH and RIF, this technique is a practical clinical tool for rapid detection of INH or RIF resistant isolates, especially among individuals at risk of MDR TB, for instance, those experiencing treatment relapse or treatment failure.[76]

5.11. *Loop-mediated isothermal amplification*

The Loop-Mediated Isothermal Amplification assay (Eiken Chemical Company) relies on a novel form of nucleic acid amplification with sufficient efficiency that enough DNA is generated to enable detection by visual inspection of fluorescence.[77] The method has been evaluated on a limited basis and has been shown to have high sensitivity for smear-positive specimens but low sensitivity for smear-negative specimens.[77]

5.12. *Oligonucleotide microarray*

Oligonucleotide microarray technology allows for the simultaneous detection of multiple genetic sequences, which can be used to detect either conserved sequences for detection of microorganisms and/or detection of mutations in sequences that confer drug resistance of an isolate. One of these assays, The TB-Biochip (Engelhardt Institute of Molecular Biology), has been evaluated for the ability of the system to detect rifampin resistance in MTB.[78] In a small study comparing the microarray with conventional AST, the assay showed a sensitivity of 80% for detecting rifampin resistance.[78]

5.13. *Xpert MTB/RIF*

GeneXpert MTB/RIF (Cepheid) is, a semi-automated molecular test, based on molecular beacon technology to detect DNA sequences amplified in a hemi-nested real time-PCR assay. In the same multiplex reaction five different hybridization probes are used. Each nucleic acid probe is complementary to a different target sequence within the *rpo*B gene of RIF-susceptible MTB and is labeled with a differently colored fluorophore. Together, these overlapping probes span the entire 81 bp RIF resistance determining region (RRDR) of the *rpo*B gene.[79] In a partial evaluation, the assay was shown to be 100% sensitive for detecting smear-positive isolates but only 71.7% sensitive for detecting smear-negative culture-positive isolates.[80] In a big pitch trial, the assay was shown to be 98.2% sensitive for the identification of culture-positive isolates but only 72.5% sensitive for the identification of smear-negative culture positive isolates; the test had a reported specificity of 99.2%.[81] The advantage of this test lies both in its increased sensitivity compared to sputum smear alone (sensitivity >98% on smear positive, >70% on smear negative samples), and in its improved time to diagnosis and treatment. So, this is a rapid molecular assay that can be used close to the point of care by operators with minimal technical expertise, enabling diagnosis of TB and simultaneous assessment of RIF resistance to be completed within 2 h. Moreover, this can be accomplished using unprocessed sputum samples as well as clinical specimens from extrapulmonary sites. One obvious disadvantage to this system is the inability to test for and detect INH resistance. Other potential disadvantages include cost but it has advantage over line-probe assays for lesser laboratory infrastructure and training of personnel.

6. Conclusions

Conventional DST is still gold standard for the detection of DR-TB but are cumbersome and has long turnaround time (TAT). Automated liquid culture DST methods have less TAT and are highly accurate but are not a replacement for conventional methods as most are not reliable when used with smear-negative specimens and needs sophisticated instruments and trained personnel. High cost of these tests is another disadvantage. Molecular methods look for genetic determinants of resistance rather than the resistance phenotype so have very less turnaround time but highly expensive. Further the association between phenotypic and molecular DST remains challenging due to insufficient knowledge of the mutations underlying drug resistance. So we can conclude that there is still lack of a 100% sensitive and specific method for the diagnosis of DR-TB. Therefore, more attention and research is needed to develop effective and cheap methods in different settings to overcome this worldwide problem.

Conflicts of interest

All authors have none to declare.

REFERENCES

1. Zhang Y, Yew WW. Mechanisms of drug resistance in *Mycobacterium tuberculosis*. *Int J Tuberc Lung Dis*. 2009;13:1320–1330.
2. Buck M, Schnitzer RJ. The development of drug resistance of *M. tuberculosis* to isonicotinic acid hydrazide. *Am Rev Tubercul*. 1952;65:759–760.
3. Mitchison DA, Nunn AJ. Influence of initial drug resistance on the response to short-course chemotherapy of pulmonary tuberculosis. *Am Rev Respir Dis*. 1986;133:423–430.
4. Prasad R. Multidrug and extensively drug-resistant TB (M/XDR-TB): problems and solutions. *Indian J Tuberc*. 2010;57:180–191.
5. Canetti G, Froman S, Grosset J, Hauduroy P, Langerova M, Mahler HT. Mycobacteria: laboratory methods for testing drug sensitivity and resistance. *Bull Worl Heal Org*. 1963;9:565–578.
6. Kent PT, Kubica GP. *Public Health Mycobacteriology: A Guide for the Level III Laboratory*. Atlanta, GA: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention; 1985:96–103.
7. Richter E, Rüsch-Gerdes S, Hillemann D. Drug-susceptibility testing in TB: current status and future prospects. *Expert Rev Respir Med*. 2009;3:497–510.
8. Canetti G, Fox W, Khomenko A, et al. Advances in techniques of testing mycobacterial drug sensitivity, and the use of sensitivity tests in tuberculosis control programmes. *Bull World Health Organ*. 1969;41:21–43.
9. The WHO/IUATLD. *Global Project on Anti-tuberculosis Drug Resistance Surveillance*. Report 3. WHO/HTM/TB/2004.343. Geneva, Switzerland: WHO; 2004.
10. Roberts GD, Goodman NL, Heifets L, et al. Evaluation of the BACTEC radiometric method for recovery of mycobacteria and drug susceptibility testing of *Mycobacterium tuberculosis* from acid-fast smear-positive specimens. *J Clin Microbiol*. 1983;18:689–696.
11. Heifets LB, Cangelosi GA. Drug susceptibility testing of *Mycobacterium tuberculosis*: a neglected problem at the turn of the century. *Int J Tuberc Lung Dis*. 1999;3:564–581.
12. Pfyffer GE, Bonato DA, Ebrahimzadeh A, et al. Multicenter laboratory validation of susceptibility testing of *Mycobacterium tuberculosis* against classical second-line and newer antimicrobial drugs by using the radiometric BACTEC 460 technique and the proportion method with solid media. *J Clin Microbio*. 1999;37:3179–3186.
13. Tiwari RP, Hattikudur NS, Bharmal RN, Kartikeyan S, Deshmukh NM, Bisen PS. Modern approaches to a rapid diagnosis of tuberculosis: promises and challenges ahead. *Tuberculosis*. 2007;87:193–201.
14. Piersimoni C, Scarparo C, Callegaro A, et al. Comparison of MB/Bact alert 3D system with radiometric BACTEC system and Löwenstein-Jensen medium for recovery and

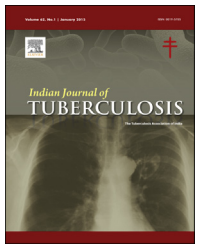
identification of mycobacteria from clinical specimens: a multicenter study. *J Clin Microbiol*. 2001;39:651–657.
15. Pfyffer GE, Welscher HM, Kissling P, et al. Comparison of the mycobacteria growth indicator tube (MGIT) with radiometric and solid culture for recovery of acid-fast bacilli. *J Clin Microbiol*. 1997;35:364–368.
16. Palomino JC, Traore H, Fissette K, Portaels F. Evaluation of Mycobacteria Growth Indicator Tube (MGIT) for drug susceptibility testing of *Mycobacterium tuberculosis*. *Int J Tuberc Lung Dis*. 1999;3:344–348.
17. Johansen IS, Thomsen VO, Marjamaki M, Sosnovskaja A, Lundgren B. Rapid, automated, nonradiometric susceptibility testing of *Mycobacterium tuberculosis* complex to four first-line antituberculous drugs used in standard short-course chemotherapy. *Diagn Microbiol Infect Dis*. 2004;50:103–107.
18. Rusch-Gerdes S, Pfyffer GE, Casal M, Chadwick M, Siddiqi S. Multicenter laboratory validation of the BACTEC MGIT 960 technique for testing susceptibilities of *Mycobacterium tuberculosis* to classical second-line drugs and newer antimicrobials. *J Clin Microbiol*. 2006;44:688–692.
19. Woods GL, Fish G, Plaunt M, Murphy T. Clinical evaluation of Difco ESP Culture System II for growth and detection of mycobacteria. *J Clin Microbiol*. 1997;35:121–124.
20. Devasia RA, Blackman A, May C, et al. Fluoroquinolone resistance in *Mycobacterium tuberculosis*: an assessment of MGIT 960, MODS and nitrate reductase assay and fluoroquinolone cross-resistance. *J Antimicrob Chemother*. 2009;63:1173–1178.
21. Moore DA, Evans CA, Gilman RH, et al. Microscopic-observation drug-susceptibility assay for the diagnosis of TB. *N Engl J Med*. 2006;355:1539–1550.
22. Palomino JC, Martin A, Portaels F. Rapid colorimetric methods for the determination of drug resistance in *Mycobacterium tuberculosis*. *Res Adv Antimicrob Agents Chemother*. 2005;4:29–38.
23. Yajko DM, Madej JJ, Lancaster MV, et al. Colorimetric method for determining MICs of antimicrobial agents for *Mycobacterium tuberculosis*. *J Clin Microbiol*. 1995;33:2324–2327.
24. Collins L, Franzblau SG. Microplate alamar blue assay versus BACTEC 460 system for high-throughput screening of compounds against *Mycobacterium tuberculosis* and *Mycobacterium avium*. *Antimicrob Agents Chemother*. 1997;41:1004–1009.
25. Palomino JC, Portaels F. Simple procedure for drug susceptibility testing of *Mycobacterium tuberculosis* using a commercial colorimetic assay. *Eur J Clin Microbiol Infect Dis*. 1999;18:380–383.
26. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods*. 1983;65:55–63.
27. Mshana RN, Tadesse G, Abate G, Miorner H. Use of 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide for rapid detection of rifampin-resistant *Mycobacterium tuberculosis*. *J Clin Microbiol*. 1998;36:1214–1249.
28. Foongladda S, Roengsanthia D, Arjrattanakool W, Chuchottaworn C, Chaiprasert A, Franzblau SG. Rapid and simple MTT method for rifampicin and isoniazid susceptibility testing of. *Mycobacterium Tuberc Int J Tuberc Lung Dis*. 2002;6:1118–1122.
29. Wilson SM, al-Suwaidi Z, McNerney R, Porter J, Drobniewski F. Evaluation of a new rapid bacteriophage-based method for the drug susceptibility testing of. *Mycobacterium Tuberc Nat Med*. 1997;3:465–468.
30. McNerney R. Phage replication technology for diagnosis and susceptibility testing. In: Parish T, Stocker NG, eds. Mycobacterium tuberculosis *protocols. Methods in Molecular Medicine*. Totowa, NY: Humana Press; 2001:145–154.
31. Jacobs WRJ, Barletta RG, Udani R, et al. Rapid assessment of drug susceptibilities of *Mycobacterium tuberculosis* by means of luciferase reporter phages. *Science*. 1993;260: 819–822.
32. Banaiee N, Bobadilla-del-Valle M, Riska PF, et al. Rapid identification and susceptibility testing of *Mycobacterium tuberculosis* from MGIT cultures with luciferase reporter mycobacteriophages. *J Med Microbiol*. 2003;52:557–561.
33. Mejia GI, Castrillon L, Trujillo H, Robledo JA. Microcolony detection in 7H11 thin layer culture is an alternative for rapid diagnosis of *Mycobacterium tuberculosis* infection. *Int J Tuberc Lung Dis*. 1999;3:138–142.
34. Hausdorfer J, Sompek E, Allerberger F, Dierich MP, Rüsch-Gerdes S. E-test for susceptibility testing of *Mycobacterium tuberculosis*. *Int J Tuberc Lung Dis*. 1998;2:751–755.
35. Palomino JC, Martin A, Von Groll A, Portaels F. Rapid culture-based methods for drug-resistance detection in *Mycobacterium tuberculosis*. *J Microbiol Methods*. 2008;75:161–166.
36. Angeby KA, Klintz L, Hoffner SE. Rapid and inexpensive drug susceptibility testing of *Mycobacterium tuberculosis* with a nitrate reductase assay. *J Clin Microbiol*. 2002;40:553–555.
37. Musa RH, Ambroggi M, Souto A, Angeby KA. Drug susceptibility testing of *Mycobacterium tuberculosis* by a nitrate reductase assay applied directly on microscopy-positive sputum samples. *J Clin Microbiol*. 2005;43:3159–3161.
38. Gupta A, Sen MR, Mohapatra TM, Anupurba S. Evaluation of the performance of nitrate reductase assay for rapid drug-susceptibility testing of *Mycobacterium tuberculosis* in North India. *J Health Popul Nutr*. 2011;29:20–25.
39. Gupta A, Anupurba S. Direct drug susceptibility testing of *Mycobacterium tuberculosis* against primary anti-TB drugs in northern India. *J Infect Dev Ctries*. 2010 Nov 24;4:695–703.
40. Viader-Salvadó JM, Garza-González E, Valdez-Leal R, del Bosque-Moncayo MA, Tijerina-Menchaca R, Guerrero-Olazarán M. Mycolic acid index susceptibility method for *Mycobacterium tuberculosis*. *J Clin Microbiol*. 2001;39:2642–2645.
41. Pai S, Esen N, Pan X, Musser JM. Routine rapid Mycobacterium species assignment based on speciesspecific allelic variation in the 65-kilodalton heat shock protein gene (hsp65). *Arch Pathol Lab Med*. 1997;121:859–864.
42. Victor TC, van Helden PD. Detection of mutations in *Mycobacterium tuberculosis* by a dot blot hybridization strategy. In: Mycobacterium tuberculosis *Protocols. Methods in Molecular Medicine*. vol. 54. New Jersey: Humana Press; 2001:155–164.
43. Loman NJ, Pallen MJ. XDR-TB genome sequencing: a glimpse of the microbiology of the future. *Future Microbiol*. 2008;3:111–113.
44. Zhao JR, Bai YJ, Wang Y, Zhang QH, Luo M, Yan XJ. Development of a pyrosequencing approach for rapid screening of rifampin, isoniazid and ethambutol-resistant *Mycobacterium tuberculosis*. *Int J Tuberc Lung Dis*. 2005;9:328–332.
45. Jureen P, Engstrand L, Eriksson S, Alderborn A, Krabbe M, Hoffner SE. Rapid detection of rifampin resistance in *Mycobacterium tuberculosis* by pyrosequencing technology. *J Clin Microbiol*. 2006;44:1925–1929.
46. Engström A, Morcillo N, Imperiale B, Hoffner SE, Juréen P. Detection of first- and second-line drug resistance in *Mycobacterium tuberculosis* clinical isolates by pyrosequencing. *J Clin Microbiol*. 2012;50:2026–2033.
47. Bravo LT, Tuohy MJ, Ang C, et al. Pyrosequencing for rapid detection of *Mycobacterium tuberculosis* resistance to rifampin, isoniazid, and fluoroquinolones. *J Clin Microbiol*. 2009;47:3985–3990.
48. Mokrousov I, Narvskaya O, Limeschenko E, Otten T, Vyshnevskiy B. Detection of ethambutol-resistant *Mycobacterium tuberculosis* strains by multiplex allele-specific PCR assay targeting *embB*306 mutations. *J Clin Microbiol*. 2002;40:1617–1620.

49. Mokrousov I, Otten T, Filipenko M, et al. Detection of isoniazid-resistant *Mycobacterium tuberculosis* strains by a multiplex allele-specific PCR assay targeting *katG* codon 315 variation. *J Clin Microbiol*. 2002;40:2509–2512.
50. Mokrousov I, Otten T, Vyshnevskiy B, Narvskaya O. Allele-specific rpoB PCR assays for detection of rifampin-resistant *Mycobacterium tuberculosis* in sputum smears. *Antimicrob Agents Chemother*. 2003;47:2231–2235.
51. Yang Z, Durmaz R, Yang D, et al. Simultaneous detection of isoniazid, rifampin, and ethambutol resistance of *Mycobacterium tuberculosis* by a single multiplex allele- specific polymerase chain reaction (PCR) assay. *Diagn Microbiol Infect Dis*. 2005;53:201–208.
52. Evans J, Segal H. Novel multiplex allele-specific PCR assays for the detection of resistance to second-line drugs in *Mycobacterium tuberculosis*. *J Antimicrob Chemother*. 2010;65:897–900.
53. Gupta A, Prakash P, Singh SK, Anupurba S. Rapid genotypic detection of *rpoB* and *katG* gene mutations in *Mycobacterium tuberculosis* clinical isolates from Northern India as determined by MAS-PCR. *J Clin Lab Anal*. 2013;27:31–37.
54. Gryadunov D, Mikhailovich V, Lapa S, et al. Evaluation of hybridisation on oligonucleotide microarrays for analysis of drug-resistant *Mycobacterium tuberculosis*. *Clin Microbiol Infect*. 2005;11:531–539.
55. Telenti A, Imboden P, Marchesi F, Schmidheini T, Bodmer T. Direct, automated detection of rifampin-resistant *Mycobacterium tuberculosis* by polymerase chain reaction and single-strand conformation polymorphism analysis. *Antimicrob Agents Chemother*. 1993;37:2054–2058.
56. Williams DL, Spring L, Gillis TP, Salfinger M, Persing DH. Evaluation of a polymerase chain reaction based universal heteroduplex generator assay for direct detection of rifampin susceptibility of *Mycobacterium tuberculosis* from sputum specimens. *Clin Infect Dis*. 1998;26:446–450.
57. Abebe G, Paasch F, Apers L, Rigouts L, Colebunders R. Tuberculosis drug resistance testing by molecular methods: opportunities and challenges in resource limited settings. *J Microbiol Meth*. 2011;84:155–160.
58. Antonova OV, Gryadunov DA, Lapa SA, et al. Detection of mutations in *Mycobacterium tuberculosis* genome determining resistance to fluoroquinolones by hybridization on biological microchips. *Bull Exp Biol Med*. 2008;145:108–113.
59. Morgan M, Kalantri S, Flores L, Pai M. A commercial line probe assay for the rapid detection of rifampicin resistance in *Mycobacterium tuberculosis*: a systematic review and meta analysis. *BMC Infect Dis*. 2005;5:62. http://dx.doi.org/10.1186/1471-2334-5-62.
60. Bwanga F, Hoffner S, Haile M, Joloba ML. Direct susceptibility testing for multi drug resistant tuberculosis: a meta-analysis. *BMC Infect Dis*. 2009;9:67–81.
61. Hillemann D, Weizenegger M, Kubica T, Richter E, Niemann S. Use of the genotype MTBDR assay for rapid detection of rifampin and isoniazid resistance in *Mycobacterium tuberculosis* complex isolates. *J Clin Microbiol*. 2005;43:3699–3703.
62. Hillemann D, Rüsch-Gerdes S, Richter E. Application of the Genotype MTBDR assay directly on sputum specimens. *Int J Tuberc Lung Dis*. 2006;10:1057–1059.
63. Huyen MNT, Tiemersma EW, Lan NT, et al. Validation of the GenoType MTBDRplus assay for diagnosis of multidrug resistant tuberculosis in South Vietnam. *BMC Infect Dis*. 2010;10:149. http://dx.doi.org/10.1186/1471-2334-10-149.
64. Hillemann D, Rüsch-Gerdes S, Richter E. Feasibility of the GenoType MTBDRsl assay for fluoroquinolone, amikacin-capreomycin, and ethambutol resistance testing of *Mycobacterium tuberculosis* strains and clinical specimens. *J Clin Microbiol*. 2009;47:1767–1772.
65. Cherkasova E, Laassri M, Chizhikov V, et al. Microarray analysis of evolution of RNA viruses: evidence of circulation of virulent highly divergent vaccine-derived polioviruses. *Proc Natl Acad Sci USA*. 2003;100:9398–9403.
66. Denkin S, Volokhov D, Chizhikov V, Zhang Y. Microarray-based pncA genotyping of pyrazinamide-resistant strains of *Mycobacterium tuberculosis*. *J Med Microbiol*. 2005;54:1127–1131.
67. Shimizu Y, Dobashi K, Yoshikawa Y, et al. Five-antituberculosis drug-resistance genes detection using array system. *J Clin Biochem Nutr*. 2008;42:228–234.
68. Espasa M, González-Martín J, Alcaide F, et al. Direct detection in clinical samples of multiple gene mutations causing resistance of *Mycobacterium tuberculosis* to isoniazid and rifampicin using fluorogenic probes. *J Antimicrob Chemother*. 2005;55:860–865.
69. Saribas Z, Yurdakul P, Alp A, Gunalp A. Use of fluorescence resonance energy transfer for rapid detection of isoniazid resistance in *Mycobacterium tuberculosis* clinical isolates. *Int J Tuberc Lung Dis*. 2005;9:181–187.
70. Varma-Basil M, El-Hajj H, Colangeli R, et al. Rapid detection of rifampin resistance in *Mycobacterium tuberculosis* isolates from India and Mexico by a molecular beacon assay. *J Clin Microbiol*. 2004;42:5512–5516.
71. Edwards KJ, Metherell LA, Yates M, Saunders NA. Detection of *rpoB* mutations in *Mycobacterium tuberculosis* by biprobe analysis. *J Clin Microbiol*. 2001;39:3350–3352.
72. Mokrousov I, Narvskaya O, Otten T, Limeschenko E, Steklova L, Vyshnevskiy B. High prevalence of katG Ser315Thr substitution among isoniazid-resistant *Mycobacterium tuberculosis* clinical isolates from northwestern Russia, 1996 to 2001. *Antimicrob Agents Chemother*. 2002;46:1417–1424.
73. Leung ET, Kam KM, Chiu A, et al. Detection of katG Ser315Thr substitution in respiratory specimens from patients with isoniazid-resistant *Mycobacterium tuberculosis* using PCR-RFLP. *J Med Microbiol*. 2003;52:999–1003.
74. Herrera-León L, Molina T, Saíz P, Sáez-Nieto JA, Jiménez MS. New multiplex PCR for rapid detection of isoniazid-resistant *Mycobacterium tuberculosis* clinical isolates. *Antimicrob Agents Chemother*. 2005;49:144–147.
75. Kox LF, van Leeuwen J, Knijper S, Jansen HM, Kolk AH. PCR assay based on DNA coding for 16S rRNA for detection and identification of mycobacteria in clinical samples. *J Clin Microbiol*. 1995;33:3225–3233.
76. Park YK, Shinb TS, Ryua S, et al. Comparison of drug resistance genotypes between Beijing and non-Beijing family strains of *Mycobacterium tuberculosis* in Korea. *J Microbiol Meth*. 2005;63:165–172.
77. Boehme CC, Nabeta P, Henostroza G, et al. Operational feasibility of using loop-mediated isothermal amplification for diagnosis of pulmonary tuberculosis in microscopy centers of developing countries. *J Clin Microbiol*. 2007;45:1936–1940.
78. Caoili JC, Mayorova A, Sikes D, Hickman L, Plikaytis BB, Shinnick TM. Evaluation of the TB-Biochip oligonucleotide microarray system for rapid detection of rifampin resistance in *Mycobacterium tuberculosis*. *J Clin Microbiol*. 2006;44:2378–2381.
79. Lawn SD, Nicol MP. Xpert® MTB/RIF assay: development, evaluation and implementation of a new rapid molecular diagnostic for tuberculosis and rifampicin resistance. *Future Microbiol*. 2011;6:1067–1082.
80. Helb D, Jones M, Story E, et al. Rapid detection of *Mycobacterium tuberculosis* and rifampin resistance by use of an on-demand, nearpatient technology. *J Clin Microbiol*. 2010;48:229–237.
81. Boehme CC, Nabeta P, Hillemann D, et al. Rapid molecular detection of tuberculosis and rifampin resistance. *N Engl J Med*. 2010;363:1005–1015.

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.journals.elsevier.com/indian-journal-of-tuberculosis/



Original Article

Isolation of *Mycobacterium tuberculosis* from sputum of tribal, non-tribal pulmonary tuberculosis patients of Andaman & Nicobar islands by conventional culture method and assessment of first line anti-tuberculosis drug susceptibility patterns

A. Mandal [a,*], G. Parthasarathy [b], S.P. Burma [c], A.P. Sugunan [d], P. Vijayachari [e]

[a] Technical Assistant, Regional Medical Research Centre (ICMR), P.B.No-13, Dollygunj, Port Blair 744 101, Andaman & Nicobar Islands, India
[b] Surg. CDR, JD (Pensions), DG AFMS Office, New Delhi, India
[c] Joint Secretary & State TB Officer, State TB Control Society (RNTCP), Directorate of Health Services, GB Pant Hospital, Port Blair, Andaman & Nicobar Islands, India
[d] Scientist E, Regional Medical Research Centre (ICMR), P.B.No-13, Dollygunj, Port Blair 744 101, Andaman & Nicobar Islands, India
[e] Scientist G/ Director, Regional Medical Research Centre (ICMR), P.B.No-13, Dollygunj, Port Blair 744 101, Andaman & Nicobar Islands, India

ARTICLE INFO

Article history:
Received 21 September 2013
Accepted 21 September 2014
Available online 7 March 2015

Keywords:
Mycobacterium tuberculosis
MDR-TB
DOTS
Non-DOTS
Tuberculosis (TB)

ABSTRACT

Background: Drug resistance surveys have not yet conducted in these Islands and as such no data exists on drug resistance currently.
Aims: the present study was initiated with the objective of isolation and assessment of Drug resistance patterns of *Mycobacterium tuberculosis* isolates from sputum specimens collected from different categories of Tribal, Non-Tribal pulmonary tuberculosis patients treated under DOTS and Non-DOTS program by conventional culture and Proportion sensitivity (PST) method to detect patients with Multidrug resistant strains.
Methods: The investigation was hospital based laboratory surveillance study carried out for a period of 3 years at the selected hospitals of Andaman district (TB ward GB Pant Hospital at Port Blair, CHC Bamboflat at Port Blair and CHC Rangat at Rangat) and Nicobar district (CHC Nancowry at Nancowry groups of Islands), among the new cases and re-treatment cases of tuberculosis patient under DOTS program and Non-DOTS patients attended selected hospitals of Andaman & Nicobar districts chosen for the study.
Results: 83 culture positive isolates obtained (74 identified as *M. tuberculosis*) from the sputum specimen of 162 cases of tuberculosis patient by conventional culture method. 60

* *Corresponding author*. Tel.: +91 9474245545.
E-mail address: alok_mandal@yahoo.com (A. Mandal).
http://dx.doi.org/10.1016/j.ijtb.2015.02.004

M. tuberculosis isolates subjected to drug susceptibility test using PST method, 7 patients (11.67%) found to be Multidrug resistant tuberculosis (MDR-TB), resistant patterns were S + H + R + E = 1(Cat II-DOTS),H + r = 3(Cat-I DOTS = 1, Cat II-DOTS-1,Non-DOTS = 1), Rifampicin resistant alone = 2 (Non-DOTS = 1, Cat II-DOTS = 1) and R + E = 1(Cat I-DOTS). *Conclusions:* Laboratory finding suggested that nine MDR-TB strains detected in DOTS and Non-DOTS among 60 *M. tuberculosis* isolates were selected for drug susceptibility testing but two isolates detected as MDR-TB from patients was already on Second line drugs treatment were not included in the MDR-TB detection criteria. Hence 7 patients (11.67%) declared to be Multidrug resistant tuberculosis (MDR-TB). 2 MDR-TB strains with resistant patterns H + r = 1(Cat II-DOTS), Rifampicin resistant alone = 1(Non-DOTS) detected from 12 isolates of Tribal patients from Nicobar district and 5 MDR-TB strains with resistant patterns S + H + R + E = 1 (Cat II-DOTS), H + r = 2(Cat I-DOTS = 1,Non-DOTS = 1), Rifampicin resistant alone = 1 (Cat II-DOTS) and R + E = 1(Cat I-DOTS) detected from 48 isolates of Non-Tribal patients from Andaman district. To assess the MDR-TB burden in the islands, systematic drug resistant surveillance study needs to be conducted.

1. Introduction

Tuberculosis (TB) is one of the serious global public health problems that mainly affect people in the economically active age groups resulting in an immense loss to communities and countries. *Mycobacterium tuberculosis* (MTB) infects over one third of the world's population.[1] The global report on tuberculosis (TB) estimates that in 2010, there are 8.8 (8.5–9.2) million incident cases of TB resulting in 1.1 (0.9–1.2)million deaths among HIV- negative people and an additional 0.35 (0.32–0.39)million deaths from HIV-associated TB. In 2010, there were 5.7 million notifications of new and recurrent cases of TB, equivalent to 65% (63%–68%) of the estimated number of incident cases in 2010. India and China accounted 40% of the world's notified cases of TB in 2010, Africa for a further 24% and the 22 high-TB burden countries (HBCs) for 82%. Between 1995 and 2010, 55 million TB patients were treated in programmes that had adopted the DOTS stop TB strategy, and in 46 million cases the treatment was successful. This successful treatment saved almost seven million lives.[2]

Alongside these achievements, diagnosis and appropriate treatment of multidrug-resistant tuberculosis (MDR-TB) remains major challenges. Less than 5% of new and previously treated TB patients were tested for MDR-TB in most countries in 2010. The reported number of patients enrolled on treatment has increased, reaching 46,000 in 2010. However, this was equivalent to only 16% of the 290,000 cases of MDR-TB estimated to exist among notified TB patients in 2010.[2]

India is a developing country and ranked 17th among 22 high burden countries in terms of TB incidence rate. India has adapted and tested the DOTS strategy in various parts of the country since 1993. Since 1999, drug resistance surveys were initiated in different parts of the country. At present India has the highest MDR-TB burden in the world contributing approximately 30% to the global burden. The prevalence of Multi-drug resistant tuberculosis (MDR-TB) defined as resistance to Isoniazid and Rifampicin with or without resistance to other drugs, is found to be at a low level in most of the regions. Data from studies conducted by Tuberculosis Research Centre (TRC) and National Tuberculosis Institute (NTI), have found MDR-TB levels of less than 1%–3% in new cases and around 12% in re-treatment cases.[3,4]

The Andaman & Nicobar Islands, an archipelago of 527 islands in the Bay of Bengal, is administratively a Union Territory of India. The population of the territory is about 3,79,944 (2011 census) that includes six indigenous tribal groups. The Union Territory of Andaman and Nicobar Islands is divided into three districts *viz.* North and Middle Andaman, South Andaman and Nicobar. More than 98% of the population of Nicobar district is constituted by the Mongoloid tribe Nicobarese.

An intensified TB control programme was carried out among the Nicobarese of Car Nicobar Island in the year 1986. All identified cases were treated and INH chemoprophylaxis was administered to children aged 5–14 years. Prevalence of sputum positive cases was 4.1 per 1000 and prevalence of infection among children was 9.3%. Later anti-tuberculosis activities were carried out as per District tuberculosis program (DTP). The centre assessed the impact of the intensified TB control programme started in 1986 and DTP that followed on tuberculosis situation in 2001–02. The observed prevalence of infection among children was 16.4%. The estimated annual risk of TB infection (ARTI) was 2.4% and prevalence of sputum positive cases was 7.28 per 1000. These findings showed that annual risk of tuberculosis infection and prevalence of tuberculosis had increased almost two-fold during the intervening period of nearly 15 years. The Revised National TB control programme (RNTCP) based on the internationally recommended Directly Observed treatment Short-course (DOTS) strategy was introduced in the islands in the year 2005.[5] Drug resistance surveys have not yet conducted in these Islands and as such no data exists on drug resistance currently. Therefore, the present study was initiated with the objective of isolation of *M. tuberculosis* from clinical specimens collected from tribal and Non-tribal pulmonary tuberculosis patients treated under different categories of DOTS and Non-DOTS program by conventional culture and Proportion

sensitivity (PST) method to observe the drug susceptibility patterns of the clinical isolates to detect patients with multi-drug resistant strains.

2. Material & methods

This was a hospital based study carried out among chest symptomatic patients, new confirmed cases and patients on DOTS and Non-DOTS belonging to tribal and non-tribal populations of Andaman and Nicobar Islands. Patients belonging to Nicobarese tribal community were recruited at Community Health Centre (CHC), at Nancowry in Nicobar District. Non-tribal patients were recruited from the TB ward of GB Pant Hospital, Port Blair, CHC, Bamboflat and CHC, Rangat situated in Andaman district. The study was carried out during the period of December 2006 to July 2009.

Three specimens of sputum (two spot and one morning) were obtained over two consecutive days from each subject of chest symptomatic patients suspected of tuberculosis and two specimens of sputum (one spot and one morning) were collected from tuberculosis patients on anti-TB treatment follow-up cases of DOTS and Non-DOTS (as per RNTCP guidelines) were send from Andaman district to the laboratory maintaining cold-chain conditions at 4°c. Similarly transport medium (equal volume of 1% cetyl pyridinium chloride in 2% sodium chloride) was added to the all specimen collected from CHC Nancowry, Nicobar district were send to the laboratory.

Sputum smears were prepared and stained by Ziehl Neelsen (Z-N) method and examined for acid-fast bacilli. Homogenization & Decontamination of sputum specimens for culture was performed by using 4% Sodium hydroxide (NaOH) solution following Modified Petroff's method. To the sputum specimens 4% NaOH to be added in double the volume, and shake to digest the sputum, let stand for 15 min at room temperature with occasional shaking. After 15 min centrifuge at 3000 g for 15 min, carefully pour off the supernatant and to the deposits add approximately 20 ml sterile distilled water and resuspend the sediment. Centrifuge again at 3000 g for 15 min and decant supernatant and kept sediment for L-J medium inoculation. Transport medium equal volume of 1% cetyl pyridinium chloride (CPC) in 2% sodium chloride (NaCl) contained specimens were processed (as per TRC lab manual 2006) by adding 15–20 ml sterile distilled water to the specimen with CPC and mix well by inversion, centrifuge at 3000 g for 15 min. Carefully pour off the supernatant, to the sediment add approximately 20 ml sterile distilled water and resuspend the sediment. Centrifuge again at 3000 g for 15 min, decant the supernatant and kept sediment for inoculation onto L-J medium. From the sediment smears were prepared and stained by Z-N method. All Processed sputum specimens sediments were then inoculated onto Lowenstein Jensen (L-J) medium slopes in McCartney Bottles (three specimens inoculated onto six LJ slopes and for two specimens inoculated onto four LJ slopes) and incubated at 37°c for a period of 8 weeks.

Colonies on L-J medium slopes were identified as *M. tuberculosis* or Non-tuberculosis *Mycobacterium* using biochemical test panel that includes Niacin production test, susceptibility to p-Nitro Benzoic acid test (500 μg/ml concentration) and Catalase activity at 68°c/pH 7 tests.

Susceptibility of the *M. tuberculosis* isolates to first line drugs Streptomycin, Isoniazid, Rifampicin and Ethambutol was tested by proportion sensitivity test (PST) method. Dihydro-streptomycin sulphate (SIGMA) at a concentration of 4 μg/ml, Isoniazid (SIGMA) at 0.2 μg/ml, Rifampicin (SIGMA) at 40 μg/ml and Ethambutol (SIGMA) at 2 μg/ml concentration were used for susceptibility testing. Approximately 4 mg moist weight of culture was scrapped and added into 4 ml of sterile distilled water to prepare neat suspension (S) 1 mg/ml, which was then further diluted into 1×10^{-1} mg/ml ($S^{1)}$, 1×10^{-2} mg/mlS^{2} and 1×10^{-3} mg/ml (S^{3})concentrations following standard operating protocol of RNTCP. For each isolate, S was inoculated into two L-J slopes and one PNB slope without any drug, S^1 and S^2 dilutions were onto two drug free L-J slopes and one LJ slope each containing all the four drugs tested and S3 onto two L-J slopes without any drug. The standard strain H_{37}RV (procured from National Institute of Research in Tuberculosis) was used during culture, biochemical test and DST. Inoculated slopes were further incubated at 37 °C for 42 days and read twice once on the 28th day and then on the 42nd day.

If an isolates shows clear-cut resistance on the 28thday, no further reading was recorded and the test was terminated. Isolates that were found susceptible on 28thdays' reading were further taken on 42ndday and the report was based on the later reading only. For each strain, the number of organisms resistant to each drug concentration was expressed as a percentage of the number of organisms growing on the drug free slopes.[6]

3. Results

A total of 162 patients were screened during the period 2006–2009, of which 78 patients were from Andaman District (Non-tribal patient) and the remaining 84 from Nicobar District. The 84 patients included from Nicobar District belonged to the indigenous mongoloid tribe called Nicobarese (tribal patient). Out of 162 sputum samples, 83 culture positives were obtained, 69 from the patients of Andaman and 14 from the patients of Nicobar. The culture positivity rate in Andaman and Nicobar Districts were 88.5% and 16.7% respectively. This difference in the culture positivity rate between Andaman District and Nicobar district was statistically significant ($\chi^2 = 83.44$, $p < 0.0000$). The culture contamination rate 3% was observed.

The break-up of patients by health facility and category of disease/treatment is summarized in Table 1. More than 50% of the patients were on Category I treatment. The overall culture positivity was 51.2%. All the four patients who were on second line drugs and the only case with extra-pulmonary tuberculosis were positive in culture. Culture positivity was lowest among patients on Category III treatment (Table 2).

Among the 83 culture positive isolates, 74 were identified as *M. tuberculosis*, two were non-tuberculosis *Mycobacteria* and the remaining seven remained unidentified due to insufficient culture growth on LJ slopes. Out of the 74 *M.* tuberculosis isolates, 60 (isolates of DOTS patients = 56, Non-DOTS = 4) were found to be more than 1 + culture growth suitable to carried out first line drug susceptibility testing and remaining 14 isolates culture growth was obtained below

Table 1 – Patients and isolates by treatment/disease category and health facility of recruitment.

Hospital	New case	On treatment						Total
		Cat I	Cat II	Cat III	2nd Line drugs	Non-DOTS	Extra pulmonary	
Andaman district								
CHC, Rangat	9	3	1	1	0	0	0	14
GB Pant Hospital	4	40	9	0	3	5	0	61
CHC, Bambooflat	0	1	0	0	1	0	0	2
INHS dhanvantari	0	0	0	0	0	0	1	1
Total	**13**	**44**	**10**	**1**	**4**	**5**	**1**	**78**
Nicobar district								
CHC, Nancowry	7	45	14	12	0	6	0	84
TOTAL A&N	**20**	**89**	**24**	**13**	**4**	**11**	**1**	**162**
Isolates	12	46	10	3	4	7	1	83
Positivity (%)	60.0	51.7	41.7	23.1	100.0	63.6	100.0	51.2

1 + growth, hence not selected for the DST. 56 isolate of DOTS patients obtained from new cases six and remaining 50 from re-treatment cases. 37 (61.7%) were sensitive to all the four drugs (Non-DOTS = 2, DOTS = 35). Thirteen (21.7%) isolates were resistant to Streptomycin, 12 (20.0%) to Isoniazid, nine (15.0%) to Rifampicin and five (8.3%) to Ethambutol (included into MDR pattern, other resistant patterns and mono-drug resistant patterns). Six (10%) isolates were resistant to Streptomycin alone while mono-drug resistance to Isoniazid, Rifampicin and Ethambutol was observed in two (3.3%) isolates each. Among the six isolates tested from new cases, two (33.3%) were resistant to Streptomycin. Resistance to no other drug was observed in these six isolates from new cases, indicating that all other drug resistance detected was acquired resistance. Fifty-four isolates were from patients who were already on anti-tuberculosis medication and based on the numbers of isolates among these that showed resistance to each of the four drugs the prevalence of acquired resistance to S, H, R and E were 20.3%, 22.2%, 16.7% and 9.3% respectively.

A total of 11 (18.3%) isolates were resistant to more than one drug and together these isolates showed five different patterns involving resistance to more than one drug. These drug resistance patterns were R + H, R + H + S, R + H + S + E, S + H and R + E. 7 (11.67%) of these isolates qualified as per the criteria for categorizing as multi-drug resistant (MDR) strains as these were resistant to both Rifampicin and Isoniazid and two (3.33%) isolate was found to be resistant to Rifampicin alone also qualified for MDR strains. The patterns obtained were from tribal patients Rifampicin alone = 1(Non-DOTS) and H + R = 1(cat II-DOTS). Similarly the patterns observed among Non-tribal patients S + H + R + E = 2, (CatII-DOTS-1, one patient already in second line drugs treatment), Rifampicin alone = 1(Cat II-DOTS), H + R = 2(each from cat I-DOTS and Non-DOTS), H + R + S = 1(DOTS but the patient already in second line drugs treatment) and R + E = 1(Cat I-DOTS). Two isolates detected as MDR-TB from patients was already on Second line drugs treatment from Andaman district were not included in the MDR-TB detection criteria.

Out of the 60 isolates tested for drug susceptibility, 12 were from Nicobar District and the remaining 48 were from Andaman District. In Nicobar District, resistance to S, R and H were found in 2 (16.67%), 1 (8.33%) and 1 (8.33%) isolates respectively while these were found in 10 (20.8%), 7 (14.6%) and 9 (18.8%) isolates respectively. Ethambutol resistance was not observed in any of the 12 isolates tested from Nicobar District, while it was observed in 2 (4.1%) of the 48 isolates tested from Andaman District. Among the 12 isolates of tribal tested from Nicobar District, two (16.7%) was multi-drug resistant (Non-DOTS = 1, DOTS = 1) while among the 48 isolates tested from Andaman District, five (8.33%) were declared to be multi-drug resistant (Non-DOTS = 1, DOTS = 4). The details of DST patterns were tabulated in Tables 3 and 4.

4. Discussion

No prior data on drug resistance in *M. tuberculosis* isolates of Andaman and Nicobar Islands existed because there were no facilities for culture and drug sensitivity testing of *M. tuberculosis* in the Islands. This facility was set up recently and as a

Table 2 – Selected 60 *M. tuberculosis* isolates obtained from different category wise treatment patient, registered under different hospitals for drug susceptibility testing (DST) are tabulated.

Hospital	New case	On treatment						Total
		Cat I	Cat II	Cat III	2nd Line drugs	Non-DOTS	Extra pulmonary	
Andaman district								
CHC, Rangat	0	3	0	0	0	0	0	03
GB Pant Hospital	0	33	7	0	1	3	0	44
CHC, Bambooflat	0	0	0	0	1	0	0	01
INHS dhanvantari	0	0	0	0	0	0	0	00
Total	**0**	**36**	**7**	**0**	**2**	**3**	**0**	**48**
Nicobar district								
CHC, Nancowry	6	2	1	2	0	1	0	12
TOTAL A&N	**6**	**38**	**8**	**2**	**2**	**4**	**0**	**60**

Table 3 – Summary of DST patterns of Andaman districts for 48 isolates of *M. tuberculosis* (along with patient's category wise treatment).

Hospitals	Number of isolates selected for DST along with patient's category wise treatment	DST patterns of the isolates
Andaman district		
CHC Rangat	3 isolates of cat I treatment patient	• Mono-drug ethambutol resistant-1 • Mono-drug streptomycin resistant-1 • MDR (R + E) resistant-1
GB Pant Hospital	33 isolates of cat I treatment patient	• All drug susceptible-27 • MDR (H + R) resistant-1 • Mono-drug streptomycin resistant-3 • Other patterns (S + H) resistant-2
	7 isolates of cat II treatment patient	• All drug susceptible-2 • MDR (S + H + R + E) resistant-1 • MDR (R) resistant-1 • Mono-drug ethambutol resistant-1 • Mono-drug isoniazid resistant-1 • Other patterns (S + H) resistant-1
	1 s line drug treatment patient	• MDR (S + H + R + E) resistant-1
	3 Non-DOTS treatment patient	• All drug susceptible-2 • MDR (H + R) resistant-1
CHC, Bambooflat	1 s line drug treatment patient	• MDR (H + R + S) resistant-1

preliminary inquiry into the drug-resistance status of *M. tuberculosis* isolates, the present study was undertaken.

The possible reasons for the difference in culture positivity rate in Andaman (88.5%) and Nicobar districts (16.7%) was due to strict adherence to DOTS programme and treatment regimens by tribal patients under RNTCP programme due to which smear negative and culture negative conversion rate is higher observed during the treatment course.

Prevalence of resistance to the four first-line drugs tested was in the range of 8%–22%. The only primary resistance detected was that against Streptomycin observed in 33% of the isolates from new cases. Although the prevalence of primary Streptomycin resistance observed in the present study was much higher than the median prevalence of primary resistance to at least one drug (10.7%) observed in the second WHO/IUATLD global project on drug resistance surveillance,[7] the number of new cases tested in the present study was too low for the estimate to be precise and the comparison to be meaningful. The prevalence of acquired resistance to the four first-line drugs was in the range of 9%–22% and was all higher than the median prevalence of acquired resistance to the respective drugs observed in the second WHO/UATLD project.

Present study indicated that 8%–10% of the isolates were multi-drug resistant in the islands. However, none of the MDR isolates were from new cases, indicating that the study did not detect any primary multi-drug resistance. The prevalence of acquired multi-drug resistance estimated is 11.67% among DOTS (Cat I and Cat II drug treatment) and Non-DOTS patients which are slightly higher than the median prevalence of multi-drug resistance observed in the WHO/IUALTD surveillance.[7] Mono-drug resistant to streptomycin, isoniazid, ethambutol and other patterns of resistance (S + H) was observed among retreatment cases of cat I, cat II and cat III DOTS patients.

This is the first attempt to estimate prevalence of drug resistance among *M. tuberculosis* isolates from Andaman and Nicobar Islands and the results indicate that drug resistance is comparable to or slightly higher than the prevalence observed in mainland India and the median prevalence in world-wide surveys. Tuberculosis is a major public health problem in the islands, particularly among the Nicobares tribe, who were found to have almost double the prevalence and annual risk of infection of tuberculosis in the last survey carried out during 2001–02.[5]

Table 4 – Summary of DST patterns of Nicobar districts for 12 isolates of *M. tuberculosis* (along with patient's category wise treatment).

Hospitals	Number of isolates selected for DST along with patient's category wise treatment	DST patterns of the isolates
Nicobar district		
CHC, Nancowry	6 isolates of new cases	• All drug susceptible-4 • Mono-drug streptomycin resistant-2
	2 isolates of cat I treatment patient	• All drug susceptible-1 • Other patterns (S + H) resistant-1
	1 isolates of cat II treatment patient	• MDR (H + R) resistant-1
	2 isolates of cat III treatment patient	• All drug susceptible-1 • Mono-drug isoniazid resistant-1
	1 Non-DOTS treatment patient	• MDR (R) resistant-1

RNTCP was introduced in the islands much later that in other parts of India. The high prevalence and ARI observed among the Nicobarese was a factor that emphasized the need to introduce RNTCP in the islands. The present observation of rather high prevalence of MDR strains among *M. tuberculosis* isolates in the islands, now, emphasizes the need to introduce DOTS plus strategy to address the problem of MDR tuberculosis.

5. Conclusion

In the present study, *M. tuberculosis* was isolated from sputum specimen of both tribal and non-tribal pulmonary tuberculosis patients under DOTS treatment and new diagnosed cases before initiation of DOTS treatment of Andaman & Nicobar districts by conventional culture method. The commonest drug susceptibility patterns in both tribal and non-tribal patients under DOTS treatment among isolated *M. tuberculosis* strains were all drug susceptible, Multi-drug resistant, other resistant (S + H) and mono-drug resistant to Isoniazid (H) patterns observed. Mono-drug resistant to streptomycin (S) and Ethambutol (E) patterns only observed in non-tribal patients. However, drug susceptibility patterns of isolated *M. tuberculosis* strains among new diagnosed cases of tribal patient before initiation of DOTS treatment were all drug susceptible and mono-drug resistant to Streptomycin (S) patterns observed. This study indicates that all form of resistant strains (35%) and all form of susceptible strains (61.67%) is existing among the *M. tuberculosis* isolates assessed for the first line drug susceptibility testing.

Conflicts of interest

All authors have none to declare.

Acknowledgement

We are thankful to State TB society (RNTCP) Andaman & Nicobar Islands, Directorate of Health Service laboratory/ medical personnel and other staff for their co-operation and providing support during the study. We are also thankful to S. Seena Kumari (laboratory technician, project) for her co-operation and help during the study.

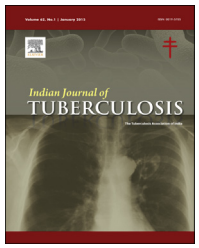
REFERENCES

1. Dolin PJ, Raviglinone MC, Kochi A. Global tuberculosis incidence and mortality during 1990–2000. *Bull World Health Organ*. 1994;72:213–220.
2. WHO Report 2011/Global Tuberculosis Control. (www.who.int/tb/publications/global_report/2011/gtbr11_main.pdf).
3. Mahadev B, Kumar P, Agarwal SP, Chauhan LS, Srikantaramu N. Surveillance of drug resistance to anti-tuberculosis drugs in districts of Hoogli in West Bengal and Mayurbhanj in Orissa. *Indian J Tuberc*. 2005;52:5–10.
4. Paramasivan CN, Venkataraman P, Chandrsekaran V, Bhat S, Narayan PR. Surveillance of drug resistance in tuberculosis in two districts of South India. *Int J Tuberc Lung Dis*. 2002;6:479–484.
5. Murhekar MV, Kolappan C, Gopi PG, Chakraborty AK, Sehgal SC. Tuberculosis situation among tribal population of Car Nicobar, India, 15 years after intensive tuberculosis control project and implementation of national tuberculosis programme. *Bull World Health Organ*. 2004 November;82:836–843.
6. Venkataraman P, Paramasivan CN. *Bacteriological methods in laboratory diagnosis of tuberculosis*. Chetpet, Chennai 600 031: Tuberculosis Research Centre (ICMR); 2006.
7. Zignol M, Gemert WV, Falzon D, et al. Surveillance of anti-tuberculosis drug resistance in the world: an updated analysis, 2007–2010. *Bull World Health Organ*. 2012;90:111–119D.

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.journals.elsevier.com/indian-journal-of-tuberculosis/



Original Article

Directly observed treatment short course for tuberculosis. What happens to them in the long term?

Marina Rajan Joseph [a,*], *Roshan Anna Thomas* [b], *Sanjeev Nair* [c], *Shibu Balakrishnan* [d], *S. Jayasankar* [e]

[a] *Professor of Community Medicine, M.O.SC. Medical College, Kolenchery, India*
[b] *Senior Lecturer Community Medicine, M.O.SC. Medical College, Kolenchery, India*
[c] *Assistant Professor, Department of Pulmonary Medicine, Govt. Medical College, Trivandrum, India*
[d] *Medical Consultant RNTCP, Office of The WHO Representative for India, India*
[e] *State TB Officer, Chest Physician, Directorate of Health Services, Kerala, India*

ARTICLE INFO

Article history:
Received 9 December 2014
Accepted 23 December 2014
Available online 16 March 2015

Keywords:
Tuberculosis treatment
DOTS outcome
Relapse after DOTS
Quality of RNTCP
Residual respiratory problem

ABSTRACT

Background: Though Directly Observed Treatment Short course (DOTS) is found effective in many controlled trials, few studies have examined its effectiveness under programmatic conditions. DOTS based Revised National TB Control Programme (RNTCP) was initiated in Ernakulam district of Kerala state in June 2000. It now covers all of India. It now seems appropriate to do an evaluation of RNTCP at field level.

Aim: This study aims to document impact of DOTS in providing productive life to tuberculosis patients and measure rate of clinical recurrence under program conditions.

Methods: Retrospective cohort study using interview with structured, peer reviewed and validated questionnaire among cohort of new smear positive patients registered in RNTCP from January 2002 to December 2003 and declared cured/Treatment completed. We have contacted 1173 patients (62.2% of the cohort) for the study at their homes by devising a strategy to identify and trace patients from address given in TB registers.

Results: Mean age of identified patients is 51.9 years. 82.4% were males. 79% patients report full supervision in the intensive period. After seven years 64.1% are healthy, work and earn; 29.8% report residual respiratory problems; 0.3% of symptomatic patients were diagnosed with smear positive pulmonary tuberculosis. Relapse calculated as worst case scenario for full target population (dead and migrated inclusive) is 9.27%. Age specific mortality is 4–6 times higher than in a comparable general population.

Conclusions: DOTS treatment under program conditions makes a measurable reduction in tuberculosis morbidity. Though high proportion of patients remains productive after DOTS, a significant proportion complains of residual respiratory symptoms. Age specific mortality of Post tuberculosis patients is high compared to general population. Close follow up irrespective of duration of symptoms may help to determine the causes of high residual morbidity and mortality rates.

* *Corresponding author*. Tel.: +91 9446138580.
E-mail address: marina.rajan@gmail.com (M.R. Joseph).
http://dx.doi.org/10.1016/j.ijtb.2015.02.005

1. Introduction

India has the highest number of tuberculosis patients in the world.[1] Since the National Tuberculosis control program established in the year 1962 did not achieve a remarkable reduction of Tuberculosis burden in India, a major review done by the Government of India (GOI) in collaboration with the World Health Organization (WHO) and Swiss International Development Agency (SIDA)[2] developed and implemented the new strategy - RNTCP since 1997 in a phased manner after pilot testing in 1992. DOTS is the heart of RNTCP. The RNTCP covered the whole of India in 2006. It seems quite appropriate now to evaluate the impact of DOTS over a long timeframe.

Kerala is a unique southern state of India with health indicators much better than other states. The state with a population of 33,387,677 has an effective health system and was one of the first to implement DOTS based RNTCP. Ernakulam district located in central Kerala with a population density of 1053/km^2 (2727/sq mi) implemented RNTCP in June 2000. The notification rates for "New Sputum smear positive cases" in Ernakulam district in 2002 and 2003 were 33/100,000 and 36/100,000 per year respectively, and cure rates were 88.8% and 88.2% respectively (Personal Communication DTO Ernakulam).

A systematic review by Cox et al ,[3] concluded that, despite glowing reports of treatment success from several countries, few studies have actually examined the effectiveness of DOTS strategy under the routine programmatic conditions. Hence the need for a study to assess the impact of standard DOTS regimen in the field. It is assumed that a retrospective evaluation of the health status of the cohorts of patients, registered in the first few years of the program, which have now completed more than 7 years after cure/treatment completion would reflect the long term effectiveness of DOTS under program conditions. The objectives of this study are.

1. To assess the health status and productivity of category I pulmonary tuberculosis patients declared 'cured' and treatment completed under the RNTCP in the cohort registered between 1st January 2002 and 31st December 2003, in Ernakulam district, 7 years after treatment.
2. To find out the rate of clinical recurrence of Tuberculosis within 7 years among them.
3. To identify risk factors for recurrence of Tuberculosis if any among these patients.

2. Methodology

Study design: Retrospective cohort study.

Location: The area covered by the District TB program of Ernakulam District including 7 Tuberculosis Units (TU).

Study subjects: All new sputum positive patients registered in the program from 1st January 2002 to 31st December 2003 were potential study subjects.

Sample size: A pilot study conducted in Vadavucode Block of Ernakulam District showed a recurrence rate of 10.6% in four years. Therefore expecting a recurrence rate of 8.2–13% the minimum sample size required to detect this recurrence rate with 95% confidence was calculated to be 1244. To get this sample size, patients registered from January 1st 2002 to December 31st 2003 were included in this study.

Ethical Clearance- Obtained from the institutional ethical council of the Principal Investigator.

2.1. Definitions

RNTCP: Revised National Tuberculosis Control Program of India.

DOTS: Directly Observed Therapy – Short Course as practiced under RNTCP in India (i.e. thrice weekly biphasic regime with INH, Rifampicin, Ethambutol and Pyrazinamide).

Long term: For this study long term has been defined as seven years after treatment completion.

Sputum smear positive patients: Patients registered as new sputum positive tuberculosis cases in category 1 under RNTCP.

Cure- Initially sputum smear positive patient who has completed treatment and had negative sputum smears on two occasions, one of which is at the end of the treatment.

Treatment completed- Initially sputum smear positive patient who has completed treatment with negative smears at end of the intensive phase/two months in the continuation phase, but none at the end of treatment.

A case of TB recurrence: Any patients diagnosed as having Tuberculosis or treated for Tuberculosis subsequent to being declared 'cured' or treatment completed under RNTCP.

Indicator for present health: Any subject capable of doing personal daily chores, working and earning is considered as being healthy and productive.

2.2. Tools

A semi-structured, peer reviewed and validated questionnaire designed on the basis of the objectives outlined earlier.

2.3. Data collection

In RNTCP after a patient has been declared cured the system depends on voluntary return of patients with any clinical symptom. To register a patient in retreatment category, only history of previous antituberculous treatment given by the patient is required. Hence in this study a system of tracing patients using the addresses from tuberculosis registers independently by the researchers was developed. Grass root level health workers in the health care system, post men, local taxi and autorickshaw drivers helped in tracing addresses. Social workers trained as research assistants conducted the interview.

Research assistants after collecting addresses of patients from the TB registers maintained by each Treatment Unit in the District, traced patients according to the given address and verified their identity with history of Tuberculosis treatment in the given period, or with patient identity cards wherever available. After documenting witnessed verbal consent, patients were interviewed using the semi-structured questionnaire. Information on details about the present health status including employment, other associated morbidities, history

and supervision of treatment, any missed doses, recurrence of cough after treatment and follow up of cough were collected. In case of patients who died before the interview, details of time of death and causes of death were collected by a verbal autopsy from informants and verified from death certificates wherever available.

3. Results

3.1. Study sample and retrieval

According to the treatment registers 1886 sputum positive category I patients registered between 1st January 2002 and 31st December 2003 were declared cured or treatment completed. This study identified 1173 persons (62.2%) (Fig. 1) by tracing given addresses. This is 94.3% of the calculated sample size (1244). Table 1. Shows age sex distribution of the sample.

3.2. Survival and mortality

The age specific mortality rates of the sample and reference population are shown in Table 3. Among 1173 patients, 266 (22.7%) died before the survey. Exact date of death was found in 214 patients by verbal autopsy. Their mean survival period after treatment is 4.4 years. Exact cause of death was available in 167 patients. Among the 167 patients, 29 (17.3%) died due to respiratory causes.

3.3. Supervision

79% patients reported fully supervised drug intake on all days of the intensive period. 81% of the patients reported that DOT provider went to them for providing treatment. 19% reported either patient went to DOT provider or mixed ways of supervision.

3.4. Smoking

48% of patients with residual problems and 52% without problems were smokers.

3.5. Relapse

Out of 271 persons (Table 4) who experienced residual chest symptoms within seven years after DOTS cure, 64 (23.6%) had productive cough and re-examined sputum for Tuberculosis; 50 (18.5%) received appropriate treatment for cough. 3 (1%) were found Sputum positive for tuberculosis and retreated using DOTS; 1 received treatment for extra pulmonary relapse. Relapse has been calculated as best scenario and worst case scenarios (Table 5).

4. Discussion

Considering the difficult situation and long follow up period of seven years 62.2% is very good retrieval. Samples drawn from hospital records, and treatment registries with inbuilt follow-up systems,[4,5] or those with much shorter follow up periods, have reported higher patient retrieval than this. The age sex distribution of the sample is comparable to other studies reported from Kerala[6] 85:15[7] 68.4: 31.6[8] 81:19. From January 2008–December 2011 it is 70.3:29.7 in Ernakulam District (Personal communication from DTO Ernakulam). Bangalore Study reports 70.5% males and mean age is much lower than this sample (35 and 27 each for males and females).[5]

4.1. Health outcome of patients after seven years

In this study 61.4% of patients, report no associated morbidity. No HIV and Hepatitis B has been self reported in this cohort (Table 4). 64.1% are healthy and work to earn a living (Table 2). Most patients (99.5%) who are able to work continue with their

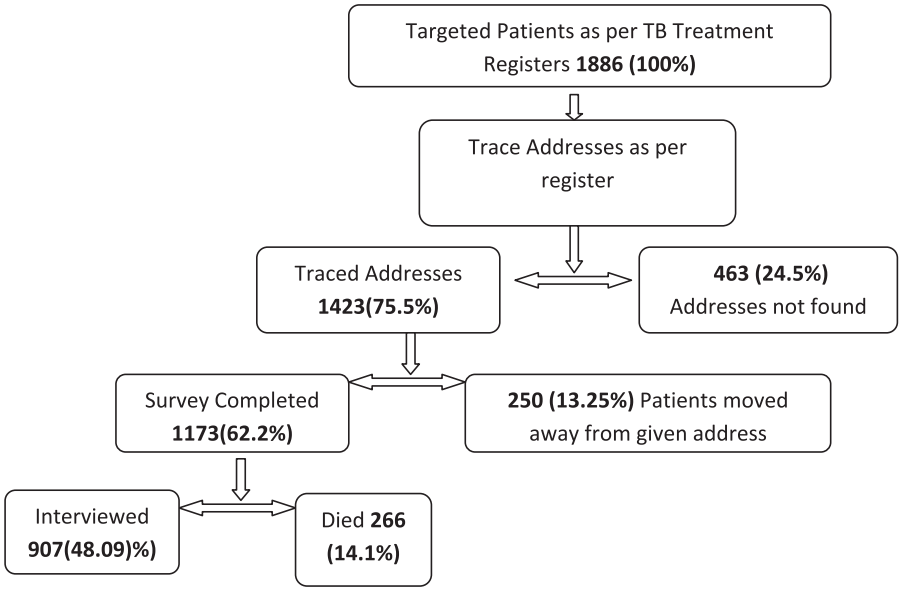


Fig. 1 – Flow Chart Showing the process of tracing patients for Interview and the sample size available.

Table 1 – Age and sex distribution of the 1423 patients traced (Proportions and Confidence limits within brackets).

Number of subjects	All identified patients		Alive and available for interview		Died		Migrated from previous address	
Total	1423		907	(63.7%)	266	(18.7%)	250	(17.6%)
Age	51.9	±15.5	51.9	±14.5	60.6	±12.6	42.4	±16.2
Sex (F)	270	(18.6%)	168	(18.5%)	22	(8.3%)	74	(29.6%)

pretreatment employment. Good majority of the patients who received DOTS enjoy clinical cure and post treatment well-being lasting more than seven years. The high cure rate achieved with DOTS[9,6,10,11,12] coupled with this finding, documents that DOTS achieves significant reduction in morbidity burden due to tuberculosis. 79% of patients interviewed reported full supervision in intensive phase of their treatment. The systematic review by Cox et al[3] has also identified that when quality of services is good, effectiveness of DOTS is very high.

4.2. *Mortality*

This is probably the first study providing data on age specific mortality rates in cured TB patients treated under RNTCP in India. The study group showed an overall mortality rate of 42.7 per 1000 person years, much higher than all cause mortality rates in Kerala in a similar population (7.97 per 1000 person years).[15] The age specific mortality rates in DOTS cured is high in most age groups (6 times higher in 20–50 yrs and 3 times higher in the older age groups) (Table 3).[16, 17] The mean survival period after DOTS cure (4.4 years) among the dead is much less compared to the present life expectancy of 34 and 37 years in Kerala for men and women respectively at the age of 40 years.[14] Age above 40 years has been identified as a significant risk factor for death among tuberculosis patients.[13]The mean age of the total identified patients was 51.9 (sd 15.5) and that of patients dyeing within seven years after DOTS cure (Table 1) is 60.6 years in this study.

Table 2 – Outcome after seven years among the sample studied (n = 1173).

No.	Outcomes	Numbers	Percentage	95% Confidence limits
1	Died before interview	266	22.7	20.3–25.1
2	**Healthy and working to earn**	**753**	**64.1**	**61.3–66.9**
3	Not working due to poor health after Tuberculosis treatment	43	3.7	
4	Healthy but not working due to other reasons	111	9.5	7.92–11.02
5	**Total**	**1173**	**100**	

Bold has been used to highlight the words being the most important point.

4.3. *Residual respiratory problems after tuberculosis*

Breathlessness or chronic cough after tuberculosis is present among 29.87% of patients (Table 4) though many of them are able to work for a livelihood. This is comparable to the findings from TRC Chennai reporting 29% residual respiratory problems, 14–18 years after treatment with short course regimens.[18] A study from Thanjavur reports post tuberculosis asthma in 76.3%[19] patients. Studies elsewhere have also reported pulmonary tuberculosis as an independent risk factor for obstructive lung disease.[20] There are reports in the literature on autophagy as a cause for lung tissue damage[21] in pulmonary tuberculosis. Other immunologic factors have also been implicated in the lung pathology resulting after tuberculosis.[22] These findings on mortality and residual respiratory problems illustrate that DOTS alone is not enough to prevent the damages Tuberculosis does to the young population. To rule out relapse or re-infection by mycobacterium in post tubercular bronchiectasis, sputum examination irrespective of duration of cough is mandatory. Follow up microscopy, and comparative studies on lung function among patients started on treatment early and late in the disease stages, studies on the virulence of different strains of mycobacteria, evaluation the immunologic processes and their interaction with drug treatment of tuberculosis etc may give clues on the reasons for residual breathlessness after tuberculosis treatment. In

Table 3 – Mortality after cure in the study patients compared to that of a similar Kerala Population.

Age groups	Number of patients	Deaths	Proportion of deaths	Mortality rate per 1000 per year	Mortality per 1000 per year in Kerala (ref no:16)
Upto 20yrs	3	1	33.3%	53.5	
21 to 30 yrs	87	4	4.6%	6.3	1.02
31 to 40 yrs	146	10	6.8%	9.4	2.05
41 to 50 yrs	235	41	17.4%	25.0	4.41
51 to 60 yrs	303	57	18.8%	26.9	10.21
61 to 70 yrs	256	95	37.1%	57.9	25.95
71 to 80 yrs	118	51	43.2%	68.4	49.95
81 and above	25	7	28.0%	40.8	82.73
Total	1173	266	–	–	–

Table 4 – Current pattern of reported morbidities among the survivors (n-907).

No	Morbidities	Numbers	Percentage	95% CI
1	No Associated illness	557	61.4	58.26–63.64
2	Diabetes mellitus	82	9	
3	Hypertension	40	4.4	
4	Self reported HIV	0		
5	Malignancy	0		
6	Self reported Hepatitis B	0		
7	**Any Residual respiratory problem**	**271**	**29.87**	**26.84–32.9**
8	• Breathlessness	238	26.24	
9	• Chronic cough	11	1.2	
10	• Poor health after Tb	43	4.7	
11	Multiple morbidities present	26	2.86	

Bold has been used to highlight the words being the most important point.

this cohort prevalence of smoking was found equal among persons with and without residual problems.

In recent years with WHO initiatives and effective National programs in place serious disabilities after tuberculosis treatment has reduced to a great extent. As the TB transmission falls through control strategies, minimizing sequelae gains more importance. Hence in tuberculosis control and research better understanding of the pathologic processes and minimizing tissue damage while treating is an important target for research and action in RNTCP.

4.4. *Magnitude of clinical relapse of tuberculosis*

0.4% were diagnosed to have relapse of tuberculosis (Table 5). Some other reports are 0.8–1.1% relapse among cured patients (clinically detected and bacteriologically confirmed) within 30 months follow up from among 12183 patients from Hong Kong[23], 5.2% relapse within 3 years of follow up from among 711 patients[24] from Brazil, 2.2% relapse per year after 16 months follow up[25] from Japan. Studies using bacteriological follow up reports much higher percentage of relapse (Chennai 12% within 18 months, Bangalore 11.4% within 2 and $^{1}/_{2}$ years, Uzbekistan- 34% within 22 months, South Africa 14% in 5.2 years).[26,5,4,27] Most of these studies have reported that recurrence is highest in the first 6 months–12 months after treatment. In this study the long interval for follow up after cure (7 years) and resultant exclusion of large number (dead patients) from the sample may be a reason for small number of reported relapses. Calculating recurrence as best case and worst case scenarios within the given data (Table 5) shows possible relapse within seven years between 7.9 and 10.6. (1.3 patients per year). This is comparable to other studies. The higher recurrence reported from Uzbekistan, is in a community with high prevalence of drug resistant tuberculosis. High prevalence of HIV infection[29] and drug resistance[28] is known to reduce treatment efficiency of DOTS. The reasons for low relapse in the present study may be the low prevalence of drug resistance (2%)[7] and HIV infection (Table 3) coupled with high supervision rate of DOTS, which ensures good patient compliance, in this community.[30]

4.5. *Risk factors for clinical relapse*

To exclude known risk factors for relapse like defaulting, only patients who completed treatment and were declared cured were followed up in this study. Table 6 shows that other known risk factors like loopholes in supervision, smoking, and contact history[24,27] have been observed in this study also. A Randomized Clinical Trial from Brazil[31] illustrates the importance contact tracing and close supervision in bringing about reduction in incidence of tuberculosis in a community through DOTS strategy. Nested case control study from Hong Kong[20] specifically looking at risk factors for relapse identifies many host factors as risk factors. Among treatment related risk factors though short duration thrice weekly regimen seems a risk factor, they substantiate the benefit of DOT during initial phase in a subgroup analysis. They quote other studies to substantiate their observations[32,33]. A detailed analysis of a larger number of relapse cases from a larger treatment cohort might throw more light on these factors.

5. Conclusion

DOTS treatment under program conditions is making a measurable reduction in tuberculosis morbidity in the long term. In situations where DOTS strategy is practiced with good treatment supervision, clinical relapse is low and the long term cure for patients is very high. These findings support the program strategy[34] of no routine follow up for cured patients.

In spite of very high proportion of patients remaining productive and healthy after DOTS, significant proportion of

Table 5 – Showing Relapse Rate of Tuberculosis (using different Analytical scenarios).

Different analytical scenarios	Relapse in number	%	95% Cl
Best Case Scenario			
1. As reported among interviewed group	4/907	0.44%	
2. Taking all respiratory causes of Death as Relapses	33 (29 + 4)/1173	3.6	2.4–4.8
Worst Case Scenario			
1. Calculating expected Respiratory causes of death among all patients in whom cause of death could not be verified and counting them as relapses	- Expected respiratory causes of death = 29*/167 = 17.3% 0.173X812 (463 + 250 + 99) = 141		
2. Expected Relapses	(141 + 33) = 175/1886	9.27	7.93–10.61

Table 6 – Showing details and information on Risk factors in Persons who had Relapse of TB.

Affected persons	Age	Sex	Type of TB	Treatment unit	Risk factors
Person 1	36years	F	Pulmonary	Muvattupuzha	Patient goes to DOT provider, Not fully supervised, Contact History
Person 2	46 years	M	Pulmonary	Muvattupuzha	Patient goes to DOT provider, Smoker, Diabetes, Hypertension, Contact history
Person 3	47 years	M	Pulmonary	Ernakulam	Patient Go to DOT Provider Not fully supervised
Person 4	58 years	M	Extra Pulmonary	Perumbavoor	Patient Go to DOT Provider, contact history, Quit smoker, Teetotaler

patients complains of residual symptoms like breathlessness. This needs further evaluation. This may be pointing towards the need among symptomatic patients for follow up lung care, irrespective of duration of symptoms after DOTS cure. The high age specific mortality among cured TB patients warrants closer follow up, for determining causes of mortality and increase survival.

6. Limitations

Since this study has assessed clinical relapse only by history and voluntary direct smear microscopy in symptomatic subjects, chances of missing some cases of relapse remains. This study being retrospective follow up after seven years, recall memory of the persons cannot be fully relied upon. A prospective study of larger cohort with mandatory regular sputum smear follow up may give better inferences.

Conflicts of interest

All authors have none to declare.

Acknowledgement

The authors are grateful to all the patients and informants who willingly cooperated with this task. We acknowledge with gratitude the cooperation extended by local people including district health staff, the DTO Dr. Sreedharakumar, postal staff, and taxi and auto rickshaw drivers. The committed work of the project coordinator Ms. Rincy, research assistants Mr. Renjith, MR. Rijesh, Ms. Neena and Ms. Sabitha in the field, the administrative support of the M.O.S.C. Medical College Kolenchery and financial support from the State Task force have made this project possible. We thank them all.

REFERENCES

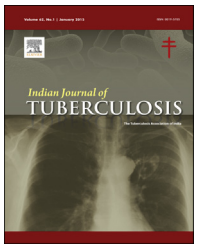
1. Central TB Division, Directorate General of Health Services, Ministry of Health and Family Welfare, Nirman Bhawan, New Delhi - 110 108. TB INDIA 2011, Revised National TB Control Programme: Annual Status Report. http://www.tbcindia.org. [Online] Central TB Division, Directorate General of Health Services, Ministry of Health and Family Welfare, Nirman Bhawan, New Delhi - 110 108, March 16, 2011. [Cited: April 26, 2011.] http://www.tbcindia.org/pdfs/RNTCP%20TB%20India%202011.pdf.
2. S.P. Agarwal, Sophia Vijay, P. Kumar, L.S. Chauhan. History of TB Control. www.tbcindia.org. [Online] [Cited: October 6, 2011.] http://www.tbcindia.org/history.asp.
3. Cox Helen S, Morrow Martha, Deutschmann Peter W. Long term efficacy of DOTS regimens for tuberculosis: systematic review. February, s.l. : bmj.com *BMJ*. 2008;4:336–484. http://dx.doi.org/10.1136/bmj.39463.640787.BE. February.
4. Cox Helen, Kebede Yared, Allamuratova Sholpan, et al. Tuberculosis recurrence and mortality after successful treatment: impact of drug resistance [Online] *plosmedicine.Org*. October 2006. http://dx.doi.org/10.1371/journal.pmed.0030384 [Cited: April 29, 2011.]http://www.plosmedicine.org/article/info:doi/10.1371/journal.pmed.0030384.
5. Vijay Sophia, Balasangameswara VH, Jagannatha PS, Saroja VN, Kumar P. Treatment outcome and two & half years follow-up status of. *Indian J Tuberc*. 2004;51:108–208.
6. Balasubramanian VN, Oommen K, Samuel R. DOT or not? direct observation of anti-tuberculosis treatment. *Int J Tuberc Lung Dis*. 2000;4:409–413.
7. Joseph MR, Shoby CT, Amma GR, Chauhan LS, Paramasivan CN. Surveillance of antituberculosis drug resistance in Ernakulam district, Kerala state, south India. *Int J Tberc Lung Dis*. 2007;4:443–449.
8. Joseph MR, Oarathel SP, Eapen CK. Integrating private health care in National Tuberculosis programme, Experience from Ernakulam, Kerala. *Indian J Tubercs*. 2001;48:17–19.
9. Gopi PG, Subramani R, Santha T, et al. Performance of a dots programme: administrative and technical. *Indian J Tuberc*. 2006;53:123–134.
10. Dye Christopher, Watt Catherine J, Bleed Daniel M, Mehran Hosseini S, Raviglione Mario C. Evolution of tuberculosis control and prospects for reducing tuberculosis incidence, prevalence, and deaths globally. *JAMA*. 2005;293:2767–2775.
11. Prasad R, Verma SK, Shrivastava P, Kant S, Kushwaha RAS, Kumar S. A follow up study on revised national tuberculosis control programme (rntcp): results from a single centre study. *Lung India*. 2008;25:142–144. http://dx.doi.org/10.4103/0970-2113.45277.
12. Tahir Mohammad, Sharma SK, Rohrberg Duncan-smith, Gupta Deepak, Singh* UB, Sinha PK. DOTS at a tertiary care center in northern India: successes,challenges & the next steps in tuberculosis control. *Indian J Med Res*. 2006;123:702–706.
13. Pardeshi Geeta. Survival analysis and risk factors for death in tuberculosis patients on directly observed treatment-short course. *Indian J Med Sci*. May 2009;63:180–188, 2009.
14... Sauvaget C, Ramadas K, Fayette JM, Thomas G, Thara S, Sankaranarayanan R. Socio-economic factors & longevity in a cohort of Kerala State, India. *Indian J Med Res*. 2011;133:479–486.

15. Soman CR, Shahulhameed S, Ramankutty V, et al. Cohort profile: the PROLIFE study in Kerala, India. *Int J Epidemiol*. 2011;40:10–11.
16. Soman CR, Kutty VR, Safraj S, Vijayakumar K, Rajamohanan K, Ajayan K. PROLIFE Study Group: all-cause mortality and cardiovascular mortality in Kerala state of India: results from a 5-year follow-up of 161,942 rural community dwelling adults. *Asia Pac J Public Health*. 2011 Nov;23:896–903. Epub 2010 May 10.
17. Kutty VR, Vijayakumar K; Respiratory mortality in Kerala state of India – data analysed from PROLIFE study – personal communication, unpublished data.
18. Banu Rekha VV, Ramachandran Rajeswari, Kuppu Rao KV, Rahman F, Adhilakshmi AR, Kalaiselvi D. Assessment of long term status of sputum positive pulmonary TB. *Indian J Tuberc*. 2009;56:132–140.
19. Rajasekaran S, Savithri S, Jeyaganesh D. Post-tuberculosis bronchial asthma. Jul *Indian J Tuberc*. 2001 Jul;48:139–142.
20. Lee SW, Kim YS, Kim DS, Oh YM, Lee SD. The risk of obstructive lung disease by previous pulmonary tuberculosis in a country with intermediate burden of tuberculosis. *J Korean Med Sci*. 2011;26:268–273, 2011.
21. Haspel JA, Choi AM. Autophagy: a core cellular process with Emerging links to pulmonary disease. Dec1 *Am J Respir Crit Care Med*. 2011;184:1237–1246 [Epub ahead of print], Aug 11 2011.
22. Cao J, Liping Z, Dairong L, et al. IL-27 is elevated in COPD and PTB patients and induces human bronchial epithelial cells to produce CXCL10.: Chest. *CHEST*. 2012;141:121–130. http://dx.doi.org/10.1378/chest.10-3297.
23. Chang Kwok C, Leung Chi C, Yew Wing W, Ho Suzanne C, Tam Cheuk M. A Nested Case–Control study on treatment-related risk factors for early relapse of tuberculosis. *Am J Respir Crit Care Med*. 2004;170:1124–1130. http://dx.doi.org/10.1164/rccm.200407-905OC.
24. d'Arc Lyra Batista Joanna. Maria de Fátima Pessoa Militão de Albuquerque, Ricardo Arraes de Alencar Ximenes, and Laura Cunha Rodrigues. 4,. Smoking increases the risk of relapse after successful tuberculosis treatment. *Int J Epidemiol*. 2008;37:841–851. http://dx.doi.org/10.1093/ije/dyn113.
25. Wada M, Yoshiyama T, Ogata H, Ito K, Mizutani S, Sugita H. Six-months chemotherapy (2HRZS or E/4HRE) of new cases of pulmonary tuberculosis–six year experiences on its effectiveness, toxicity, and acceptability]. *Kekkaku*. 1999;74:353–360.
26. Thomas A, Gopi PG, Santha T, et al. Predictors of relapse among pulmonary tuberculosis patients treated in a DOTS programme in South India. *Int J Tuberc Lung Dis May*. 2005;9:556–561.
27. Verver S, Warren RM, Beyers N, et al. Rate of reinfection tuberculosis after successful treatment is higher than rate of new tuberculosis. *Am J Respir Crit Care Med*. June 15 2005;171:1430–1435.
28. Marcos A, Espinal MD, PH, et al. Standard short-course chemotherapy for drug-resistant TuberculosisTreatment Outcomes in 6 countries. 19. *JAMA*. 2000;283:2537–2545. http://dx.doi.org/10.1001/jama.283.19.2537.
29. Sonnenberg P, Murray J, Glynn JR, Shearer S, Kambashi B, Godfrey-Faussett P. HIV-1 and recurrence, relapse, and reinfection of tuberculosis after cure: a cohort study in South African mineworkers. *Lancet*. 2001, Nov.17;358:1687–1693. PMID: 11728545.
30. Picon PD, Bassanesi SL, Caramori ML, Ferreira RL, Jarczewski CA, Vieira PR. Risk factors for recurrence of tuberculosis. *J Bras Pneumol*. 2007;33:572–578.
31. Cavalcante SC, Durovni B, Barnes GL, et al. Community-randomized trial of enhanced DOTS for tuberculosis control in Rio de Janeiro, Brazil. *Int J Tuberc Lung Dis*. 2010 Feb;14:203–209.
32. Tam CM, Chan SL, Kam KM, Goodall RL, Mitchison DA. Rifapentine report at 5 years: prognostic value of various measures and isoniazid in the continuation phase of a 6-month regimen. Final report at 5 years: prognostic value of various measures. *Int J Tuberc Lung Dis*. 2002 jan;6:3–10.
33. Benator D, Bhattacharya M, Bozeman L, Burman W, Cantazaro A, Chaisson R, et al. Tuberculosis versus rifampicin and isoniazid twice a week for treatment of drugTrials Consortium. Rifapentine and isoniazid once a week versus rifampicin and isoniazid twice a week for treatment of drugsusceptible pulmonary tuberculosis in HIV-negative pa. *Lancet*. 2002, August;17:528–534.
34. Central TB Division, Directorate General of Health Services, Ministry of Health and Family Welfare, Nirman Bhavan., Module for Senior Treatment Supervisors. [Online] June 2005. [Cited: October 6, 2011.] http://www.tbcindia.org/pdfs/module%20for%20senior%20treatment%20supervisor.pdf.

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.journals.elsevier.com/indian-journal-of-tuberculosis/



Original Article

Role of bronchoscopy in evaluation of cases with sputum smear negative pulmonary tuberculosis, interstitial lung disease and lung malignancy: A retrospective study of 712 cases

Raj Kumar [a,*], *Nitesh Gupta* [b]

[a] *Professor and Head of the Department, Department of Respiratory Allergy and Applied Immunology, National Centre of Respiratory Allergy, Asthma and Immunology, V P Chest Institute, University of Delhi, Delhi 110007, India*
[b] *Resident, Department of Respiratory Allergy and Applied Immunology, V P Chest Institute, University of Delhi, Delhi 110007, India*

ARTICLE INFO

Article history:
Received 30 October 2013
Accepted 24 December 2014
Available online 14 March 2015

Keywords:
Biopsy
Bronchoscopy
Malignancy
Sarcoidosis
Tuberculosis

ABSTRACT

Background: The introduction of flexible bronchoscope has revolutionized the field of pulmonary medicine and is a standard instrument used for diagnostic purpose. A retrospective analysis of the clinico-radiological profile, indication, biopsy procedure and complications, for patients undergoing bronchoscopy at one of the respiratory unit at a tertiary care center in India.

Methods: Retrospective analysis of 712 bronchoscopies was done in regard to demographic profile, clinical and radiological presentation and diagnostic indication. The results were analyzed on basis of bronchoscopy inspection and histopathological specimen obtained from transbronchial (TBLB), endobronchial biopsy (EBLB) and cytology specimen by transbronchial needle aspiration (TBNA). Furthermore, diagnostic yield of each biopsy procedure and their combination was evaluated.

Results: Of 712 patients undergoing bronchoscopy, the pathological diagnosis was achieved in 384 (53.93%). Of 384 diagnosed cases, the clinic-radio-pathological diagnosis of pulmonary tuberculosis in 88 (22.19%), interstitial lung disease (ILDs) in 226 (58.85%), and lung cancer in 70 (18.22%) cases. Of 116 sputum smear negative tuberculosis patients, 88 (75.86%) were diagnosed to be pulmonary tuberculosis; the contribution of BAL being 71.59%. Of 226 ILDs, sarcoidosis was most common 148/226 (65.48%). Among 70 lung cancer diagnosed cases, squamous cell carcinoma was most common (54.28%).

Conclusion: The results from current study reemphasizes on the diagnostic utility as well as safety of the bronchoscopy procedure.

* *Corresponding author*. Tel.: +91 9810146835, +91 011 27667667x144; fax: +91 011 27667420.
E-mail address: rajkumarvpci@gmail.com (R. Kumar).

http://dx.doi.org/10.1016/j.ijtb.2015.02.006
0019-5707/

1. Introduction

Bronchoscopy is the diagnostic inspection of the tracheobronchial tree. Gustav Killian performed the first bronchoscopy in 1897. In 1966, first flexible bronchoscope was introduced by Shigeto Ikeda.[1,2] Since then, the flexible bronchoscope has revolutionized the field of bronchoscopy and became the standardized instrument for diagnosis by the pulmonologists. It is an important advancement in the field of respiratory medicine, particularly for the investigation of haemoptysis or radiological appearances, such as atelectasis or non-resolving opacities. It is an alternative method to aid in diagnosis of infection where non-invasive methods fail to cite the underlying etiology; especially in cases of pulmonary tuberculosis.[3] Furthermore, fibreoptic bronchoscopy has become an important instrument for the pathological diagnosis of interstitial lung disease particularly sarcoidosis, and hypersensitivity pneumonitis.[4] Bronchoscopy is an important tool in the diagnostic pathway and staging in patients of lung cancer.[5]

Thus, a retrospective study for analysis of data, with reference to clinico-radiological profile, indications, procedure performed and complications for diagnosis in patients undergoing bronchoscopy at one of the respiratory unit at Vallabhbhai Patel Chest Institute, Delhi, India was done.

2. Materials and methods

712 bronchoscopies performed at one of the respiratory units at Vallabhbhai Patel Chest Institute, Delhi, during 1999–2013 were retrospectively analyzed. The patients who were unable to complete the procedure and unavailability of case records were excluded from the analysis.

2.1. Methods

The instruments used were fibreoptic bronchoscopes; Olympus and Fujinon. The following accessories were used during the bronchoscopy, 1) forceps for endobronchial biopsy (EBLB) and transbronchial lung biopsy (TBLB) 2) transbronchial needle for aspiration (TBNA). After complete visualization of tracheobronchial tree, bronchoalveolar lavage and bronchial aspirate were obtained wherever indicated.

The patients work up for bronchoscopy included a prior detailed clinical history and evaluation. All patients underwent a battery of investigations which included complete haemogram, random blood sugar, kidney and liver function tests and coagulation profile. The sputum investigations for AFB, pyogenic, fungus and cytology were obtained. Also, the records of other serological investigations were reviewed. The radiological evaluation included a chest x-ray and computed tomography of chest.

The patients and the family members were explained the procedure in advance and a written informed consent were taken from the patient. Patients were kept on overnight fasting and a prior skin sensitivity test for lignocaine was performed on volar aspect of forearm.

The patients were nebulized with lignocaine/lignocaine spray 10 min before the bronchoscopy and absence of gag reflex was confirmed. Cardiac monitoring and pulse oximetry was done throughout the procedure. The transtracheal injection of 4% lignocaine was used for giving topical anaesthesia and 2% lignocaine solution was given during the bronchoscopy whenever required. The bronchoscope was introduced either transnasally or orally. Firstly, supraglottic airways were inspected for any abnormalities followed by vocal cord examination for defective movement or growth. The scope was then gently advanced carefully observing for any abnormalities of mucosa, growth, bleeding spots, luminal distortion and movement during respiration. Firstly the normal side of the lung was inspected, followed by the diseased side and the aspirate/biopsy were taken whenever indicated.

The steps for performing lung biopsies (TBLB, EBLB and TBNA) and bronchoscopic aspirate/lavage were followed as described in the literature.[6–8] In cases with significant bleed following the biopsy procedure, wedging of the bronchoscope and instillation of the cold saline was done till bleeding ceases and thorough inspection of the bronchial tree was done before withdrawing the bronchoscope. The procedure was well tolerated by almost all the patients and they were explained about the possible episode of fever, hemoptysis or chest pain after the bronchoscopy. The patients were allowed to have oral feeding once the gag reflex reappears and were kept under observation for few hours and if there were no complications; discharged same day.

2.2. Statistical analysis

The data analysis was performed using SPSS statistical package version 14.0 for windows (SPSS, Chicago, IL, USA) for the purpose of percentage calculation.

3. Results

3.1. Patient demographics

A total of 712 patients underwent bronchoscopy, including 400 males and rest 312 females. The age ranged from 14 to 80 years, mean being 44.5 ± 13.6years.

3.2. Clinical data

The decision to proceed to bronchoscopy was made on the basis of the clinical history along with physical examination and radiological interpretation.

3.3. Symptoms

The predominant symptom was cough (84.26%), followed by dyspnea (68.25%), hemoptysis (16.29%) and chest pain (8.12%). The patients also suffered from fever (8.97%), weight loss (6.51%) and hoarseness of voice (2.23%).

3.4. Radiological profile

The most common radiological presentation was of interstitial/diffuse shadows (33.00%), followed by lymphadenopathy

(23.59%) and consolidation (18.39). Other common presentations were lung mass (10.25%), multiple nodules (13.34%), and cavity (10.12%). Solitary pulmonary nodule, bronchiectasis, pleural effusion and SVC obstruction were found in <1% of cases (Table 1). The lesions were bilateral in 424 (59.55%) cases and unilateral in 288 (40.45%) cases. The lymph node enlargement was present in 168 (23.59%) patients, either alone or in combination with other radiological patterns. The most common lymph node involvement was hilar lymph nodes (91.04%); right being more common than left, followed by subcarinal (62.90%) and paratracheal (32.70%).

The diagnosis of sputum smear negative pulmonary tuberculosis was suspected in 116 (16.29%) cases. Based on clinico-radiological evaluation, the presumptive diagnosis of interstitial lung diseases (ILDs) was made in 468 (65.73%) cases. Among ILDs, sarcoidosis was presumptive diagnosis in 238 (50.85%), NSIP in 118 (25.21%), IPF in 58 (12.39%), HSP in 30 (6.41%), LIP in 3 (0.6%), DIP in 2 (0.4%) patient's. Clinico-radiological evaluation was suggestive of lung malignancy in 118 (16.57%) patients. Remaining, 20 (2.8%) patients were suspected to be suffering from eosinophilic pneumonia, bronchiolitis, pulmonary alveolar proteinosis or pneumoconiosis (Table 2).

3.5. *Fibreoptic bronchoscopy findings*

The inspection of trachea-bronchial tree was normal in 405 (56.88%) cases (Table 3). In 52 (7.3%) cases, an intraluminal growth was observed while 4 (0.5%) cases showed extrinsic compression. The intraluminal growths were most commonly protruding from right upper lobe (53.82%) followed by right bronchus intermedius (24.25%) and left lower lobe bronchus (15.67%). The mucosal abnormalities in form of erythema/hyperemia (32.26%), nodules (12.16%), edema (5.68%), fibrosis (2.16%) and polyps (1.57%) were observed. The vocal cord involvement was seen in 9 (1.2%) cases; left (55.55%) being more common.

Table 1 – The distribution of radiological pattern of 712 patients.

Radiological pattern[a]	Total number of patients ($n = 712$) N (%)
Bilateral	424 (59.55)
Unilateral	288 (40.45)
Interstitial/diffuse shadows	235 (33.00)
Lymphadenopathy	168 (23.59)
Hilar	153 (91.04)
Paratracheal	53 (32.70)
Subcarinal	104 (62.90)
Others[b]	42 (25.00)
Consolidation	130 (18.39)
Multiple nodules	95 (13.34)
Lung mass	71 (10.25)
Cavity	71 (10.12)
Solitary pulmonary nodule	3 (0.4)
Bronchiectasis	15 (2.1)
Pleural effusion	7 (0.9)
SVC obstruction	1 (0.1)

[a] More than one radiological pattern was observed in patients.
[b] Includes para and pre aortic, pulmonary ligament lymphnodes.

Table 2 – Classification of patients on basis of clinical suspicion and their final diagnosis after bronchoscopy.

Diagnosis	No of suspected cases N = 712	Final diagnosis N = 384 (positive % out of suspected cases)
Interstitial lung disease	**468**	**226 (56.83)**
Sarcoidosis	238	148 (62.18)
NSIP	118	32 (27.11)
IPF	58	26 (44.82)
HSP	30	7 (23.33)
Scleroderma	7	5 (71.42)
LIP	3	1 (33.33)
DIP	2	1 (50.0)
SLE	3	1 (33.33)
RB-ILD	2	1 (50.0)
Bronchiolitis obliterans	1	1 (100.00)
PAP	2	1 (50.00)
Eosinophilic pneumonia	2	1 (50.00)
Pneumoconiosis	2	1 (50.00)
Tuberculosis	**116**	**88 (75.86)**
Lung Cancer	**118**	**70 (59.32)**
Squamous cell	60	38 (63.33)
Adenocarcinoma	18	10 (55.55)
BAC	8	2 (25.00)
Small cell carcinoma	30	20 (66.66)

The bold section corresponds to the three major groups of diseases in the study.

3.6. *Diagnostic procedures*

The bronchial aspirate was the most frequently performed procedure, done in all 712 cases. Of 712 cases, the EBLB was done in 355 (49.8%) cases, TBLB was performed in 492 (69.20%) and TBNA of lymphnode/mass were done in 132 (18.53%) cases as indicated for the diagnosis of the disease (Table 4). Out of 355 cases in which EBLB was performed, 216 (61.21%) cases yield positive results, similarly among 492 cases where TBLB was taken, 281 (57.21%) cases showed positive yield. The TBNA yield was positive in 49 (37.53%) out of 132 cases (Table 3). The combined diagnostic yield of all 3 biopsy procedures i.e. TBLB, EBLB and TBNA in confirming a pathological

Table 3 – Visualization of tracheobronchial tree on bronchoscopy.

Finding	Number	Percentage
Normal	405	56.88
Mucosal erythema	230	32.26
Nodules	86	12.16
Intraluminal growth	52	7.3
Edema	40	5.68
Fibrosis	15	2.16
Polyps	11	1.57
Vocal cord impairment	9	1.2
Extraluminal compression	4	0.5

Table 4 – The results of bronchoscopic procedures.

S. no	Procedure	Total number	No of positive results (%)	Sarcoidosis $n = 148$ N (% out of total positive results)	Tuberculosis $n = 88$ N (% out of total positive results)	Malignancy $n = 70$ N (%out of total positive results)	Others[a] $n = 68$ N (%out of total positive results)
1.	Transbronchial lung biopsy (TBLB)	492	281 (57.21)	144 (51.20)	28 (13.57)	31 (11.03)	68 (24.19)
2.	Endobronchial lung biopsy (EBLB)	355	216 (61.21)	133 (61.57)	19 (8.7)	36 (16.66)	28 (12.96)
3.	Transbronchial needle aspiration (TBNA)	132	49 (37.53)	32 (65.30)	15 (30.61)	3 (6.1)	–
6.	Bronchoalveolar Lavage	116	63 (55.12)	–	63 (100)	–	–
7.	Bronchial[b] aspirate	712	90 (12.64)	–	26 (28.88)	19 (21.11)	35 (38.88)

[a] Included ILD other than sarcoidosis (NSIP, IPF, HSP, Scleroderma, LIP, DIP, SLE, RB-ILD, Bronchiolitis obliterans, PAP, Eosinophilic pneumonia, Pneumoconiosis).
[b] Bronchial aspirate was routinely done in all cases and assessed for differential cell counts; presence of Acid fast bacilli, yield of bacterial or fungus organism and atypical cells has been considered as positive result.

diagnosis was 64.59%. The pathological diagnosis was suggestive of pulmonary tuberculosis in 88/116 (75.86%), ILD in 226/468 (56.83%) and lung malignancy in 70/118 (59.32%) suspected patients (Table 2). Of 384 diagnosed cases, the clinic-radio-pathological diagnosis of pulmonary tuberculosis was made in 88 (22.19%), interstitial lung disease (ILDs) in 226 (58.85%), and lung cancer in 70 (18.22%) cases.

Bronchoscopy was performed in suspected cases of pulmonary tuberculosis that were sputum smear negative despite the clinico-radiological presentation supporting the diagnosis of tuberculosis. They underwent biopsy and bronchoalveolar lavage from the localized segment for the purpose of diagnosis. Of 116 such patients, 88 (75.86%) were diagnosed to be pulmonary tuberculosis; the contribution of BAL being 71.59% (Fig. 1).

Amongst 226 diagnosed ILDs, sarcoidosis was most common with 148 cases (64.48%). The yield of different biopsy procedures for diagnosis of sarcoidosis was highest with TBLB (97.29%), followed by EBLB (89.86%) and conventional TBNA (21.62%); the yield increased with combined TBLB and EBLB (97.97%) (Fig. 2a, b). The diagnosis of lung malignancy was made on cytology from aspirate or from tissue biopsy by bronchoscopy procedures. The squamous cell carcinoma was the most common, 38/70 (54.28%) lung malignancy (Table 2; Fig. 3a, b).

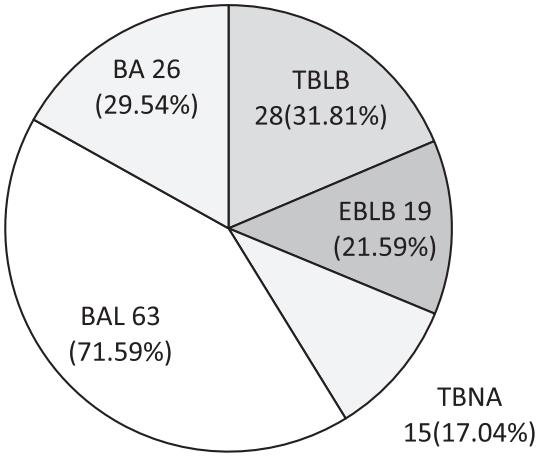


Fig. 1 – Diagnostic yield of bronchoscopic procedures in diagnosis of 88 pulmonary tuberculosis cases [N (%)]. BA–bronchial aspirate; BAl–bronchoalveolar lavage; TBLB–transbronchial lung biopsy; EBLB–endobronchial lung biopsy; TBNA–transbronchial needle aspiration.

3.7. *Histopathology*

The presence of caseating granuloma, suggestive of tuberculosis was observed in biopsy of 54/88 (61.36%) patients (Fig. 1). In 226 histopathology diagnosed ILDs, non-caseating granulomas; suggestive of sarcoidosis was reported in 148 cases (65.48%), followed by non-specific interstitial pneumonitis in 32 (14.15%). Other pathological patterns were, usual interstitial pneumonitis pattern in 26 cases (11.50%). diffuse interstitial pneumonitis 3 (1.3%) and lymphocytic interstitial pneumonitis in 1 (0.8%) case (Fig. 2a). Of histopathologically confirmed 70 patients of malignancy, squamous cell carcinoma in 38 (54.28%), adenocarcinoma in 10 (14.28%) and small cell carcinoma in 20 (28.57%) patients (Fig. 3a, b).

3.8. *Complications*

The most frequent complication was minor bleeding which subsided spontaneously in most of the cases; none required any surgical intervention. 3 cases had respiratory distress following the procedure which was managed with appropriate medical therapy. Only one patient had pneumothorax, which resolved by conservative management.

4. Discussion

The flexible bronchoscopy is an important investigation in the diagnostic pathway for patients with pulmonary infection,

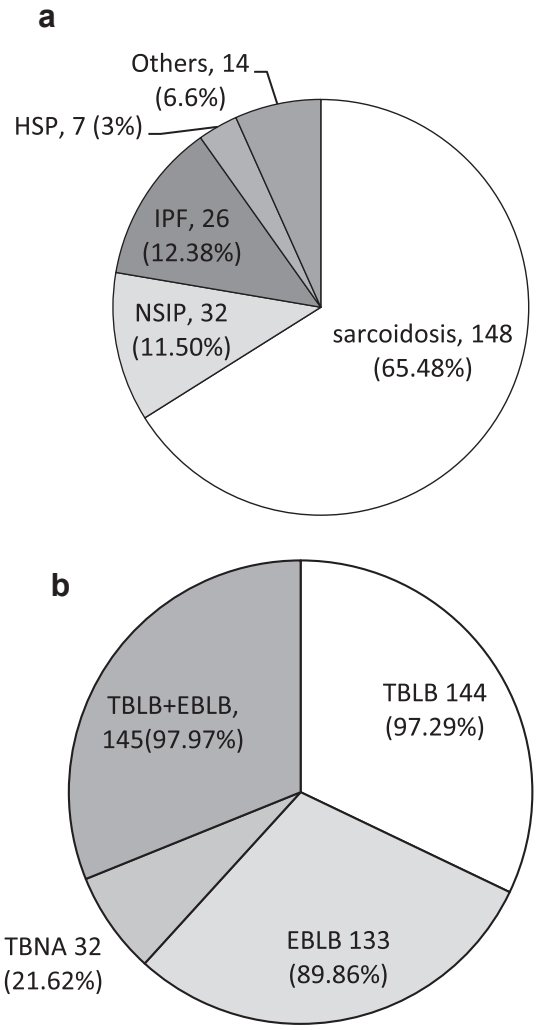


Fig. 2 – (a) Distribution of different interstitial lung diseases in 226 diagnosed cases [N (%)]. IPF–idiopathic pulmonary fibrosis; HSP–hypersensitivity pneumonisitis; NSIP–non-specific interstitial pneumonitis. (b) Diagnostic yield of bronchoscopic procedures in diagnosis of 148 sarcoidosis cases [N (%)]. BA–bronchial aspirate; BAl–bronchoalveolar lavage; TBLB–trans bronchial lung biopsy; EBLB–endobronchial lung biopsy; TBNA–transbronchial needle aspiration.

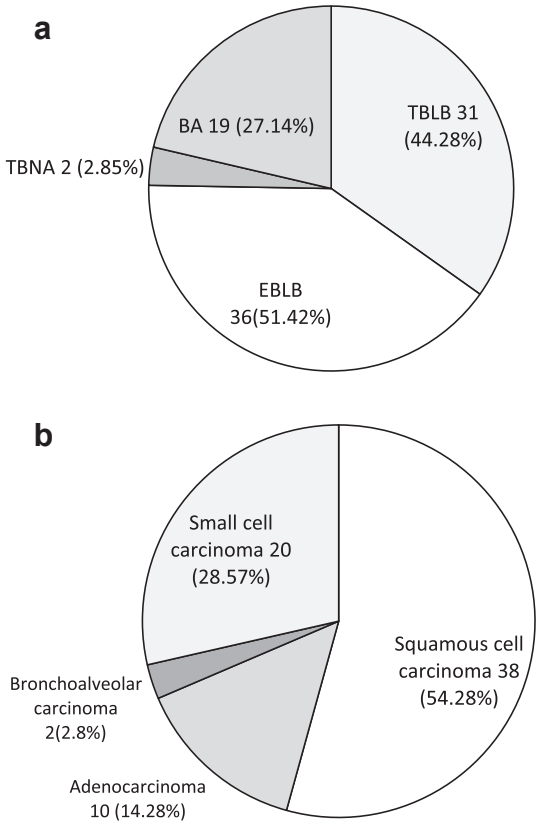


Fig. 3 – (a) Diagnostic yield of bronchoscopic procedures in diagnosis of 70 lung cancer cases [N (%)]. BA–bronchial aspirate; BAl–bronchoalveolar lavage; TBLB–trans bronchial lung biopsy; EBLB–endobronchial lung biopsy; TBNA–transbronchial needle aspiration. (b) Distribution of types of lung carcinoma in 88 diagnosed cases [N (%)].

interstitial lung disease, and potential lung cancer. There are various sampling techniques that can be done during the bronchoscopy to aid in the diagnosis.

The aim of our study was to define clinico-radiological profile of patients undergoing bronchoscopy and also to identifying the diagnostic accuracy of various sampling methods.

In current study, 712 patients underwent bronchoscopy; 400 males (56.17%) and 312 females (43.83%). The age of subjects ranged from 14 to 80 years with a mean age of 44.5 ± 13.6 years. In a study by Sinha et al[9] the males constituted 75.1% of cases with a mean age of 49 ± 15.1 years. In a study by Ravindran et al[10] 86% were male patients and mean age was 56.15 years. The most common symptom at presentation in current study was cough (84.26%), similar percentage (80.2%) was observed in another study. Other clinical symptoms were dyspnea (68.25%), hemoptysis (16.29%) and chest pain (8.12%). In his review of patients presenting with refractory cough, Sen et al[11] showed respectable yield in diagnosis by bronchoscopy and suggested it to be a reasonable procedure in carefully selected patients. However, Barnes et al[12] concluded that bronchoscopy adds little to the diagnosis of chronic cough in the context of normal or non-localizing chest radiographic or CT findings. In cases presenting with hemoptysis, bronchoscopy has central role in diagnosis, localization of bleeding site and therapeutic intervention whenever required.[13]

In current study, on radiographic evaluation, the commonest presentation was interstitial/diffuse shadows (33.00%) followed by lymph nodes enlargement (23.59%), consolidation (18.39%), nodular opacities (13.34%), lung mass (10.25%) and cavity (10.12%). Papagiannis et al,[14] in audit of 200 bronchoscopies, reported lung mass (31.5%), lymphadenopathy (14%) and interstitial pattern (2%) patients. In a study by Ravindran et al,[10] various radiologic presentations were lung mass (49.9%), consolidation (29.3%), collapse (8.7%) and interstitial pattern (1.6%).

The role of bronchoscopy in evaluation of pneumonias in early phase is limited. However, in regions where tuberculosis is endemic, bronchoscopy may prove to be a useful technique

in the evaluation of suspected cases which are sputum smear-negative.[15,16] The combined yield of bronchoscopy with procedures such as BAL, bronchial biopsies, bronchial wash and post-bronchoscopy sputum, in patients with negative sputum smears is reported to be in a range of 28.8–48.3% based on microscopy and smears for AFB, and 69.2–94% based on cultures.[3,17–19] A study by Singhal et al[20] showed the overall diagnostic yield of 62.7% (23/43). Aggarwal et al[21] reported in 19 smear-negative suspected pulmonary tuberculosis patients undergoing bronchoscopy, 2 had biopsy proven tuberculosis. Kalawat et al[22] in their study reported BAL samples positivity in 82.2% of sputum smear negative samples, culture positivity of BAL samples was 90.9% as compared to sputum culture positivity, which was 26.4%. Overall diagnosis could be established in 86.6% of patients with the help of fiber optic bronchoscopy. In current study, of 116 suspected smear-negative pulmonary tuberculosis patients, 88 were diagnosed as pulmonary tuberculosis with BAL positive in 63 (71.59%) patients and were started on anti-tubercular regimen. Of remaining 28 (24.14%) cases, antitubercular therapy was initiated on clinic-radiological suspicion and on follow-up all 28 cases responded clinically as well as radiologically.

Bronchoscopy is a useful diagnostic tool in ILDs, specifically sarcoidosis, hypersensitivity pneumonitis and organizing pneumonia.[4] TBLB is performed using biopsy forceps to obtain an alveolar tissue; which is then used as a histological specimen for suspected cases of ILD, though this tissue may be inadequate in suspected cases of idiopathic pulmonary fibrosis. In retrospective analysis of 916 patients, Kulshreshtha et al[23] reported definitive diagnosis after clinical-radiological-pathological correlation using TBLB for histopathology specimen in 55.3% cases; commonest pattern being granulomatous inflammation (30.2%) followed by interstitial pneumonitis with or without fibrosis (22.4%). In current study, of initially clinico-radiology suspected 238 sarcoidosis patients, 148 were biopsy proven overall positivity of 62.18%. In 148 sarcoidosis patients, EBLB was positive in 133 (89.86%), TBLB in 144 (97.29%), TBNA in 32 (21.62%) patients with combined yield of EBLB and TBLB being positive in 145 (97.97%) cases. The TBLB is the recommended procedure with a yield of 44–90%, dependent on the site sampled, number of biopsies and extent of parenchymal disease on radiology.[24–26] The efficacy of EBLB, TBLB and TBNA for diagnosis of sarcoidosis in the current study was similar to that observed in other studies.[10,27,28] Thus, bronchoscopy biopsy is an important investigation for prompt diagnosis in suspected cases of sarcoidosis.

The visualization of tracheobronchial tree includes assessment of mobility of vocal cords and presence and extent of an endobronchial lesion. The evaluation of pooled data of 4507 patients demonstrated an overall combined sensitivity of 88% for bronchoscopy techniques in diagnosis of lung cancer. In current study, the bronchoscopic inspection showed an intraluminal growth in 52 cases while 4 cases showed extrinsic compression. The diagnostic yield for malignancy from sample of intraluminal growth was higher (62.23%) than in absence of visible lesion (32.68%). The vocal cord palsy was observed in 9 cases; left being common. Of 70 cases, Squamous cell carcinoma was the most common (54.28%). The results were consistent with previous studies.[9,10,16,20,29]

The complications that occurred in the current study were mostly mild, none of the cases requiring any intensive care admission or surgical intervention. The complication rates observed were similar to other studies.[9,10]

5. Conclusion

The results from current study reemphasizes on the diagnostic utility and safety of the bronchoscopy procedure. In suspected cases of sputum smear negative pulmonary tuberculosis, the diagnosis of tuberculosis was established in 80% of cases, highlighting its role in diagnosis of pulmonary tuberculosis. In current medical scenario, bronchoscopy is an important tool in establishing the diagnosis of type of ILDs especially sarcoidosis. Also, the safety of biopsy procedures when done with adequate precautions and pre-bronchoscopy work-up; increases the overall yield of bronchoscopy results.

Conflicts of interest

All authors have none to declare.

REFERENCES

1. Becker HD. Gustav Killian – a biographical sketch. *J Bronchol*. 1995;2:77–83.
2. Ikeda S, Yanai N. Flexible fibrebronchoscope. *Kejo J Med*. 1968;17:1–16.
3. Jaiswal AK, Kulpati DDS, Jain NR, Singh MM. Role of bronchoscopy in the early diagnosis of suspected smear negative cases of pulmonary tuberculosis. *Ind J Tub*. 1989;36:233.
4. Wells A, Bradley B, Branley H, et al. Interstitial lung disease guideline: the British Thoracic Society in collaboration with the Thoracic Society of Australia and New Zealand and the Irish Thoracic Society. *Thorax*. 2008;63:v1–58.
5. Rivera MP, Mehta AC. Initial diagnosis of lung cancer. *Chest*. 2007;132:131S–148S.
6. Mehta AC, Jain P, eds. *Interventional Bronchoscopy: A Clinical Guide, Respiratory Medicine* 10. New York: © Springer Science+Business Media; 2013. http://dx.doi.org/10.1007/978-1-62703-395-4_2.
7. Dionísio J. Diagnostic flexible bronchoscopy and accessory techniques. *Rev Port Pneumol*. 2012;18:99–106.
8. Du Rand IA, Blaikley J, Booton R, et al. British Thoracic Society guideline for advanced diagnostic and therapeutic flexible bronchoscopy in adults. *Thorax*. 2011;66:iii3–iii21.
9. Sinha S, Guleria R, Pande JN, Pandey RM. Bronchoscopy in adults at a tertiary care centre: indications and complications. *J Indian Med Assoc*. 2004;102:152–154.
10. Anandan PT, Rajagopal TP, James PT, Ravindran C. Clinical profile of patients undergoing fibreoptic bronchoscopy in a tertiary care setting. *Indian J Bronchology*. 2006;1:58–68.
11. Sen RP, Walsh TE. Fiberoptic bronchoscopy for refractory cough. *Chest*. 1991;99:33–35.
12. Barnes TW, Afessa B, Swanson KL, Lim KG. The clinical utility of flexible bronchoscopy in the evaluation of chronic cough. *Chest*. 2004;126:268–272.
13. Prakash UBS. Bronchoscopy. In: Bone RC, Dantzker DR, George RB, Matthay RA, Reynolds HY, eds. *Pulmonary and*

Critical Care Medicine. Vol 1. St Louis: Mosby Year Book; 1993:1–18. F(5).
14. Papagiannis A, Ioannidis G, Chrysanthopoulou G, Kontakiotis T. An audit of fiberoptic bronchoscopy practice in a private hospital. *Pneumon*. 2007;20:56–62.
15. Wallace J, Deutsch A, Harrell J, Moser K. Bronchoscopy and transbronchial biopsy in evaluation of patients with suspected active tuberculosis. *Am J Med*. 1981;70:1189–1194.
16. Danek SJ, Bower JS. Diagnosis of pulmonary tuberculosis by flexible optic bronchoscopy. *Am Rev Respir Dis*. 1979;119:677–679.
17. Dasgupta KS, Mundada PS, Soni N. Diagnostic role of fibreoptic bronchoscopy in pulmonary tuberculosis. *Indian J Otolaryngol Head Neck Surg*. 2000;52:347–349.
18. de Gracia J, Curull V, Vidal R, et al. Diagnostic value of bronchoalveolar lavage in suspected pulmonary tuberculosis. *Chest*. 1988;93:329–332.
19. Bachh AA, Gupta R, Haq I, Varudkar HG. Diagnosing sputum/smear-negative pulmonary tuberculosis: does fibreoptic bronchoscopy play a significant role? *Lung India*. 2010;27:58–62.
20. Singhal S, Gaidhane AM, Khatib N, et al. Use of flexible bronchoscopy for rapid diagnosis of suspected tubercular cases in rural India. *J Infect Dev Ctries*. 2009;3:860–864.
21. Aggarwal P, Kumar R. Diagnostic yield of induced sputum and bronchoscopy in sputum smear negative pulmonary tuberculosis. *Respirology*. 2007;12:A240.
22. Kalawat U, Sharma KK, Reddy PN, Kumar AG. Study of bronchoalveolar lavage in clinically and radiologically suspected cases of pulmonary tuberculosis. *Lung India*. 2010;27:122–124.
23. Kulshrestha R, Menon BK, Kumar R, Vijayan VK. Role of a pattern-based approach in interpretation of transbronchoscopic lung biopsy and its clinical implications. *Indian J Chest Dis Allied Sci*. 2012;54:9–17.
24. Poe RH, Israel RH, Utell MJ, Hall WJ. Probability of a positive transbronchial lung biopsy result in sarcoidosis. *Arch Intern Med*. 1979;139:761–763.
25. Roethe RA, Fuller PB, Byrd RB, Hafermann DR. Transbronchoscopic lung biopsy in sarcoidosis. Optimal number and sites for diagnosis. *Chest*. 1980;77:400–402.
26. de Boer S, Milne DG, Zeng I, Wilsher ML. Does Ct scanning predict the likelihood of a positive transbronchial biopsy in sarcoidosis? *Thorax*. 2009;64:436–439.
27. Kumar R, Goel N, Gaur SN. Sarcoidosis in North Indian population: a retrospective study. *Indian J Chest Dis Allied Sci*. 2012;54:99–104.
28. Milman N, Faurschou P, Munch EP, Grode G. Transbronchial lung biopsy through the fibre optic bronchoscope. Results and complications in 452 examinations. *Respir Med*. 1994;88:749–753.
29. Hetzel J, Eberhardt R, Herth FJ, et al. Cryobiopsy increases the diagnostic yield of endobronchial biopsy: a multicentre trial. *Eur Respir J*. 2012;39:685–690.

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.journals.elsevier.com/indian-journal-of-tuberculosis/

Case Report

Multifocal tuberculous osteomyelitis: A rare presentation

Prasanna Chandiralingam [a,*], *S.M. Arvind Kumar* [b], *S. Krishna Kumar* [c], *S. Kailash* [c]

[a] *Associate Professor, Department of Orthopaedics, PSGIMS & R, Coimbatore, Tamilnadu 641004, India*
[b] *Professor, Department of Orthopaedics, PSGIMS & R, Coimbatore, Tamilnadu 641004, India*
[c] *Junior Resident, Department of Orthopaedics, PSGIMS & R, Coimbatore, Tamilnadu 641004, India*

ARTICLE INFO

Article history:
Received 27 February 2014
Accepted 12 December 2014
Available online 12 March 2015

Keywords:
Tuberculosis
Multifocal osteomyelitis
Biopsy

ABSTRACT

A 29 year old lady presented with vague right lower quadrant abdomen and thigh pain for the past 4 years. X-ray pelvis with both hips was remarkably normal, and MRI was suggestive of osteomyelitis in right ilium and proximal femur. Biopsy confirmed the lesion as tubercular. Isolated bone involvement by tuberculosis without a joint or pulmonary involvement is extremely rare in immunocompetent patients and has not been reported in literature so for. Tuberculosis should be suspected in patients presenting with multiple bone lesions, especially in endemic areas. Prompt surgical drainage and ATT forms the mainstay of treatment.

1. Introduction

Though pulmonary tuberculosis is the most common presentation approximately 10–30% of patient's exhibit extrapulmonary manifestations of the disease.[1] A smaller percentage of these cases involve the musculoskeletal system. The musculoskeletal manifestations of tuberculosis include spondylitis, arthritis, osteomyelitis and bursitis or tenosynovitis. Spinal tuberculosis, also referred to as Pott's disease, represents approximately half of all the cases of musculoskeletal tuberculosis.

Osteomyelitis is the least common musculoskeletal manifestations of tuberculosis, representing less than five percent of cases.[1] The multifocal form of tuberculous osteomyelitis is exceptional even in endemic countries.[2] A suppressed host immune response predisposes to multiple bone involvement. The multifocal skeletal form is usually seen associated with pulmonary tuberculosis.[3]

Tuberculosis osteomyelitis which does not involve a joint is uncommon and may often fail to be diagnosed.[4] Here we are presenting a case of multifocal tuberculous osteomyelitis involving the proximal Femur and Ilium without any joint involvement or pulmonary manifestation in an immunocompetent patient.

2. Case report

A 29 year old lady from an affluent family presented to us with c/o right lower quadrant abdominal pain and pain in the right thigh region for 4 years. A mother of two children, both of them delivered by cesarean section, attributed her lower

* *Corresponding author*. Tel.: +91 0422 2570170, +91 9952102589 (mobile); fax: +91 0422 2594400.
E-mail address: prasannapgi@gmail.com (P. Chandiralingam).
http://dx.doi.org/10.1016/j.ijtb.2015.02.007
0019-5707/

abdomen pain to the cesarean section and sterilization done 4 years back. The pain was dull aching, on and off and did not interfere with her routine activities. Hence, the patient did not obtain any medical evaluation apart from some analgesics as prescribed by local practitioners. Later she developed pain in the right upper aspect of thigh for the last 6 months before presenting to us, which got aggravated over the last one month. The patient had difficulty in squatting and walking. There was no H/O fever/trauma/cough with expectoration. Contact history with tuberculosis was negative. Though the patient per se did not complain of loss of appetite, she had lost 16 Kilograms over the past 4 years.

On examination she was thin built and weighed 29 Kilograms. Patient was afebrile and anaemic. Local examination revealed a swelling of 4 × 2 cm around the right anterior superior iliac spine. Skin over the swelling was normal. Tenderness was present over the right anterior superior iliac spine, iliac wing and right proximal femoral region. The right hip joint movements were full and pain free.

Blood investigations revealed an elevated ESR of 160 mm at 1 h. Haemoglobin was 6.4 g/dl and CRP was positive with a value of 51.63 and LFT was normal except for the albumin-globulin ratio which was 1:1. The serology for HIV was negative. X-ray chest was normal. Radiology of the pelvis with both hips was remarkably normal apart from a lytic to sclerotic area around the right anterior superior iliac spine (Fig. 1a). Chronic osteomyelitis was kept as a possibility and tuberculosis could not be ruled out. Hence, it was decided to proceed with a MRI, which showed features suggestive of multifocal osteomyelitis at the right anterior superior iliac spine with adjacent small loculated abscesses and intraosseous collection in the right proximal femur (Fig. 1b). To obtain a tissue diagnosis and to drain the abscess surgery was planned.

Corticotomy window was made in the right proximal femur and intramedullary pus drained out and debridement done over the right iliac wing and tissue obtained for histopathology. Biopsy came out as indistinct epithelioid granulomata suggestive of tuberculous osteomyelitis (Fig. 2a,b), which was sensitive to first line antitubercular chemotherapy. Patient was started on four drug chemotherapy including Rifampicin, Isoniazid, pyrazinamide and Ethambutol. The dosage was adjusted according to the patient's weight. She was put on daily self-supervised therapy. By the end of first week her pain had come down. The food intake improved as compared to the last four years. She was discharged on the 12th post-operative day after the sutures were removed.

3. Discussion

Tuberculosis of the bone is a well recognized clinical condition that can be diagnosed and managed by physicians and orthopaedic surgeons, often with an excellent outcome. However, the occurrence of multifocal skeletal involvement is rare, even in countries where tuberculosis is endemic. The occurrence of tuberculous osteomyelitis is rare compared with skeletal tuberculosis involving the spine or a joint. The spine is the site of bone tuberculosis in about half the cases,[5–7] and isolated bone involvement without spread to a joint often fails to attract attention. To our knowledge, multifocal tuberculous osteomyelitis without involvement of a joint has not been reported in the literature.

There are no specific radiographic features that are pathognomonic of tuberculosis of bones and joints.[8] Plain radiography in the oesteomyelitis may show soft tissue swelling, minimal periosteal reaction, osteolysis with little or no reactive change, periarticular osteoporosis and erosions. Sclerosis is less frequently seen. Sequestration in tuberculous osteomyelitis is relatively uncommon and less extensive than with pyogenic osteomyelitis.[9] Approximately fifty percent of patients with bone and joint tuberculosis have a negative finding on chest X-ray.[5]

The multifocal skeletal form is usually seen associated with pulmonary tuberculosis. A suppressed host immune response also predisposes to multifocal tuberculosis. Tuberculosis with multiple bone involvement is exceedingly rare in non-immunocompromised patients and in those with normal pulmonary findings. One type of tuberculous osteomyelitis is cystic tuberculosis, which is more commonly encountered in children than in adults. The multifocal form is more common

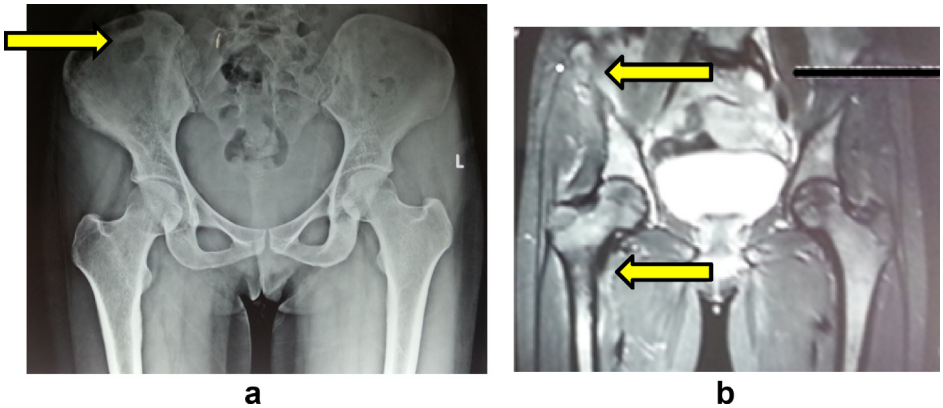


Fig. 1 – (a) X-ray pelvis with both Hips was remarkably normal except for a lytic area in the ilium (arrow) (b) MRI demonstrating multifocal osteomyelitis at the right anterior superior iliac spine with adjacent small loculated abscesses and intraosseous collection in the right proximal femur (arrow).

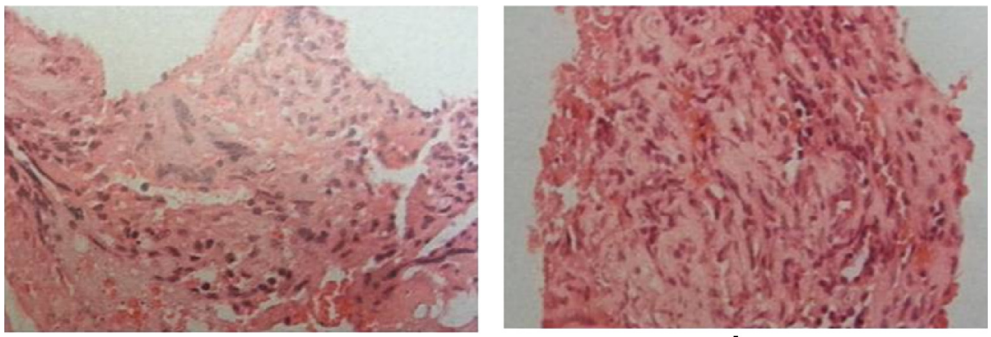


Fig. 2 – (a) and (b) H & E staining from the lesion showing indistinct epithelioid granulomata suggestive of tuberculous osteomyelitis (40× magnification).

than solitary lesions and, in a series of 13 children with histologically confirmed tuberculosis of bone, solitary cystic lesions were found in ten.[10] All of these cases had involvement of the adjacent joint. Multifocal tuberculous osteomyelitis is also known as Osteitis cystic tuberculosa multiplex.

Patients may have non specific features in radiography as in this case and MRI may not be conclusive. Hence a tissue diagnosis is mandatory for confirmation and treatment. Antitubercular chemotherapy along with judicious drainage of pus forms the mainstay of treatment.

4. Conclusion

Multifocal tuberculous osteomyelitis without involvement of a joint or pulmonary disease is a rare presentation. Patients present with vague symptoms. Radiological features are non specific so a high index of suspicion is required. A biopsy should be done in patients with multiple bone lesions to confirm the diagnosis and initiate appropriate treatment.

Conflicts of interest

All authors have none to declare.

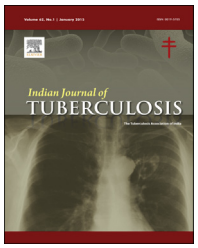
REFERENCES

1. Shrestha Om P, Sitoula Prakash, Hosalkar Harish S, Banskota Ashok K, Spiegel David A. Bone and joint tuberculosis. *Spiegel Univ Pennysylvania J*. 2010;20:23–28.
2. Moujtahid M, Essadki B, Lamine A, Bennouno D, Zryouil B. Multifocal bone tuberculosis: Apropos of a case. *Rev Chir Orthop Reparatrica Mot*. 1995;81:553–556. French.
3. Marudanayagam Ashok, Gnanadoss James J. Multifocal skeletal tuberculosis: a report of three cases. *Iowa Orthop J*. 2006;26:151–153.
4. Vohra Rajeev, Kang Harinder S, Dogra Sameer, Saggar Radha R, Sharma Rajan. Tuberculous osteomyelitis. *J Bone Joint Surg Br*. 1997;79-B:562–566.
5. Davidson PT, Horowitz I. Skeletal tuberculosis. A review with patient presentations and discussion. *Am J Med*. 1970;48:77–84.
6. Gropper GR, Acker JD, Robertson JH. Computed tomography in Pott's disease. *Neurosurgery*. 1982;10:506–508.
7. Martini M, Ouahes M. Bone and joint tuberculosis: a review of 652 cases. *Orthopaedics*. 1988;11:861–866.
8. Watts Hugh G, Lifeso Robert M. Tuberculosis of bones and joints. *J Bone Joint Surg*. 1996;78-A:288–298.
9. Vanhoenacker Filip M, Sanghvi Darshana A, De Backer Adelard I. Imaging features of extraaxial musculoskeletal tuberculosis. *Indian J Radiol Imaging*. 2009;19:176–186.
10. Rasool MN, Govender S, Niidoo KS. Cystic tuberculosis of bone in children. *J Bone Joint Surg Br*. 1994;76 B:113–117.

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.journals.elsevier.com/indian-journal-of-tuberculosis/



Case Report

Rare interstitial lung disease: Pulmonary Langerhans Cell Histiocytosis in a young non smoking Indian female

Lalita Fernandes [a,*], *Rohit Vadala* [b], *Anthony Menezes Mesquita* [c], *Pradeep Vaideeswar* [d]

[a] *Professor, Department of Pulmonary Medicine, Goa Medical College, India*
[b] *Junior Resident, Department of Pulmonary Medicine, Goa Medical College, India*
[c] *Director-Professor & HOD, Department of Pulmonary Medicine, Goa Medical College, India*
[d] *Professor (Additional), Department of Pathology, Seth GS Medical College, Mumbai, India*

ARTICLE INFO

Article history:
Available online 12 March 2015

Keywords:
LCH (Langerhans cell histiocytosis)
Interstitial lung disease
High resolution computed tomography

ABSTRACT

Adult Pulmonary Langerhans Cell Histiocytosis (PLCH) is a rare interstitial lung disease which occurs almost exclusively in smokers. A marked male predominance was initially reported, but recent studies show both men and women are equally affected due to the increasing smoking habits in women. The natural history is variable with 25% of patients having asymptomatic disease while 10–20% progress rapidly to respiratory insufficiency and death. The diagnosis is not easily recognized by clinicians or pathologists. Awareness of the clinical presentation and classical HRCT findings helps in early diagnosis and management of this disease. We report a rare case of severe PLCH in a young non smoking female with a short history who progressed rapidly to respiratory failure and died.

1. Introduction

Pulmonary Langerhans Cell Histiocytosis is an isolated form of LCH and is characterized by peribronchiolar nodular proliferation of Langerhans' cells, which gradually progresses to fibrosis with formation of irregular cysts. This results in classical High Resolution Computed Tomography (HRCT) findings that differentiates it from other interstitial lung diseases. Smoking is a risk factor. Management includes smoking cessation, corticosteroid therapy or immunosuppressive therapy. Outcome is variable with some showing spontaneous remission while a subgroup of patients develop severe disease leading to respiratory failure and death. Severe PLCH is rare[1] and hence this case is being reported.

2. Case report

A 26 years old married female with two children and housewife by occupation presented with history of occasional dry

* *Corresponding author*. TB and Chest Diseases Hospital, St. Inez, P.O. Caranzalem, Goa 403002, India. Tel.: +91 (0)9822102177 (mobile), +91 (0)8322225088 (office); fax: +91 (0)8322425007.
E-mail address: drlalitafernandes@gmail.com (L. Fernandes).
http://dx.doi.org/10.1016/j.ijtb.2015.02.008

cough, low grade fever, breathlessness, easy fatigability and loss of appetite for two weeks. There was no associated hemoptysis, chest pain, joint pain, or skin rash. There was neither a past history of tuberculosis or contact with tuberculosis nor any other relevant medical history. She was a non - smoker and non - alcoholic and she was not exposed to environmental tobacco smoke. She gave a history of using biomass fuel for cooking (chulha smoke exposure) for the last eight years with 40 hour years of exposure.

Chest X-ray showed bilateral reticulo-nodular shadows (Fig. 1). Sputum for acid fast bacilli (AFB) and Tuberculin Test were negative. She was started on DOTS Category 1 (Isoniazid, Ethambutol, Pyrazinamide and Rifampicin) as a case of Miliary Tuberculosis at her primary health centre.

Despite seven doses of anti-tubercular therapy, patient continued to be breathless with development of swelling of feet. Chest X-ray taken during this episode showed increase in diffuse reticulo-nodular shadows in the lung and was admitted for further investigations.

On physical examination, she was thinly built, afebrile with pulse rate of 108/minute, respiratory rate of 28/minute and blood pressure of 108/70 mmHg. She had facial puffiness, increased jugular venous pressure, cyanosis and bilateral edema of feet. She had no evidence of lymphadenopathy or clubbing. On systemic examination of respiratory system, crackles were heard in bilateral bases. Investigations done at admission showed hemoglobin of 10.9 gm%, total leukocyte count of 12400/cmm with neutrophil predominance, normal coagulation profile and normal renal and liver functions tests. Anti-nuclear antibodies and rheumatoid factor were negative. C-reactive protein was positive. Assays for human immunodeficiency virus1 and 2 antibodies were negative. Venous Doppler study of both lower limbs was negative for deep vein thrombosis.

HRCT thorax done showed multiple thick walled irregular cysts, multiple centrilobular nodules and peribronchial cuffing. Thick walled cysts were predominantly noted in upper and mid zones while the lower zone showed cysts and nodular distribution involving the costophrenic regions. There was no mediastinal lymphadenopathy or any pleural effusion (Fig. 2). In view of HRCT findings, a provisional diagnosis of LAM (Lymphangioleiomyomatosis) and PLCH was considered. Abdominal ultrasound was normal and renal ultrasound did not reveal any angiomyolipoma.

On the 5th day of admission, the patient developed spontaneous left-sided pneumothorax followed by right-sided pneumothorax (see Fig. 3)

CT guided lung biopsy done following left intercostal tube insertion for left spontaneous pneumothorax showed epithelioid cells, necrosis and acute inflammatory cells. She was treated with nasal oxygen and talc pleurodesis. However patient progressively deteriorated and died. Post mortem lung biopsy revealed presence of alveoli showing cystic dilatation. The interstitium showed presence of collection of lymphocytes, plasma cells, eosinophils and histiocytes. The histiocytes showed presence of moderate amount of cytoplasm and vesicular nucleus with nuclear grooving (See Fig. 4). Immunohistochemistry markers was positive for CD1a (See Fig. 5). The diagnosis of PLCH was thus confirmed.

3. Discussion

Langerhans cell was first described by Paul Langerhans in 1868. It is a bone marrow derived dendritic cell found in the skin and lung whose normal function is processing and presentation of antigens. The disease Langerhans Cell Histiocytosis results from clonal accumulation and proliferation of langerhans cell in bone, lungs, skin, lymphnodes and pituitary gland due to unknown reasons.

It is predominantly a disease of childhood and is rarely seen in adults. The Histiocyte Society founded in 1985 classified LCH into single system LCH involving one organ or system and multisystem LCH involving two or more organs or system. Acute disseminated LCH (Letterer-Siwe disease) is a severe multisystem disease affecting mostly young children with poor prognosis. Multifocal LCH seen in older children and adolescent (Hand-Schuller- Christian syndrome) almost

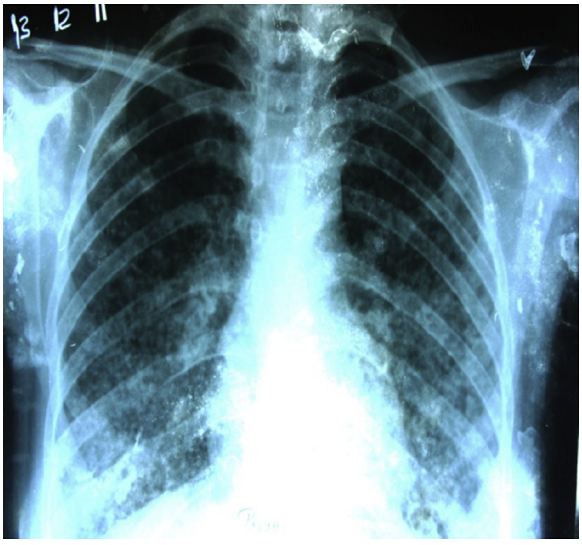


Fig. 1 – Chest X-ray showing bilateral reticulo-nodular shadows.

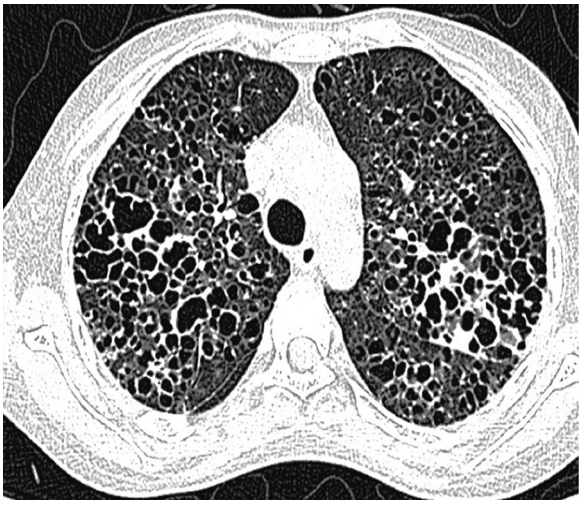


Fig. 2 – HRCT Thorax showing bilateral multiple thick walled irregular cysts.

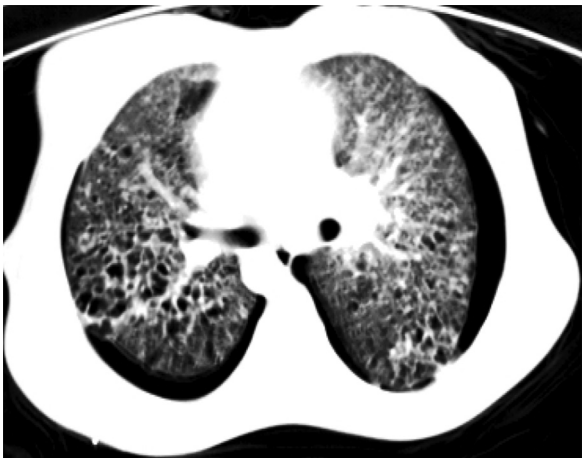


Fig. 3 – HRCT thorax Showing Bilateral Pneumothoraces and multiple thick walled cysts.

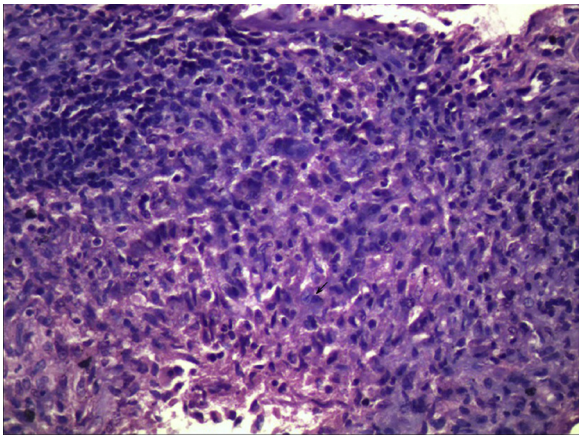


Fig. 4 – Histiocytes show presence of moderate amount of cytoplasm and vesicular nucleus with nuclear grooving. HE stained specimen at 40X.

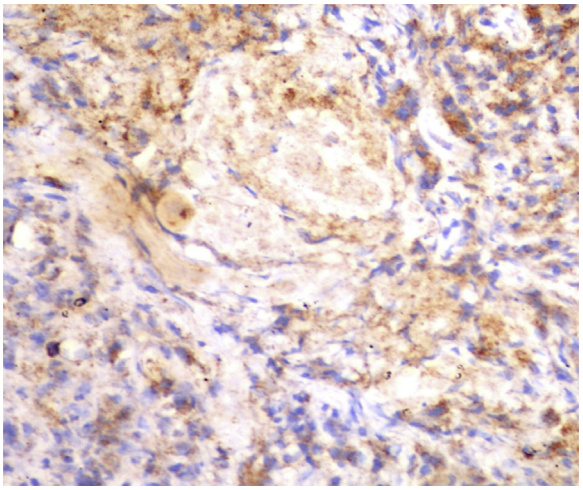


Fig. 5 – IHC staining positive for CD1a.

always involves bones. Pulmonary Langerhans Cell Histiocytosis (PLCH) is a disease in adults affecting lung in isolation or sometimes involving other organ system.

The incidence of LCH in adults is approximately 1–2 cases per million and Adult Pulmonary Langerhans Cell Histiocytosis is a rare disease. Data on prevalence is not available, but in a study on surgical lung biopsies done for diffuse lung disease, PLCH was found in 4–5%.[2] In a recent study in China on LCH, only 0.8% had PLCH. The prevalence is underestimated as 25% of patients are asymptomatic or undergo spontaneous remission. It affects adults in age group of 20–40 years and in 90% of the cases, smoking was the risk factor.[3] Our patient did not have history of smoking nor was exposed to environmental tobacco smoke, but was exposed to biomass smoke (chulha smoke). Biomass smoke contains particulate matter PM2.5, PM10 and gases similar to tobacco smoke. Biomass smoke and tobacco smoke induce similar pathological processes as noted in patients of COPD. There is no case reported in literature with association of PLCH and biomass smoke and this is probably the first. Based on the pathogenesis of PLCH we suggest that biomass smoke may have induced the disease.

Time to presentation of the disease can be six months. Respiratory symptoms include dry cough and breathlessness on exertion. Chest pain associated with pneumothorax can be the first manifestation leading to diagnosis and approximately 15% present with spontaneous pneumothorax as initial presentation.[4] Bilateral or recurrent pneumothoraces should raise a suspicion of this disease. Fever, malaise and weight loss are seen in minority of patients.

Chest x-ray shows bilateral, symmetrical, irregular predominantly upper and mid zone nodules. Costophrenic angles are typically spared but in severe disease the lower zones and costophrenic angle can be involved. As disease progresses, reticular and cystic lesions are seen. HRCT shows classical picture of nodules, cavitated nodules, irregular or bizarre shaped thick and thin walled cysts. The distribution of nodules is centrilobular affecting upper and mid lung fields and sparing of lower lung fields. In extensive disease the lung bases are involved[5] as seen in our patient indicating severe disease. Hilar lymphadenopathy and pleural effusion are rare. This HRCT pattern is diagnostic of PLCH and lung biopsy is not needed for diagnosis.[6] Pulmonary arterial hypertension can also be noted as was manifested in our patient with raised JVP and right sided cardiac failure. Tru-cut lung biopsy taken was inconclusive due to paucity of tissue obtained from the fibrosed lung.

Bronchoalveolar lavage (BAL) also shows very low sensitivity[7] and is rarely useful in the diagnosis. Although there are studies stating that identification of 5% or greater CD1a positive cells is highly suggestive of PLCH, such high levels are infrequently seen.[8] Open lung biopsy shows Langerhans cells in bronchiolar walls and epithelium surrounded by eosinophils, lymphocytes, fibroblasts and plasma cells. These Langerhan Cells have convoluted nuclear membrane and abundant cytoplasm. Immunohistochemical staining for CD1a is usually sufficient to make diagnosis. On electron microscopy Birbeck granules (tennis racquet shaped inclusions) are seen in cytoplasm.

There are no randomized clinical trials available on treatment of this disease. Some patients show spontaneous

remission on smoking cessation,[9] while some despite corticosteroid therapy/immunosuppressive therapy show poor prognosis. Rapid deterioration is seen in 10–20% of patients[10] and this patient died within two months of presentation.

4. Conclusions

Reticulo-nodular shadows on chest xray have various differential diagnosis. HRCT helps differentiate the causes of reticulo-nodular shadows. Typical HRCT findings are diagnostic of PLCH. Although this disease is mostly seen in smokers, our patient was a non smoking female with severe PLCH and biomass smoke exposure as a probable risk factor.

Conflicts of interest

All authors have none to declare.

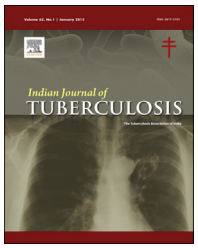
REFERENCES

1. Watanabe R, Tatsumi K, Hashimoto S, Tamakoshi A, Kuriyama T. Clinico-epidemiological features of pulmonary histiocytosis X. *Intern Med*. 2001;40:998–1003.
2. Gaensler EA, Carrington CB. Open biopsy for chronic diffuse infiltrative lung disease: clinical, roentgenographic and physiological correlations in 502 patients. *Ann Thorac Surg*. 1980;30:411–426.
3. Vassallo R, Ryu JH, Schroeder DR, Decker PA, Limper AH. Clinical outcomes of pulmonary Langerhans cell histiocytosis in adults. *N. Engl. J. Med*. 2002;346:484–490.
4. Cosgrove GP, Frankel SK, Brown KK. Challenges in pulmonary fibrosis 3: cystic lung disease. *Thorax*. 2007;62:820–829.
5. Juvet SC, Hwang D, Downey GP. Rare lung diseases III: pulmonary Langerhans cell histiocytosis. *Can Respir J*. 2010;17:e55–62.
6. Grenier P, Valeyre D, Cluzel P, Bauner MW, Lenoir S, Chastang C. Chronic diffuse interstitial lung disease: diagnostic value of chest radiography and high-resolution CT. *Radiology*. 1991;179:123–132.
7. Tazi A. Adult pulmonary Langerhans cell histiocytosis. *Eur Respir J*. 2006;27:1272–1285.
8. Daniel C, Israel- Biet D, Costabel U, Rossi GA, Wallaert B. The clinical role of BAL in pulmonary histiocytosis X. *Eur Respir J*. 1990;3:949–950, 961–969.
9. Mogulkoc N, Veral A, Bishop PW, Bayindir U, Pickering CA, Egan JJ. Pulmonary Langerhans cell histiocytosis: radiological resolution following smoking cessation. *Chest*. 1999;115:1452–1455.
10. Abbot GF, Rosado-de-Christenson ML, Franks JJ, Fraiser AA, Gavin JR. From the archives of the AIFP: pulmonary Langerhans cell histiocytosis. *Radiographics*. 2004;24:821–841.

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.journals.elsevier.com/indian-journal-of-tuberculosis/



Case Report

Cavitating lung disease due to concomitant drug resistant tuberculosis and invasive pulmonary *Aspergillosis* in a post-partum patient: A case report

Animesh Ray [a], *J.C. Suri* [b,*], *M.K. Sen* [c], *S. Chakrabarti* [c], *Ayush Gupta* [a], *Malini Capoor* [d]

[a] *Senior Resident, Department of Pulmonary, Critical Care & Sleep Medicine, Vardhman Mahavir Medical College, & Safdarjang Hospital, New Delhi, India*
[b] *Professor, Consultant Chest Physician & Head of Department, Department of Pulmonary, Critical Care & Sleep Medicine, Vardhman Mahavir Medical College, & Safdarjang Hospital, New Delhi 110029, India*
[c] *Associate Professor & Consultant Chest Physician, Department of Pulmonary, Critical Care & Sleep Medicine, Vardhman Mahavir Medical College, & Safdarjang Hospital, New Delhi, India*
[d] *Associate Professor & Senior Microbiologist, Department of Pulmonary, Critical Care & Sleep Medicine, Vardhman Mahavir Medical College, & Safdarjang Hospital, New Delhi, India*

ARTICLE INFO

Article history:
Received 27 February 2014
Accepted 12 December 2014
Available online 12 March 2015

Keywords:
Invasive pulmonary aspergillosis
Drug resistant tuberculosis
Pregnancy associated tuberculosis

ABSTRACT

Many disorders can present as cavitating lesions in the lung. In this case report, a case of mixed infection with drug resistant tuberculosis and invasive pulmonary aspergillosis in a post-partum patient has been presented.

1. Introduction

Cavitating lung lesions can arise due to various causes – both infectious and non-infectious. In most of the cases meticulous clinical examination and appropriate investigations can pinpoint the cause. Rarely in some cases might more than one cause be responsible. Pulmonary tuberculosis is a common cause of cavitating lesions in our region. Fungal infections of the lung due to *Aspergillus* species are well known. The principal forms of pulmonary aspergillosis include colonizing aspergillosis, invasive aspergillosis and allergic aspergillosis. Aspergilloma with pulmonary tuberculosis usually occurs in upper lobes in approximately 94% of cases.[1] Of the long term sequelae of pulmonary tuberculosis, chronic pulmonary aspergillosis is perhaps the most severe.[2] Clinical algorithms to discriminate.

* *Corresponding author*. Tel.: +91 011 26190958, +91 9810097259.
E-mail address: docjcsuri@gmail.com (J.C. Suri).
http://dx.doi.org/10.1016/j.ijtb.2015.02.009

Aspergillus colonization from putative invasive pulmonary aspergillosis (IPA) in critically ill patients has been described.[3] Coexistent infection with drug resistant tuberculosis and IPA are rare. We present a case of mixed infection with drug resistant tuberculosis and IPA causing cavitating pulmonary lesions and highlight the salient points of this case.

2. Case history

A 20 year old post-partum female patient who had given birth to twin babies 40 days back presented with progressively increasing shortness of breath, generalized weakness, loss of weight and loss of appetite for around 30 days and low grade fever for around 5 days. She gave a past history of pulmonary tuberculosis around 5 years back for which she was treated with four drugs anti-tubercular therapy (ATT) for 6 months. There were no complaints of cough, hemoptysis or features suggestive of sinusitis. She had been empirically started on ATT by a private practitioner 10 days ago to which there was no improvement. On examination she was conscious, alert and oriented. Her BP was 90/60 mmHg, pulse: 132/min; respiratory rate: 34/min,temperature: 100 °F, SpO_2: 86%. Arterial blood gas analysis (on room air) showed Type I respiratory failure (PaO_2: 30 mmHg; $PaCO_2$: 27 mmHg; HCO_3: 20 meq/L; pH: 7.42). The salient investigations are also listed in Table 1. Contrast enhanced computed tomography thorax (Fig. 1) was done which revealed centrilobular nodules and tree in bud opacities diffusely with large nodular opacities distributed bilaterally, but mainly in mid and lower lung fields (few showing cavitation and a peripheral halo). In view of worsening respiratory failure she had to be intubated and mechanically ventilated on the fifth day of admission. Fresh investigations were ordered to find out the cause of her deteriorating condition. Assay was positive for c-ANCA (by immunoflouroscence) and p-ANCA was borderline positive. Bronchoscopic lavage was done and its analysis revealed positivity for acid fast bacilli (AFB). A complete mycological workup was done. Broncho alveolar lavage (BAL) sample showed septate hyphae under microscopic examination. After 4 days of incubation, the culture of BAL fluid revealed growth of *Aspergillus fumigatus*. BAL sample and serum sample showed galactomannan antigen optical density index (ODI) of 2 and 1.5 respectively. Transbronchial lung biopsy specimen was sent for fungal stain and culture. Calcufluor mount (Fig. 2) and potassium hydroxide (KOH) mount of lung biopsy specimen showed septate hyphae and its culture also revealed growth of *Aspergillus fumigatus*.

Table 1 – Laboratory investigations of the patient.

Hb, TLC, DLC, Platelets	Hb: 10, TLC: 12,100/cmm, Platelets: 3 lacs/cmm
Kidney function test and serum electrolytes	WNL
Liver function test	WNL
HIV I & II	Negative
Anti-nuclear antibody	Negative
c-ANCA and p-ANCA (by immunoflouroscence)	c-ANCA (positive) and p-ANCA (borderline positive)
c-ANCA and p-ANCA (by enzyme immune-assay)	Negative
Serum galactomannan	Postive (ODI of 2)
Blood culture (bacterial and fungal)	Negative
Urine routine examination and culture	WNL
Bronchoscopic washings and biopsy	Postive (ODI of 1.5)
Gram stain and culture	Negative
Fungal stain	Negative
Acid Fast Bacilli stain	Positive
BAL galactomannan	
Mycobacterium tuberculosis culture	Postive (drug resistant)
Lung biopsy culture (fungal)	Aspergillus fumigatus
GeneXpert (from endotracheal tube secretions)	Positive; sensitive to rifampicin
Chest X-ray	Bilateral widespread cavitating nodular opacities
CT lung	Bilateral cavitating lung nodules
CT abdomen	WNL
CT sinus	WNL

Hb: Hemoglobin; TLC: Total leucocyte count; DLC: Differential leucocyte count; WNL:Within normal limit; HIV: Human immunodeficiency virus; c-ANCA: cytoplasmic Anti-neutrophilic cytoplasmic antibody; p-ANCA: perinuclear Anti-neutrophilic cytoplasmic antibody; CT: Computed tomography; ODI: Optical Density Index.

Anti-tubercular therapy (isoniazid, rifampicin, pyrazinamide and ethambutol) was continued and liposomal Amphotericin B was started in appropriate doses and was continued for 6 weeks till BAL and serum galactomannan antigen ODI were each <0.5. Within two weeks partial improvement to the above treatment was noted as there was mild decrease in intensity and frequency of fever and partial reduction in ventilatory requirements. Repeat examination of respiratory secretions after 2 weeks showed persistent copious acid fast bacilli (AFB). A GeneXpert® analysis from the patient's respiratory secretion was ordered on the second week of admission. It revealed *Mycobacterium tuberculosis* with sensitivity to rifampicin. The laboratory report of culture for *M. tuberculosis* that had been sent initially was reported as positive and showed resistance to isoniazid, ethambutol and streptomycin. Second line ATT was started on the basis of drug sensitivity pattern (cycloserine, levofloxacin, kanamycin, ethionamide, pyrazinamide and rifampicin). She responded gradually to this treatment. Her fever resolved within a week of starting second line anti-tubercular drugs and her ventilator requirements decreased. Analyses of respiratory secretions were negative for AFB from the second week of starting second line ATT. The patient showed steady improvement to treatment and she was gradually shifted from total invasive ventilation to nocturnal ventilation through tracheostomy tube after about three months of starting of second line ATT. After another two months she was shifted to nocturnal non-invasive ventilation. She was finally discharged after about eight months of total stay in the hospital. Since the patient had developed post-infection lung fibrosis with resultant Type II respiratory failure she was advised to continue nocturnal non-invasive ventilation and 24 h nasal oxygen @ 1 litre/min through nasal prongs at the time of discharge. Fig. 3 shows repeat CT scan thorax around five months after starting

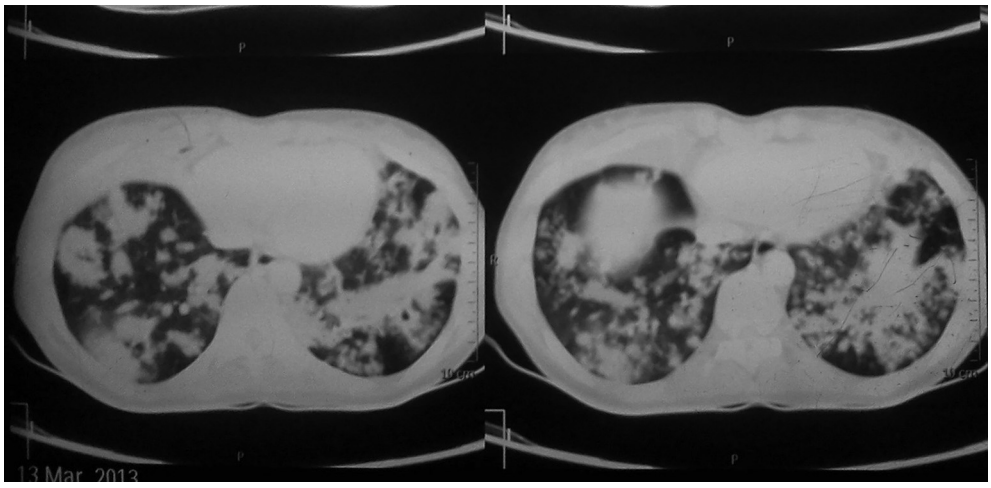


Fig. 1 – CT thorax at presentation showing bilateral diffuse nodules with evidence of cavitation in some nodules.

treatment. She remained stable on this regimen. Subsequent estimation of c-ANCA at the time of discharge was negative.

3. Discussion

In this case a diagnosis of drug resistant tuberculosis and proven invasive pulmonary aspergillosis was finally made. It was based on the criteria for definite invasive pulmonary aspergillosis (IPA) according to EORTC/MSG criteria.[4] The patient showed therapeutic response only after both the conditions were appropriately treated. This case brings up certain extra-ordinary points worth highlighting. First, mixed infection with drug resistant tuberculosis and proven invasive pulmonary aspergillosis has been very rarely reported in literature. Kumar et al. reported a case of Multi-drug resistant tuberculosis with aspergilloma and IPA in a diabetic patient who was successfully treated.[5]We believe this is the second such case report of co-existence of drug resistant tuberculosis and IPA in literature. Secondly the influence of post-partum state on the disease course and severity deserves a special mention. IPA usually occurs in the presence of classical risk factors none of which was present in this case.[6] The patient's post-partum condition, which is considered a risk factor for infections like tuberculosis, probably rendered her vulnerable to the invasive fungal infection.[7] Interestingly, past history of tuberculosis, as present in this case, might also be a risk factor for aspergillus colonization and possible invasion.[8] Thirdly as a part of work-up of cavitating lung disease c-ANCA and p-ANCA estimation was done which was positive (by immunofluorescence method). However investigations revealed no evidence of upper respiratory or renal involvement. ANCA assay was therefore repeated by the enzyme linked immune-assay (ELISA) which was negative for both anti-proteinase-3 and anti-myeloperoxidase antibodies. Transbronchial biopsy specimen also showed no evidence of vasculitis. Fungal infections and tuberculosis can rarely give rise to false positive ANCA results especially with the immunofluorescence methods.[9] In such cases ELISA methods can be useful as they are more specific. Fourthly this patient's GeneXpert® result (sensitivity for rifampicin) had to be taken in the appropriate context. GeneXpert® indicates sensitivity to rifampicin only but not to the other drugs.[10] Hence conventional culture with drug sensitivity has to be relied upon for diagnosis of drug resistant tuberculosis, as in this case. Fifthly in this case amphotericin B was used in place of voriconazole (which is considered the treatment of choice for IPA) as rifampicin has significant interactions with voriconazole.[11] Rifampicin, being an enzyme inducer, leads to increased metabolism of voriconazole thus decreasing its level in the body. So we would like to emphasize that in cases of concomitant infection with tuberculosis and aspergillosis this significant interaction has to be kept in mind. Lastly this patient's protracted course in the intensive care unit and requirement of prolonged ventilator support, without which her survival might not have been

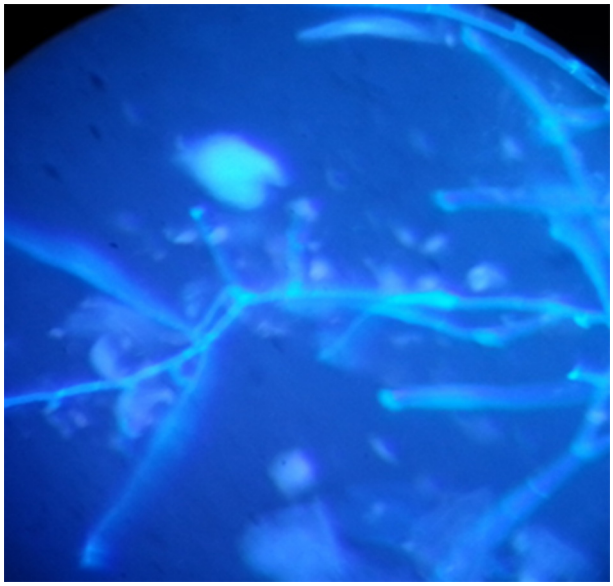


Fig. 2 – Calcofluor white staining of lung biopsy specimen showing multiple septated hyphae of Aspergillus fumigatus.

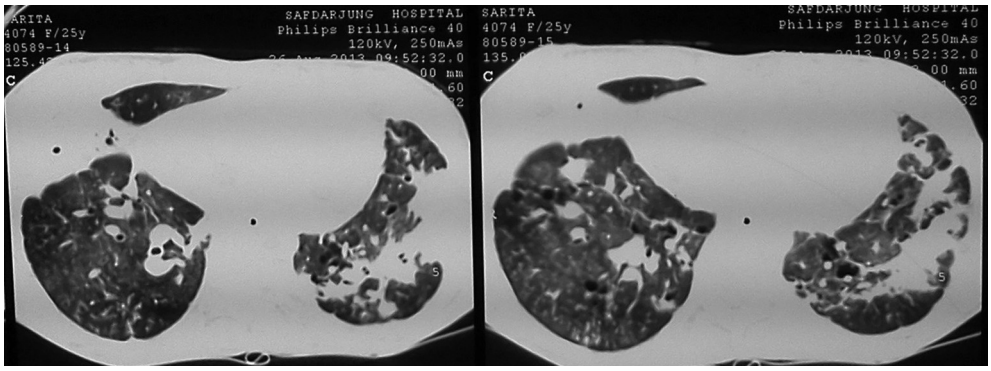


Fig. 3 – CT thorax 5 months after initiation of treatment showing marked decrease in the number of nodules with evidence of fibrosis in the lung parenchyma.

possible, underscores the role of critical care services in the management of such obstinate infections as also the need for the same in general tuberculosis hospitals.

To conclude we would like to reiterate that in critically ill tuberculosis patients who have risk factors like post-partum state and past tuberculosis; and who are not responding to standard therapy, drug resistant tuberculosis and concomitant invasive fungal infection is to be considered early. In this regard selection of appropriate investigations, treatment with appropriate drugs and provision of sustained intensive care support is of paramount importance.

4. Summary

Cavitation in the lung can arise due to a large number of causes. In some cases more than one cause can co-exist. We describe a case of cavitating pulmonary lesions caused by concomitant proven invasive pulmonary aspergillosis and drug resistant pulmonary tuberculosis in a post-partum patient and highlight the diagnostic and therapeutic challenges it posed.

Conflicts of interest

All authors have none to declare.

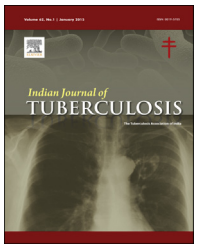
REFERENCES

1. Garros GJ, Ruiz de Gordejuela E, Vara Quadrado F. Pulmonary Aspergillomas. Analysis of 31 patients. *Arch Bronconeumol.* 1994;30:424–432.
2. Denning DW, Pleuvry A, Cole DC. Global burden of chronic pulmonary aspergillosis as a sequel to pulmonary tuberculosis. *Bull WHO.* 2011;89:864–872.
3. Blot SI, Taccone FS, Van den Abeele A-M, et al. A clinical algorithm to diagnose invasive pulmonary aspergillosis in critically ill patients. *Am J Respir Crit Care Med.* 2012;186:56–64.
4. De Pauw B, Walsh TJ, Donnelly JP, et al. Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group EORTC/MSG) Consensus Group. *Clin Infect Dis.* 2008 Jun 15;46:1813–1821.
5. Kumar AA, Shantha GP, Jeyachandran V, et al. Multidrug resistant tuberculosis coexisting with aspergilloma and invasive aspergillosis in a 50 year old diabetic woman: a case report. *Cases J.* 2008 Nov 8;1:303.
6. Kousha M, Tadi M, Soubani AO. Pulmonary aspergillosis: a clinical review. *Eur Respir Rev.* 2011 Sep 1;20:156–174.
7. Zenner D, Krullshaar ME, Andrews N, Abubakar I. Risk of tuberculosis in pregnancy: a national, primary care- based cohort and self-controlled case series study. *Am J Respir Crit Care Med.* 2012;185:779–784.
8. Panda B, Rosha D, Verma M. Pulmonary tuberculosis: a predisposing factor for colonizing and invasive aspergillosis of lungs. *Ind J Tub.* 1998;45:221.
9. Vahid B, Wildemore B, Nguyen C, Marik P. Positive c-ANCA and cavitary lung lesion: recurrence of Wegener Granulomatosis or Aspergillosis? *South Med J.* 2006 Jul;99:753–756.
10. Helb D, Jones M, Story E, et al. Rapid detection of *Mycobacterium tuberculosis* and rifampin resistance by use of on-demand, near-patient technology. *J Clin Microbiol.* 2010;48:229–237.
11. FDA Antiviral Drugs Advisory Committee. *Briefing Document for Voriconazole (Oral and Intravenous Formulations)*; 2001. Available from: http://www.fda.gov/ohrms/dockets/ac/01/briefing/3792b2_01_Pfizer.pdf.

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.journals.elsevier.com/indian-journal-of-tuberculosis/



Response to Dr Daley's article: Do not ignore the private sector

Radhika Banka [a,*], *Zarir Udwadia* [b]

[a] *DNB Trainee, Department of Pulmonary Medicine, P D Hinduja National Hospital and Medical Research Centre, Veer Savarkar Marg, Mahim, Mumbai 400016, India*

[b] *Consultant, Department of Pulmonary Medicine, P D Hinduja National Hospital and Medical Research Centre, Veer Savarkar Marg, Mahim, Mumbai 400016, India*

The review by Dr Daley[1] provides an informative insight to the current MDR TB (Multi Drug Resistant Tuberculosis) scenario in India. However, official figures may not be representative of ground reality because large numbers of Tuberculosis patients choose to bypass the RNTCP (Revised National Tuberculosis Control Program), opting instead for care with private practitioners. These patients are slipping under the radar and in fact, scandalously, notification of Tuberculosis was only made compulsory after the first Totally Drug Resistant (TDR) TB Cases were reported in 2011.[2]

In a community based door to door survey conducted by Satyanarayan in thirty districts across the country, they found that nearly half of TB cases were on treatment from 'outside DOTS/RNTCP' sources and hence were missed by the TB notification system.[3]

Results from the National Family Health Survey-3 revealed that the private sector was the preferred source of healthcare (70% of urban households and 63% of rural households) for patients diagnosed with TB, although there is very variable quality of treatment provided by private practitioners.[4]

The WHO slogan for World TB Day on 24 March, 2014 was – 'reach the missing three millions'. Out of the estimated three million incident TB cases missing from notification globally, nearly a million are from India. More than a third of the TB cases in the country are unable to avail the public health program services. Most of these missed cases might have been diagnosed and treated in other health care sectors, including the large private sector in India and these cases largely remain unnotified to the program.[5]

WHO has estimated that in 2009, 99,000 cases of MDR TB emerged in the country including those outside RNTCP. Among these, 64,000 were estimated to have emerged from TB cases notified to RNTCP, which leaves almost a third of cases being notified outside RNTCP.[6]

Thus whilst we agree with the author that the RNTCP is a robust public health program which has reached out to over 15 million people in the last decade, the private sector cannot be wished away. The sheer number of Indian TB patients visiting private practitioners mandates the importance of Public Private Program Models. The dysfunctional relationship between private and public sectors is, in our opinion fuelling India's MDR-TB Crisis.[7]

Clearly the way ahead involves public-private mix (PPM) and currently apart from a few pilot studies such as the WHO Stop TB Partnership, involvement of NGOs, private practitioners, IMA (Indian Medical Association) and projects such as Akshya (Union) as a part of RNTCP for "universal coverage", this has yet to be developed and expanded.[8,9]

Conflicts of interest

All authors have none to declare.

Editor's note

We definitely agree with the views expressed in the letter. Although RNTCP is making efforts to involve private sector by introduction of various schemes, lot more has to be done in this direction from private sector as well as by non-governmental sector including private practitioners as their social responsibility.

* *Corresponding author*. Tel.: +91 022 24447353, +91 9869403861, +91 9757262410.
E-mail address: radhika.banka@gmail.com (R. Banka).

http://dx.doi.org/10.1016/j.ijtb.2015.02.029

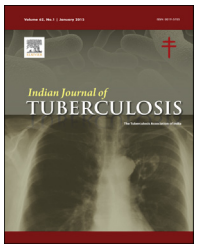
REFERENCES

1. Daley CL. Global scale-up of the programmatic management of multidrug-resistant tuberculosis. *Indian J Tuberc.* 2014;61:108–115.
2. Udwadia ZF, Amale RA, Ajbani KK, Rodrigues C. Totally drug-resistant tuberculosis in India. *Clin Infect Dis.* 2012;54: 579–581.
3. Ministry of Health and Family Welfare. National Family Health Survey (NFHS-3), 2005-06. Available from: http://www.measuredhs.com/pubs/pdf/SR128/SR128.pdf Last accessed from 21.06.14
4. Satyanarayana S, Nair SA, Chadha SS, et al. From where are tuberculosis patients accessing treatment in India? Results from a cross-sectional community based survey of 30 districts. *PloS One.* 2011;6:e24160. http://dx.doi.org/10.1371/journal.pone.0024160. Epub 2011 Sep 2.
5. World Health Organisation. *Message from WHO Representative to India*; 2014. Available from: http://www.searo.who.int/india/mediacentre/events/2014/tb_day/en/. Last accessed from 21.06.14.
6. Revised National Tuberculosis Control Programme. *Guidelines on Programmatic Management of Drug Resistant TB (PMDT) in India.* New Delhi: Central TB Division; 2012 [Chapter 1]: Background & Framework For Effective Control of Multi Drug-Resistant Tuberculosis 4 p.
7. Udwadia ZF, Pinto LM. Tuberculosis management by private practitioners in Mumbai, India: has anything changed in two decades? *PloS One.* 2010 Aug 9;5:e12023. http://dx.doi.org/10.1371/journal.pone.0012023.
8. World Health Organisation. *Public-private Mix for TB Care and Control a Toolkit.* Geneva: Stop TB Partnership; 2010.
9. TB India. *Revised National TB Control Programme Annual Status Report.* New Delhi: Central TB Division; 2013 [Chapter 9]: Partnership.

Available online at www.sciencedirect.com

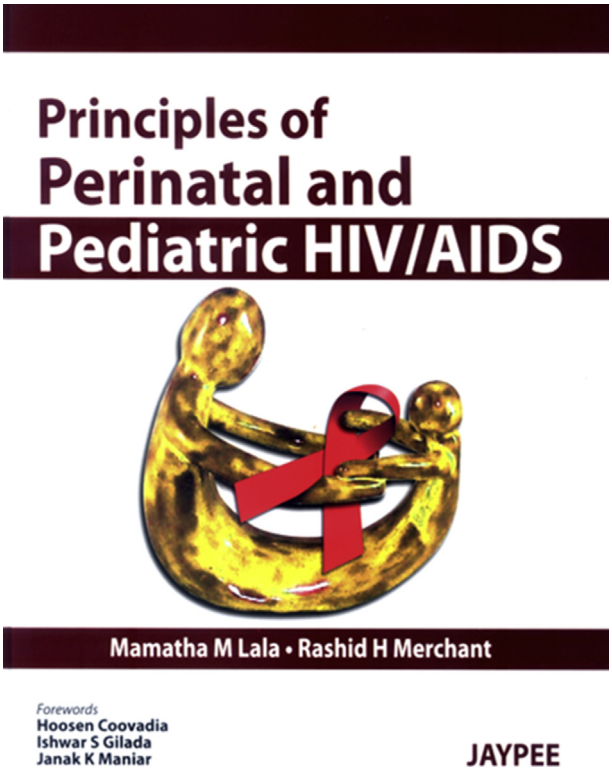
ScienceDirect

journal homepage: http://www.journals.elsevier.com/indian-journal-of-tuberculosis/



Book review

Principles of Perinatal and Pediatric HIV/AIDS, Mamatha M. Lala, Rashid H. Merchant. Jaypee Publishers, 4838/24, Ansari Road, Daryaganj, New Delhi 110002, India



This is a multiauthor book, which has 47 chapters dealing with perinatal and pediatric HIV care. The book is dedicated to children who have lost their lives to HIV epidemic, is well written and has clear presentations from experts from all over the world. The book is very comprehensive and is divided into six sections, which includes basic sciences, clinical manifestations in children, HIV in newborn and adolescents, management issues and some significant miscellaneous issues like ethical and legal aspects. Different sections are well written and informative and are compiled with key messages at the end of each chapter. Since tuberculosis has epidemiological dimensions in our country and TB-HIV co-infection has been very high in many states, more chapters on HIV-TB co-infection and its impact in children could have been useful additions.

The book is recommended for postgraduates, physicians, pediatricians, obstetricians and researchers for understanding the complexities occurring due to HIV disease in pediatric and perinatal age groups. This hardcover bound book consists of 627 pages with excellent illustrations both in tabular form and in photographic plates and will be a useful treasure of knowledge for Medical College and Hospital libraries.

V.K. Arora
Executive Editor, IJT, India
E-mail address: vijaykumar1945@gmail.com

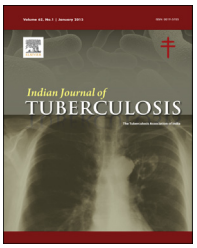
Available online 19 March 2015

0019-5707/$ – see front matter
http://dx.doi.org/10.1016/j.ijtb.2015.02.010

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.journals.elsevier.com/indian-journal-of-tuberculosis/



Abstracts

Pulmonary changes of pleural TB: Up-to-date CT imaging

Jeong Min Ko; Hyun Jin Park; Chi Hong Kim. *Chest* 2014; **146 (6)**:1604–11

Background: The objective of this study was to evaluate pulmonary abnormalities of pleural TB by CT scanning and to determine CT scan findings for the development of the paradoxical response (PR).

Methods: CT scans were performed for 349 patients with pleural TB (between 2008 and 2013). We excluded 34 patients with coexisting pulmonary disease (n = 13) or a totally collapsed lung (n = 21). We analyzed CT scans focusing on pulmonary abnormalities such as the presence of consolidation, cavitation, interlobular septal thickening, and micronodules and their distribution. In addition, we recorded the development of PR during follow-up and statistically analyzed differences in clinical and CT scan findings between patients with and without PR.

Results: A total of 270 of 315 patients (86%) had pulmonary abnormalities. Common CT scan findings were micronodules (n = 209 [77%]), interlobular septal thickening (n = 202 [75%]), and consolidation (n = 120 [44%]). Cavitation was seen in 49 patients (18%). Among 209 with micronodules, the nodules were in the subpleural region (n = 146 [70%]), peribronchovascular interstitium (n = 113 [54%]), and centrilobular region (n = 64 [31%]). PR occurred in 81 patients (26%), and patients with PR tended to be young, male, and without underlying disease ($P < .05$ by t test, Pearson χ^2 test). Subpleural micronodules were more common in patients with PR than in those without PR (Pearson χ^2, $P = .025$).

Conclusions: Pulmonary abnormalities are very common in pleural TB. The most common CT scan findings were micronodules in the subpleural and peribronchovascular interstitium and interlobular septal thickening, suggesting the lymphatic spread of TB. In addition, PR is not rare in patients with pleural TB, especially in young, previously healthy, male patients who show subpleural nodules on initial CT scans.

Long-term effects of a program to increase physical activity in smokers

Leandro C. Mantoani; Karina C. Furlanetto; Demétria Kovelis; Mahara Proença; Juliana Zabatiero; Gianna Bisca; Andréa Morita; Fabio Pitta. *Chest* 2014; **146 (6)**:1627–32

Background: Programs aimed at increasing physical activity in daily life (PADL) have generated growing interest to prevent the deleterious effects of physical inactivity. Recent literature has shown that a short-term protocol using pedometers increased PADL in smokers with normal lung function. However, the long-term effects of such a protocol were not yet studied. The objective of this study was to evaluate the results of 1-year follow-up after a program aimed at increasing PADL in smokers with normal lung function.

Methods: Twenty-four smokers were followed (15 men; mean [interquartile range (IQR)], 51 [41–57] years of age; BMI, 26 [22–29] kg/m^2; 20 [20–30] cigarettes/d). Subjects were assessed at baseline, immediately after completion of the program, and 1 year later for PADL, lung function, 6-min walking distance (6MWD), smoking habits, quality of life, anxiety, and depression. The 5-month program used pedometers and informative booklets as interventions.

Results: The gains achieved after the program were maintained in the long term: steps/d (postprogram vs 1-year follow-up, mean [IQR]: 10,572 [9,804–12,237] vs 10,438 [9,151–12,862]); 6MWD (625 [530–694] m, 88 [81–97] % predicted vs 609 [539–694] m, 89 [81–96] % predicted), anxiety (34 [26–41] points vs 35 [36–47] points) and depression (6 [2–9] points vs 5 [2–11] points) ($P > .05$ for all). One year after the program, 20% of the subjects had quit smoking.

Conclusions: In smokers with normal lung function, improvements in daily physical activity, exercise capacity, anxiety, and depression obtained through a 5-month program aimed at increasing physical activity are sustained 1 year after completion of the program. Furthermore, such a program can contribute to smoking cessation in this population.

Children exposed to multidrug-resistant tuberculosis at a home-based day care centre: A contact investigation

Garcia-Prats, A. J.; Zimri, K.; Mramba, Z.; Schaaf, H. S.; Hesseling, A. C. *The International Journal of Tuberculosis and Lung Disease* 2014; **18 (11)**:1292–98

Setting: In high tuberculosis (TB) burden settings, day care centres may be an underestimated source of exposure of children to infectious drug-susceptible (DS) and drug-resistant (DR) TB cases.

Objective: To describe the results of a contact investigation of children exposed to an adult with DR-TB at a South African home-based day care centre.

Design: Retrospective descriptive community-based cohort study.

Results: *Mycobacterium tuberculosis* resistant to isoniazid (INH), rifampicin and amikacin was cultured from the sputum of an adult index case residing in a home-based day care centre. Of 38 children aged <15 years identified during routine contact investigation, consent was obtained for 34; the median age was 3.9 years (IQR 2.9–5.2); 23/34 were aged <5 years, none were human immunodeficiency virus infected. The median contact score was

4/10, 8 had a reactive tuberculin skin test (<10 mm) and none had TB. Of the 34 study children, 24 received 6 months of DR-TB preventive therapy comprising ofloxacin, ethambutol and high-dose INH; 21 completed 12 months' follow-up and none developed TB.
Conclusions: TB at day care centres may result in many exposed young children with high TB contact scores, similar to household contact investigations. Active identification and initiation of preventive treatment may be able to avert DR-TB cases.

Initial experience of bedaquiline use in a series of drug-resistant tuberculosis patients from India

Udwadia, Z. F.; Amale, R. A.; Mullerpattan, J. B. *The International Journal of Tuberculosis and Lung Disease* 2014; **18 (11)**:1315–18
Drug-resistant tuberculosis (DR-TB) is a major problem both in India and worldwide. Newer drugs such as TMC-207 (bedaquiline) may have an important role to play in making up an effective drug regimen in such cases. There have been a few reports of bedaquiline use in a non-trial setting from Europe. Our series of five patients is the first series of DR-TB patients from India to receive bedaquiline. All five patients showed striking improvement, with microbiological conversion and an absence of notable adverse effects (e.g., prolonged QTcF), indicating the potential impact of this drug in such a population.

The disconnect between a national tuberculosis drug resistance survey and treatment outcomes: A lost opportunity

Click, E. S.; Chirenda, J.; Kibias, S.; Menzies, H. J.; Oeltmann, J. E.; Sentle, C.; Muribe, T.; Lere, T. D.; Makombe, R.; Bamrah, S.; Moore, B. K.; Cain, K. P. *The International Journal of Tuberculosis and Lung Disease* 2014; **18 (11)**:1319–22(4)
We linked results from the Fourth Botswana National Drug Resistance Survey (DRS), 2007–2008, to patient records from the national Electronic Tuberculosis Registry to determine treatment outcomes. Of 915 new patients, 651 (71%) had treatment data available. Completion or cure was achieved for 10/15 (67%, 95%CI 42–85) with isoniazid monoresistance, (6/16, 38%, 95%CI 18–61) with multidrug resistance, while 73% (391/537, 95%CI 69–76) were susceptible to first-line drugs. The analysis was limited because of unavailable treatment records and undocumented outcomes. Prospective analyses following DRSs should be considered to ensure adequate outcome data.

What is the role for Xpert® MTB/RIF in high-resource settings? Experience from a central London hospital

Gupta, R. K.; Lawn, S. D.; Booth, H.; Morris-Jones, S. *The International Journal of Tuberculosis and Lung Disease* 2014; **18 (11)**:1323–6
The role of Xpert® MTB/RIF for tuberculosis (TB) diagnosis remains to be clearly delineated in high-resource settings. At a London hospital, we evaluated a policy of selective assay use, with testing restricted to defined sub-groups of patients. Management was directly influenced in 30% of patients studied, including 'ruling-in' a TB diagnosis (leading to initiation of treatment for TB or for potential multidrug-resistant TB); negative assay results also helped support decisions for cessation of empirical anti-tuberculosis treatment or the safe initiation of other treatments such as immunosuppressant drugs. The benefits and pitfalls of this assay's use within high-resource settings are discussed.

Detection and management of drug-resistant tuberculosis in HIV-infected patients in lower-income countries

Ballif, M.; Nhandu, V.; Wood, R.; Dusingize, J. C.; Carter, E. J.; Cortes, C. P.; McGowan, C. C.; Diero, L.; Graber, C.; Renner, L.; Hawerlander, D.; Kiertiburanakul, S.; Du, Q. T.; Sterling, T. R.; Egger, M.; Fenner, L.; for the International epidemiological Databases to Evaluate AIDS (IeDEA).*The International Journal of Tuberculosis and Lung Disease* 2014; **18 (11)**:1327–36
Setting: Drug resistance threatens tuberculosis (TB) control, particularly among human immunodeficiency virus (HIV) infected persons.
Objective: To describe practices in the prevention and management of drug-resistant TB under antiretroviral therapy (ART) programs in lower-income countries.
Design: We used online questionnaires to collect program-level data on 47 ART programs in Southern Africa ($n = 14$), East Africa ($n = 8$), West Africa ($n = 7$), Central Africa ($n = 5$), Latin America ($n = 7$) and the Asia-Pacific ($n = 6$ programs) in 2012. Patient-level data were collected on 1002 adult TB patients seen at 40 of the participating ART programs.
Results: Phenotypic drug susceptibility testing (DST) was available in 36 (77%) ART programs, but was only used for 22% of all TB patients. Molecular DST was available in 33 (70%) programs and was used in 23% of all TB patients. Twenty ART programs (43%) provided directly observed therapy (DOT) during the entire course of treatment, 16 (34%) during the intensive phase only, and 11 (23%) did not follow DOT. Fourteen (30%) ART programs reported no access to second-line anti-tuberculosis regimens; 18 (38%) reported TB drug shortages.
Conclusions: Capacity to diagnose and treat drug-resistant TB was limited across ART programs in lower-income countries. DOT was not always implemented and drug supplies were regularly interrupted, which may contribute to the global emergence of drug resistance.

Elevated hepcidin at HIV diagnosis is associated with incident tuberculosis in a retrospective cohort study

Minchella, P. A.; Armitage, A. E.; Darboe, B.; Jallow, M. W.; Drakesmith, H.; Jaye, A.; Prentice, A. M.; McDermid, J. M. *The International Journal of Tuberculosis and Lung Disease* 2014; **18 (11)**:1337–9
Hepcidin inhibits ferroportin-mediated iron efflux, leading to intracellular macrophage iron retention, possibly favoring *Mycobacterium tuberculosis* iron acquisition and tuberculosis (TB) pathogenesis. Plasma hepcidin was measured at human immunodeficiency virus (HIV) diagnosis in a retrospective HIV-prevalent, antiretroviral-naïve African cohort to investigate the association with incident pulmonary and/or extra-pulmonary TB. One hundred ninety-six participants were followed between 5 August 1992 and 1 June 2002, with 32 incident TB cases identified. Greater hepcidin was associated with significantly increased likelihood of TB after a median time to TB of 6 months. Elucidation of iron-related causal mechanisms and time-sensitive biomarkers that identify individual changes in TB risk are needed.

Impact of three empirical anti-tuberculosis treatment strategies for people initiating antiretroviral therapy

Van Rie, A.; Westreich, D.; Sanne, I. *The International Journal of Tuberculosis and Lung Disease* 2014; **18 (11)**:1340–6

Background: Early mortality in people initiating antiretroviral treatment (ART) remains high. Empirical anti-tuberculosis treatment strategies aim to reduce early mortality by initiating anti-tuberculosis treatment in individuals at high risk of death from undiagnosed TB.
Methods: Using data from 16 913 individuals starting ART under program conditions, we simulated the impact of three empirical treatment strategies (two clinical trials and a pragmatic approach), assuming that 50% of early deaths and 100% of incident TB are averted in those eligible.
Results: Compared to starting anti-tuberculosis treatment on clinical or mycobacteriological grounds, 4.4–31.4% more individuals were eligible for anti-tuberculosis treatment, 5.5–25.4% of deaths were averted and 10.9–57.3% of incident TB cases were prevented under empirical anti-tuberculosis treatment strategies. The proportion receiving any anti-tuberculosis treatment during the first 6 months of ART increased from the observed 24.0% to an estimated 27.5%, 40.4% and 51.3%, under the PrOMPT, REMEMBER and pragmatic approach, respectively.
Conclusion: The impact of empirical anti-tuberculosis treatment strategies depends greatly on the eligibility criteria chosen. The additional strain placed on anti-tuberculosis treatment facilities and the relatively limited impact of some empirical TB strategies raise the question as to whether the benefits will outweigh the risks at population level.

Role of adenosine deaminase and the influence of age on the diagnosis of pleural tuberculosis

Abrao, F. C.; de Abreu, I. R. L. Bruno; Miyaki, D. H.; Busico, M. A. M.; Younes, R. N. *The International Journal of Tuberculosis and Lung Disease* 2014; **18 (11)**:1363–9
Objective: 1) To determine factors affecting adenosine deaminase (ADA) levels in pleural fluid (PF), and 2) to establish the optimal ADA cut-off level for a Brazilian population.
Design: ADA levels in PF of 309 patients were analysed to investigate pleural effusion. All patients were evaluated for age, sex and presence of tuberculosis (TB) based on a positive pleural biopsy. Differences in ADA levels between groups were analysed using Kruskal-Wallis one-way analysis of variance. Logistic regression analysis was also carried out to predict the occurrence of TB. ADA cut-off levels were selected using the receiver operating characteristic (ROC) curve.
Results: The mean PF ADA level was significantly higher in the tuberculous pleural group than in non-tuberculous pleural patients (63.3 ± 29 IU/l vs. 19 ± 31 IU/l, $P < 0.001$). There was a significant correlation between PF ADA levels and age: for patients aged <45 years, the ROC curve for ADA had an area under the curve of 0.91. An ADA level of 29 IU/l resulted in a sensitivity of 88.6% and specificity of 91.5%.
Conclusions: There is a significant negative correlation between PF ADA level and age. The use of a lower ADA cut-off reduces the number of false-negative results.

A four-year nationwide molecular epidemiological study in Estonia: Risk factors for tuberculosis transmission

Toit, K.; Altraja, A.; Acosta, C. D.; Viiklepp, P.; Kremer, K.; Kummik, T.; Danilovitš, M.; Van den Bergh, R.; Harries, A. D.; Supply, P. *Public Health Action* 2014; **4 (S 2)**:S34–S40
Setting: Estonia has a high proportion of multidrug-resistant tuberculosis (MDR-TB). It is important to link molecular and epidemiological data to understand TB transmission patterns.
Objective: To use 24-locus variable numbers of tandem repeat (VNTR) typing and national TB registry data in Estonia from 2009 to 2012 to identify the distribution of drug resistance patterns, *Mycobacterium tuberculosis* isolate clustering as an index for recent transmission, socio-demographic and clinical characteristics associated with recent transmission, and the distribution of transmission between index and secondary cases.
Design: A retrospective nationwide cross-sectional study.
Results: Of 912 cases with isolate and patient information, 39.1% of isolates were from the Beijing lineage. Cluster analysis identified 87 clusters encompassing 69.1% of isolates. The largest cluster comprised 178 isolates from the Beijing lineage, of which 92.1% were MDR- or extensively drug-resistant TB (XDR-TB). Factors associated with recent transmission were polyresistant TB, MDR- and XDR-TB, human immunodeficiency virus positivity, Russian ethnicity, non-permanent living situation, alcohol abuse and detention. XDR-TB cases had the highest risk of recent transmission. The majority of transmission cases involved individuals aged 30–39 years.
Conclusion: Recent TB transmission in Estonia is high and is particularly associated with MDR- and XDR-TB and the Beijing lineage.

Pattern of primary tuberculosis drug resistance and associated treatment outcomes in Transnistria, Moldova

Dolgusev, O.; Obevzenco, N.; Padalco, O.; Pankrushev, S.; Ramsay, A.; Van den Bergh, R.; Manzi, M.; Denisiuk, O.; Zachariah, R. *Public Health Action* 2014; **4(2)**:S64–S66
This cohort study assessed drug susceptibility testing (DST) patterns and associated treatment outcomes from Transnistria, Moldova, from 2009 to 2012. Of 1089 newly registered tuberculosis (TB) patients with available DST results, 556 (51%) had some form of drug resistance, while 369 (34%) had multidrug-resistant TB (MDR-TB). There were four cases of extensively drug-resistant TB. MDR-TB patients had poor treatment success (45%); human immunodeficiency virus positivity and a history of incarceration were associated with an unfavourable treatment outcome. This first study from Trans-nistria shows a high level of drug-resistant TB, which constitutes a major public health problem requiring urgent attention.

Sputum smear conversion and treatment outcomes for tuberculosis patients with and without diabetes in Fiji

Prasad, P.; Gounder, S.; Varman, S.; Viney, K. *Public Health Action* 2014; **4(3)**:159–63
Settings: Three tuberculosis (TB) treatment centres under the Fiji National Tuberculosis Programme.
Objectives: To determine the prevalence of diabetes mellitus (DM) among TB patients for the period 2010–2012, and to evaluate sputum smear conversion and anti-tuberculosis treatment outcomes, comparing patients with and without DM.
Design: A retrospective descriptive study using routinely collected data from the TB register and in-patient folders.
Results: Of 577 TB patients identified, information on DM was available for 567 (98%), of whom 68 (12%) had DM. Smear status at 2 months was available for 254 (82%) patients with sputum smear-positive pulmonary TB. The sputum smear conversion rate (from positive to negative) was equivalent in TB patients with and without DM (78% vs. 80%, $P = 0.66$). Anti-tuberculosis treatment

outcome information was available for 462 patients; the difference in outcome comparing successfully treated patients with those unsuccessfully treated was not statistically significant (91% in TB patients with DM vs. 84% in TB patients without DM, $P = 0.06$).
Conclusion: DM is common among TB patients in Fiji. Sputum smear conversion rates were not different in TB patients with and without DM; no difference in treatment success between the two groups was observed.

Changing from single-drug to fixed-dose combinations: Experience from Fiji

Mahadeo, R.; Gounder, S.; Graham, S. M. *Public Health Action* 2014; **4(3)**:169–73
Background: Fixed-dose combinations (FDCs) of first-line anti-tuberculosis drugs were introduced in Fiji in 2011, and there have been concerns about treatment response.
Objective: To evaluate the treatment response to FDCs among tuberculosis (TB) patients.
Methods: A retrospective cohort study was undertaken of treatment outcomes of new TB cases registered from January 2010 to April 2013 and weighing ≥30 kg. Sputum smear conversion of new sputum smear-positive cases and end-of-treatment outcomes of all cases were evaluated for those receiving FDCs and compared to outcomes with previous use of single-drug preparations.
Results: Among new TB patients, 240 received single-drug preparations and 259 received FDCs for the full duration of treatment. The groups were similar in terms of demographic and clinical characteristics. Treatment outcomes were available for 95% of cases. Unknown outcomes were more common in those receiving FDCs. When known, end-of-treatment outcome was the same in the two treatment groups and did not differ between TB types. Sputum smear conversion after the 2-month intensive phase of treatment was similar in the two treatment groups: 95% and 97%, respectively.
Conclusion: The introduction of FDCs in Fiji for the treatment of TB cases has not been associated with changes in treatment response.

Preliminary validation of an instrument to assess social support and tuberculosis stigma in patients' families

Arcencio, R. A.; de Almeida Crispim, J.; Touso, M. M.; Popolin, M. P.; Rodrigues, L. B. B.; de Freitas, I. M.; Yamamura, M.; Santos Neto, M. *Public Health Action* 2014; **4(3)**:195–200
Setting: Ribeirao Preto, Sao Paulo, Brazil.
Objective: To develop and validate a preliminary instrument for assessing social support and tuberculosis (TB) stigma in families of TB patients.
Design: A literature review on social support and TB stigma was used to generate the theoretical domains for the instrument. A focus group was then conducted with TB patients and their families to revise the domains. Reviewers were invited to judge the appropriateness of the items in the instrument. A cross-sectional survey was carried out among 110 family members to assess the factorial structure using principal component analysis and confirmatory factor analysis to assess construct validity. Reliability was assessed in terms of internal consistency using Cronbach's alpha.
Results: After semantic validation and a pilot study, 23 items were selected for the scale. Examination of the factorial structure of the 16 items that were factorable using principal component analysis led to the extraction of two factors. The 16-item instrument was assessed for construct validity with confirmatory factor analysis, which confirmed a model with four items for each dimension.
Conclusion: The study analysed the psychometric properties of an instrument that is still in its preliminary stages. Other studies on a similar scale in the Brazilian setting are required.

Factors affecting treatment outcomes in drug-resistant tuberculosis cases in the Northern Cape, South Africa

Elliott, E.; Draper, H. R.; Baitsiwe, P.; Claassens, M. M. *Public Health Action* 2014; **4(3)**:201–203
The Northern Cape Province has low cure rates (21%) for multi-drug-resistant tuberculosis (TB). We audited the programme to identify factors affecting treatment outcomes. Cases admitted to two drug-resistant TB units from 2007 to 2009 had data extracted from clinical folders. Unfavourable treatment outcomes were found in 58% of the 272 cases. A multivariable regression analysis found that male sex was associated with unfavourable outcome ($P = 0.009$). Weight at diagnosis ($P < 0.001$) and oral drug adherence ($P < 0.001$) were also associated with an unfavourable outcome; however, injectable drug adherence was not ($P = 0.395$). Positive baseline smear and human immunodeficiency virus positive status were not associated with unfavourable outcome. Shorter, more patient-friendly regimens may go a long way to improving adherence and outcomes.